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Giant Vesicles: Imitating the Cytological Processes of Cell Membranes

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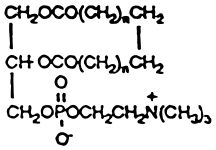
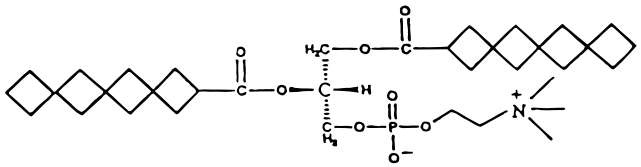
Introduction

The creation of life was, no doubt, intimately connected to the development of the membrane and its lipid constituents. Only after the membrane had evolved could the cell components be retained within a confined space; could nutrients diffuse in, and waste out, at controlled rates; could protective barriers be established against chemical and biological assaults; could membrane−membrane contacts maintain the integrity and limit the growth of multicellular populations; could membrane fusion direct fertilization and viral attack; and could membrane fission help steer the course of cell division. It is in admiration of the cell membrane that we write this Account.

Admittedly, the primary membrane component (the lipid) seems simple—even dull—compared to proteins and nucleic acids. But this view is unfair because the prosaic lipid does not operate as a single entity as do enzyme or RNA molecules; lipids instead form "molecular communities" via self-assembly. These communities are anything but simple or dull. They undergo fusion, fission, endocytosis, and budding; they incorporate channels that open and close; they allow the creation of pH gradients that drive ATP formation; they provide the framework for trapping light energy; they shuttle proteins from organelle to organelle; they are involved in regulating the activity of embedded enzymes; and the component lipid molecules undergo diverse relocations such as rotation, lateral diffusion, flip-flop, and phase transitions. Thus, the lipid is a particularly lively biological entity which, when aggregated into bilayers, is capable of remarkable chemical and physical processes that are vital to life.

This Account has been written by specialists in bioorganic chemistry and in biophysics. Each mode of thinking is, obviously, helpful to the membrane field for developing its full potential. Bioorganic chemists contribute nonquantitative skills including the synthesis of new lipids and other membrane-active compounds. Shown below are two examples of new lipids synthesized in the laboratory of one of the authors.[1,2]





Biophysicists, on the other hand, contribute a quantitative or theoretical slant. Shown below is an equation derived by the second author for the vibrational amplitude A of a membrane as a function of U and f (the amplitude and frequency of an applied ac field, respectively).[3]

$$A = (U/a)[b + c/(1 + df)]/f$$

But the reader should have no fear; we will present neither synthetic schemes nor derivations of equations. Instead, the plan is to prepare a text readable by people of diverse expertise—all the time focusing on a relatively new and highly useful membrane system: the giant vesicle.

Giant vesicles are closed membranes of spherical shape separating a water compartment from the bulk water in which the vesicle is suspended (Figure 1).[4] The membrane consists of double-tailed amphiphiles (e.g., phospholipids) that self-assemble into bilayers. Substantial amounts of additives such as cholesterol can also be present. Giant vesicles vary in diameter from 10 to 200 μm, and therein lies their most unique property: visibility under the light microscope. Other prominent members of the vesicle family (i.e., the 30−50 nm "SUV" (small unilamellar vesicle) and the 100−200 nm "LUV" (large unilamellar vesicle)) are only submicroscopic in nature.[5,6] Despite their invisibility, SUV and LUV systems have commanded the vast majority of attention in the past.

Unfortunately, SUV and LUV systems have several disadvantages: (a) SUVs and LUVs usually display a rather broad size distribution. This can present an experimental problem if the various sizes within a population do not have identical properties. (b) SUVs and LUVs, being of

Fredric M. Menger is Candler Professor of Chemistry at Emory University. His secretary claims he is a renegade living comfortably inside a scientist. This may be traceable to the first Menger of record, a knight in the Middle Ages who had disputes with the Vatican. It is not generally known that his full name would be "Fredric M. Menger von Wolfensgrüen" were it not for the historical fact that his grandfather Carl (founder of the Austrian school of economics) shunned the appellation. Karl Menger, the son of Carl, was a professor of mathematics at the University of Vienna, but he left Vienna in 1935 to become a professor at the University of Notre Dame in South Bend where Fred was born. Perhaps owing in part to his diverse legacy, grandson Menger has recently taken an excursion away from chemistry and completed two-thirds of a book entitled "The Thin Bone Vault" describing his ideas on the evolution of the human mind.

Miglena I. Angelova is an associate professor at the Institute of Biophysics in Sofia. She received her Ph.D. in 1988 with Dr. D. S. Dimitrov (now at the NIH) and has spent extended time at the CRPP/CNRS in Bordeaux with Professor P. Bothorel. She was the first to observe electroformation of giant vesicles as discussed in this Account.

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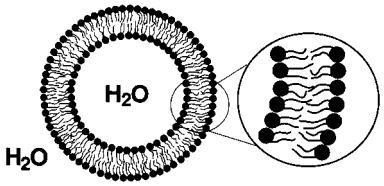


FIGURE 1. Structure of a unilamellar (i.e., one bilayer) vesicle. Giant vesicles have diameters of 10−200 μm.

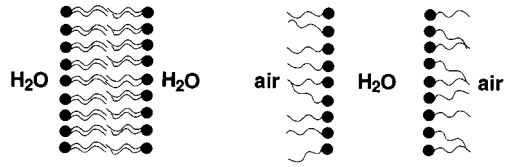


FIGURE 2. Comparison of a lipid bilayer (left) with a soap bubble film (right) showing completely different structures.

small diameter, possess a much higher curvature than do cell membranes. Since curvature affects lipid packing,[7] SUVs and LUVs are poor models for biological membranes. (c) Much information about SUVs and LUVs is difficult to obtain owing to their invisibility. It is not always easy, for example, to differentiate SUV fusion and SUV aggregation. Similarly, SUV/LUV systems do not lend themselves to studies of membrane injury and healing properties. (d) Although it is possible to uniformly bathe a sample of SUVs in a particular reagent of interest, it is not possible to expose one section of a SUV to a reagent and, thereby, create a localized asymmetry.

Giant vesicles have none of the above disadvantages. Under the microscope one can select any size of giant vesicle one wants. Membrane curvature is much more similar to that of cells. Injury and healing experiments, among others, now become possible via direct observation. And it is simple to inject a high concentration of a reagent at one point on a giant vesicle in order to create localized damage. In fact, reagents can be injected directly into the vesicle interior should this be desirable.[8] These are just a few reasons why one of us, after years of SUV and LUV experimentation,[9] has switched to giant vesicles. Meanwhile the other author, persuaded of their virtues, has investigated giant vesicles from the very beginnings of her research.[10]

The main body of this Account begins with a discussion of how to prepare giant vesicles. As will be seen, the authors have used different approaches. The Account then continues with a section on key properties of giant vesicles; this will include brief historical information. We end with sections on some favorite experiments of ours and the prospects for the future.

One final point by way of introduction: Giant vesicles should by no means be confused with "bubbles". Bubbles are largely aqueous films stabilized by surfactant molecules oriented in a manner "opposite" that in a lipid bilayer (Figure 2).[11] In contrast to soap bubbles, giant vesicles closely model biological membranes. Indeed, we will show how giant vesicles undergo "cytomimetic" processes including fusion, fission, endocytosis, growth, healing, and adhesion.

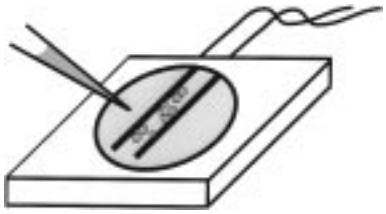


FIGURE 3. Diagram of the electroformation apparatus in which giant vesicles form on Pt wires under an external ac electric field.

Preparation

The "giant unilamellar vesicle" or "GUV" is the most desirable type of giant vesicle owing to its single bilayer shell. Multilamellar ("onion-like") vesicles are more complicated and less amendable to theoretical analysis. Classical methods for preparing GUVs all have disadvantages including an unwanted production of multilamellar vesicles, fibers, "sausages", and undefined lipid clumps. Among several published methods (drying−rehydration,[12] dialysis,[13] freeze−thaw,[14] and solid hydration[4]), F.M.M. selected solid hydration for initial studies. Since GUV photographs given herein have been prepared by solid hydration, the details of this particular procedure will be presented. It should be mentioned forthwith, however, that both laboratories are now using "electroformation", a method which requires a modest investment in equipment but which gives GUV populations virtually free from other morphologies. Electroformation will also be discussed in detail.

In the solid hydration method, a phospholipid or phospholipid mixture is dissolved in $CHCl_3$/MeOH, whereupon the solvent is removed under reduced pressure. Deionized water is added to the dry film and vortexed. Subsequent freezing and lyophilization affords a white fluffy powder. Giant vesicle formation is initiated by smearing 0.1 mg or less of the powder inside a Teflon O-ring cemented onto a microscope slide. Approximately 0.5 mL of water is used to cover the powder, and the sample is allowed to hydrate for at least 30 min at 20 °C (or at a temperature that exceeds the transition temperature T_m of the lipid).

Solid hydration has three appealing attributes: (a) It is simple and requires no special equipment. (b) In our hands it produces fewer "unidentified swimming objects" than do many other methods. (c) Vesicles are confined to a small region near the bottom of the slide from where they originate. Despite these advantages, we now recommend an extraordinarily clean and reliable alternate method developed by M.I.A.: electroformation.

Electroformation, invented by Angelova and Dimitrov,[15] utilizes a cell shown in Figure 3 containing two 0.5 mm diameter platinum wires spaced 0.5 cm apart. A 1 μL droplet of lipid solution (0.2−1.5 mg/mL in 9:1 $CHC1_3$/MeOH or Et_2O/MeOH) is deposited on the center of the wires. Evaporation of the solvent produces a film of 10−50 lipid bilayers on the platinum surface. An initial voltage of 0.2 V is then applied at an ac frequency of 10 Hz, and the cell (held at a temperature above the T_m of the lipid) is filled with water or aqueous solution. Keeping the

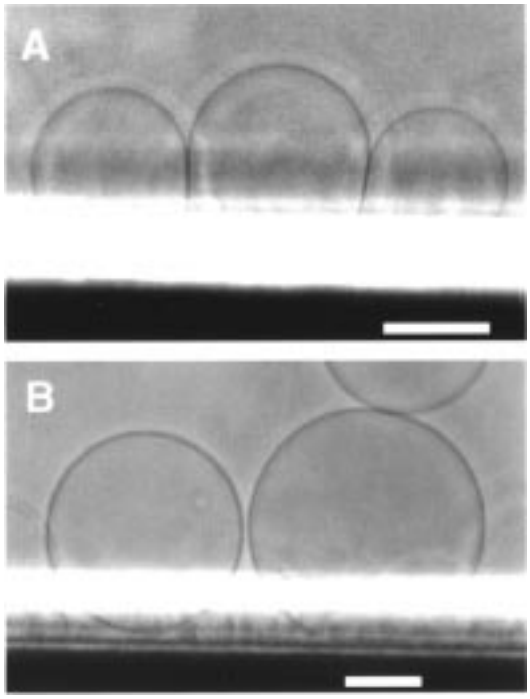


FIGURE 4. (A) Beginning stage of phospholipid vesicle formation on a Pt wire. (B) Fully formed GUVs. The bright horizontal line is a "shadow" of the Pt wire observed by phase-contrast microscopy. Bar = 50 μm.

frequency constant, the voltage is increased to 1−4 V depending upon the particular system. After a few minutes, mushroom-like structures are seen to bud from the wire (Figure 4A). Lateral fusion between these 10 μm structures produces large unilamellar spheres lined up like pigeons on a telephone line (Figure 4B). Further application of the ac field at gradually decreasing frequencies causes the vesicles to oscillate at greater amplitude and to detach from the wire except for a virtually invisible "umbilical cord". Such vesicles remain in proximity of the wire either as isolated entities or else in contact with one or more partners, both types being useful in various experiments. If one so wishes, a GUV may be completely removed from a wire by gentle pulling with a micropipet under suction. Electroformation, which requires 1 or 2 h, gives high-quality, 10−100 μm GUVs that do not further evolve once the voltage is terminated. They are stable for days or even weeks, especially if stored at low temperatures to reduce phospholipid hydrolysis.

The mechanism of GUV electroformation in an ac field has not been fully established. Since the growing vesicles were observed to vibrate at the same frequency as the applied field, the electric field may serve to create a gentle mechanical agitation that assists in the formation, fusion, and detachment of the vesicles.[16] Interestingly, however, a mechanical vibration of the coated wire, achieved with the aid of a piezoelectric device, does not generate GUVs. Thus, very likely it is electroosmotic motion of the water, creating an oscillating density gradient in a direction perpendicular to the wire, that induces bilayer separation and vesicle growth. One thing is clear: Electrofusion (i.e., the electrical breakdown and rehealing of membranes) is not a source of giant vesicles. Such a mechanism would require much stronger fields applied perpendicularly to the vesicle contacts.[17]

Electroformation can also be carried out using as electrodes two thin, conductive, and optically clear indium tin oxide coated glass plates separated by a 0.3 mm thick silicone spacer.[18] A patch of dry lipid film is formed on the bottom plate by depositing and drying a 2.5 μL drop of a lipid solution (0.5 mg in 1 mL of 9:1 chloroform/methanol). Similar to the wire apparatus, an ac field of 1 V and 10 Hz is applied between the plates, and lipid swelling is allowed to proceed for 2 h. The glass plate cell is useful when GUVs must be transferred by micropipet procedures.

In a recent paper, giant vesicles were prepared by rapidly evaporating under reduced pressure the organic solvent of a lipid suspended in water/$CHCl_3$/MeOH.[19] Although the vesicles were claimed to be unilamellar on the basis of electron microscopy (EM), the exact nature of the vesicles remains inconclusive owing to the magnification being too low to resolve single bilayers and to artifacts inherent to EM in the absence of freeze-fracturing. The paper does point out correctly that electroformation is limited to low ionic strengths (i.e., below 10 mM NaC1).[20] We have observed, however, electroformed vesicles in 50 mM sucrose and in various buffers (e.g., 10 mM Tris or 2-morpholinoethanesulfonic acid). Another professed disadvantage of electroformation cited in ref 19, namely, that the voltage cannot exceed 2 V, seems contrived since exceeding 2 V is unnecessary to secure excellent GUVs whose structure, as proved below, is unilamellar.

Properties

A phospholipid vesicle of 40 μm diameter (a convenient size with which to work) has an internal volume of 34 pL and about 1.4×10^{10} molecules in its bilayer. By way of comparison, a red blood cell and an amoeba are 7 and 100 μm in diameter, respectively.

Electroformation was shown to work well with a wide variety of phospholipids and phospholipid mixtures (although each system requires small adjustments in cell parameters, particularly the film thickness and voltage). These include (a) pure 1-palmitoyl-2-oleoylphosphatidylcholine (POPC); (b) pure 1-stearoyl-2-oleoylphosphatidylcholine (SOPC); (c) POPC (90%) + cholesterol (10%); (d) POPC (80%) + 1-palmitoyl-2-oleoylphosphatidylglycerol (10%) + cholesterol (10%); (e) POPC (80%) + cholesterol (10%) + fluorescein-labeled egg phosphatidylethanolamine (10%); (f) SOPC (90%) + dilauroylphosphatidylglycerol (10%); and (g) egg lecithin. Charged lipid films, as in (d), form more rapidly than neutral ones.

Perhaps the most important property of any vesicle is the number of bilayers. Phase-contrast light microscopy can distinguish a "unilamellar" vesicle from a multilamellar vesicle owing to the dense aspect of the latter.[4] Unilamellar is placed in quotes here because phase-contrast microscopy cannot really distinguish a single bilayer from a few bilayers. Giant vesicles are sometimes claimed to be GUVs (i.e., truly unilamellar), but this is often based on citations to past work of others who have,

in turn, likewise assumed their vesicles to be unilamellar. Strong evidence for unilamellarity in electroformed vesicles has been obtained by analyzing the thermal fluctuations of their membranes.[18] Stated simply, waves generated on the vesicle surface via Brownian motion of water molecules, observed visually under the light microscope, provide amplitude and wavelength data that generate a "bending elasticity modulus", a parameter that depends on the membrane thickness. It was found that the great majority of electroformed vesicles all have an identical bending elasticity, a result which is most reasonably taken to signify unilamellarity (one bilayer). Freeze-fracture electron microscopy, in which more than 90% of electroformed vesicles were cross-fractured to expose their interiors, showed that all were unilamellar.[21]

Freshly prepared vesicles are perfectly spherical, and they possess no visible thermal undulations, indicating the presence of a membrane tension. On standing for hours or days, depending upon the temperature, electroformed vesicles begin to undulate. It has been proposed that such undulations are caused by low levels of "lysolipids" (i.e., phospholipid which has lost one chain via hydrolysis to become a potent surfactant).[21] Undulations can also be triggered, for example, by exposing vesicles to small external sugar gradients. If one imposes even larger osmotic pressures (and this must be done gradually so as to avoid vesicle collapse), then the GUVs are transformed into flaccid vesicles with discoid or obloid shapes which, eventually, are transformed into small spheres.

Important photobleaching experiments of the Devaux group in Paris[21] demonstrated "vesicle connectivity" among GUVs assembled at the Pt wire of an electroformation cell. Thus, egg phosphatidylcholine, admixed with a small percentage of fluorescent-labeled phospholipid, produced a cluster of fluorescent GUVs, a few of which were bleached with a laser beam. The bleached vesicles recovered their fluorescence within 2 min, suggesting that the fluorescent lipid can move from vesicle to vesicle quickly. Since the phospholipid has no water solubility, its hopping across a water layer is not a viable possibility. Instead, the vesicles must be interconnected, possibly via invisible tethers. When the GUVs were removed from the Pt wire by suction, placed in a cluster, and partially photobleached, no fluorescent recovery occurred. Presumably the intervesicular tethers had been broken during the transfer. We now routinely remove GUVs from the wire prior to experimenting with them.

Svetina and Seks were among the first to propose that surface-area asymmetry between the two layers (or "leaflets") of a bilayer induces shape changes in the membrane.[22] Theoretical considerations have shown that less than 1% lipid redistribution from one GUV leaflet to the other suffices to trigger important alterations in surface curvature.[23] Submicroscopic vesicles are by comparison far less responsive to membrane asymmetries caused by lipid translocations and other environmental factors.

An interesting morphological change, attributable to membrane asymmetry, was observed with GUVs made from a synthetic cationic lipid, didodecyldimethylammonium bromide (DDAB).[4] When these GUVs were exposed to an external injection of 0.25 M NaOAc, the vesicles began to disintegrate immediately. This was not solely due to osmotic stress because 0.25 M NaCl failed to produce the effect. A better explanation invokes both the Svetina–Seks idea and the fact that strongly hydrated anions, such as acetate, bind only "loosely" to cationic membrane surfaces. Thus, when excess acetate was added externally to DDAB vesicles, the acetate ion-exchanged with bromide to produce an outer leaflet that was more highly dissociated from its counterions than was the inner leaflet (Figure 5). Owing to the resulting headgroup–headgroup repulsion, the outer leaflet expanded relative to the inner one, creating an asymmetry that would be expected to promote curvature. The result was the expulsion of small vesicles and the eventual disappearance of microscopically visible structures.

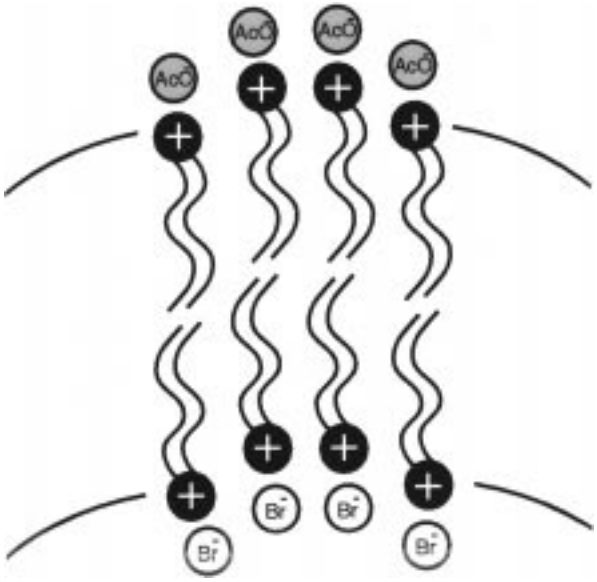


FIGURE 5. Asymmetrically solvated GUV, with acetate on the outside and bromide on the inside, leading to a curvature increase.

The shape of a vesicle can also be modified by imposing an asymmetric lipid composition. Thus, when a flaccid GUV is exposed externally to a single-tailed lyso/phospholipid, the compound will initially penetrate only into the outer leaflet. Before the lysolipid has had an opportunity to "flip-flop" into the inner leaflet, the GUV will transform into two smaller connected spheres via a "budding" process.[21] Such an increase in spontaneous curvature, reflecting the compositional asymmetry of the membrane, has been examined theoretically by several groups.[24,25]

Temperature effects on membrane morphology have also been explained in terms of the Svetina–Seks model. For example, a GUV composed of dimyristoylphosphatidylcholine (DMPC) changed from spherical (27.2 °C) to an ellipsoid (36.0 °C) to various pear shapes (37.5–40.9 °C).[26] All these morphologies were stable and reversible. A further temperature increase of 0.1 °C to 41.0 °C created what appears to be an attached "bud" on the verge of becoming a separate daughter vesicle (Figure 6). It was speculated that temperature-dependent shape transitions

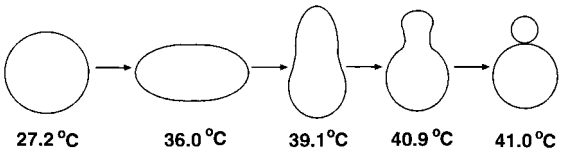


FIGURE 6. Shape transformations of a phospholipid GUV as a function of temperature.

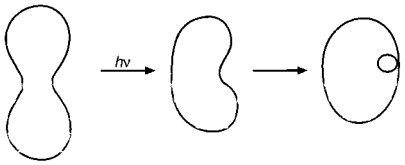
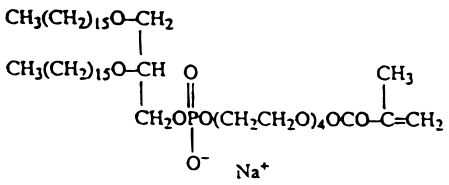


FIGURE 7. A photochemically induced endocytosis of a GUV composed of two phospholipids, one of which is polymerizable.

can be qualitatively explained in terms of the two leaflets of the bilayers having slightly different thermal expansitivities.

Giant vesicles, prepared from a 4:1 mixture of DMPC and the polymerizable phospholipid shown below, were UV irradiated to induce polymerization.[27]



In a sort of photochemically induced endocytosis, the vesicles invaginated as schematically portrayed in Figure 7. Two assumptions were invoked to explain this phenomenon: (a) The polymerizable lipid concentrates preferentially in the outer leaflet where there is slightly more room to accommodate the bulky headgroup. (b) Polymerization diminishes the total molecular area occupied by the membrane additive. If both these factors are operative, then polymerization will shrink the outer leaflet more than the inner one, and the composite bilayer distorts inwardly to create structures with increased curvature.

Although GUV membranes are easily deformed when not under tension, they possess a considerable tensile strength. Membrane stretching is resisted by the van der Waals attraction among the roughly parallel hydrocarbon chains in the bilayers. In apparent contradiction to this assertion, we have been able to insert a micropipet into a vesicle and to inject water so as to substantially expand the GUV volume like a balloon.[8] On closer inspection, however, one sees on the surface of the GUV (which was made by solid hydration and not by the "cleaner" electroformation method) a clump of amorphous lipid which, no doubt, was supplying lipid to the growing GUV bilayer.

Injecting into a phospholipid GUV, first carried out in our laboratory, is not a difficult procedure as it turns out. Thus, one first fills a micropipet of $<1\ \mu$m outer diameter with a 300−1000 fL plug of sample. The pressure on the pipet, provided by a "picoinjector", should be set sufficiently high so as to prevent the plug from moving inwardly by capillary action and yet not so high as to expel

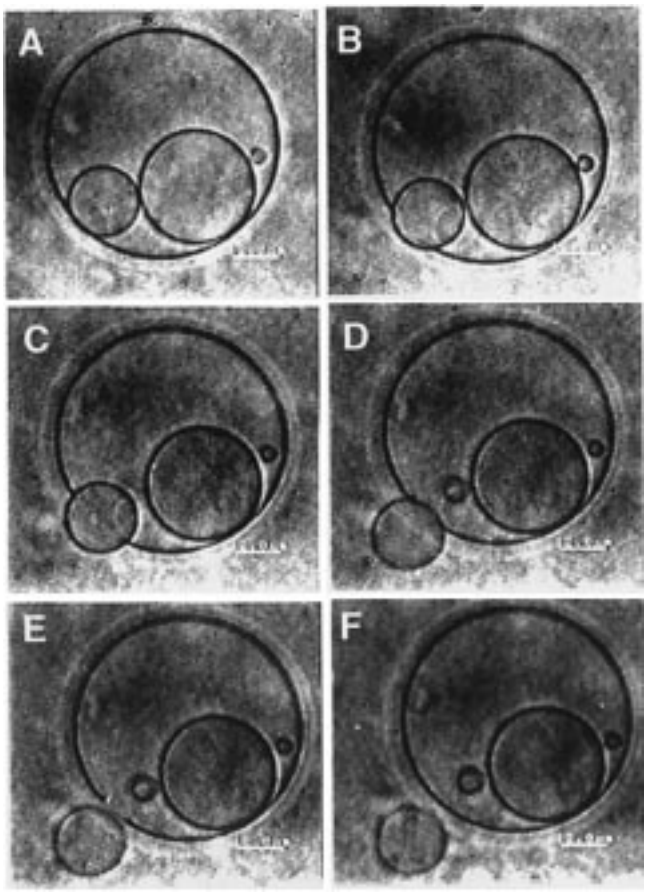


FIGURE 8. "Birthing" of a vesicle induced by octyl glucoside (phase-contrast microscopy; scale bar = 12 μm). The process takes 12 s from start to finish. Note gap in large vesicle in (E).

the sample. Once inside the vesicle interior (a feat accomplished with the aid of a "micromanipulator"), the micropipet is subjected to a 3 psi increase in pressure which shoots the sample plug into the GUV. Penetrating the membrane, which is sometimes a problem, can often be facilitated by spraying the GUV with a small portion of the sample prior to entry of the micropipet. Note that many procedures helpful for GUV research (e.g., phase-contrast microscopy, making micropipets, filling them, directing them into vesicles, etc.) cannot be described here in detail, but they are readily learned and carried out using commercially available equipment well-known to cytologists.

Healing of damaged membranes has been a major interest of ours for biomedical reasons. Here again GUVs are more useful than the popular submicroscopic vesicles where damage and repair cannot be directly visualized. Most of our initial experiments in the area have shown a fast healing of holes that are created in GUVs by physical or chemical means. For example, a GUV membrane, weakened by exposure to octyl glucoside, will allow smaller vesicles inside the GUV to pass through its bilayer into the exterior medium (Figure 8).[4] This "birthing process" creates, of course, a hole in the GUV which heals instantaneously. Since the edge of a hole exposes hydrocarbon chains to the water, there is a strong tendency for the defect to seal itself.

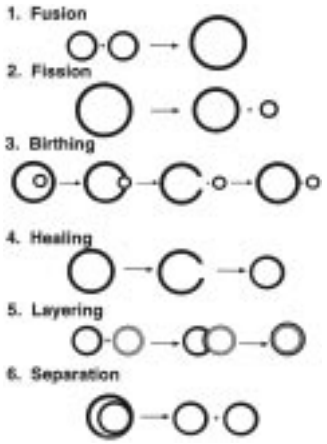


FIGURE 9. Cytomimetic processes observed in our research.

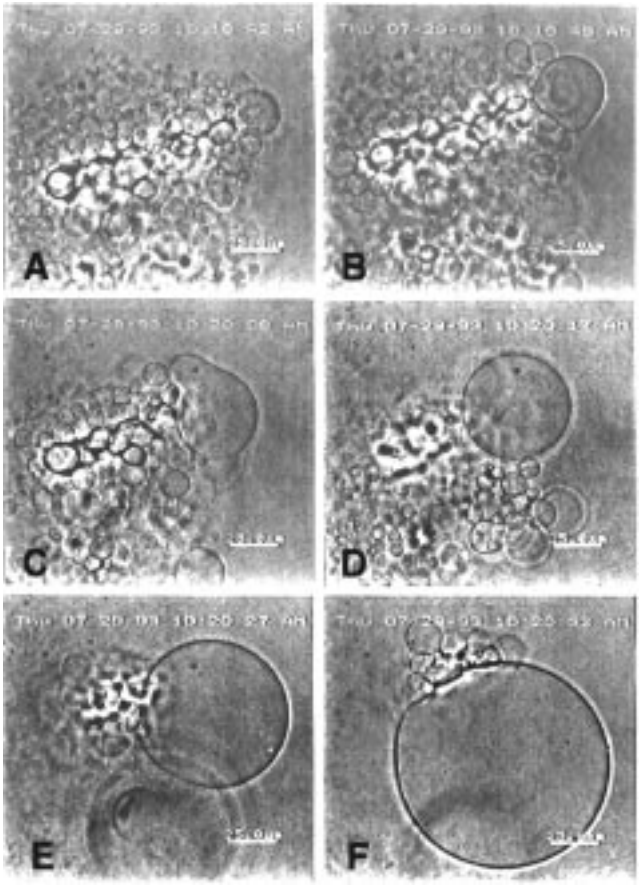


FIGURE 10. Sodium cholate-induced foraging of a DDAB GUV. The entire process takes less than 1 min. Scale bar = 25 μm.

Partial polymerization of lipids within the bilayers can enhance the lifetime of pores. For example, a GUV was exposed to a 1 ms electric pulse (ca. 0.5 kV/cm).[28] A hole formed on the side of the GUV facing the anode, with lipid material being ejected from the membrane (a process called "electroporation"). The GUV resealed itself within 0.7 s and restored its original spherical shape in 3.7 s. If, however, the GUV was comprised of a partially polymerized lipid, the structural rigidity of the membrane was enhanced, and the electroporation-created puncture wounds remained and were stable for up to 20 min.

An electroformed GUV, containing a substance within its cavity, was adsorbed onto a highly porous surface (i.e., a pollen grain).[29] Thereupon the GUV became permeable to the substance which departed the GUV and entered the porous surface. Apparently, portions of the GUV membrane, which lay unsupported over the pores, sagged into those pores. Consequently, the membrane was stretched at various points and rendered permeable.

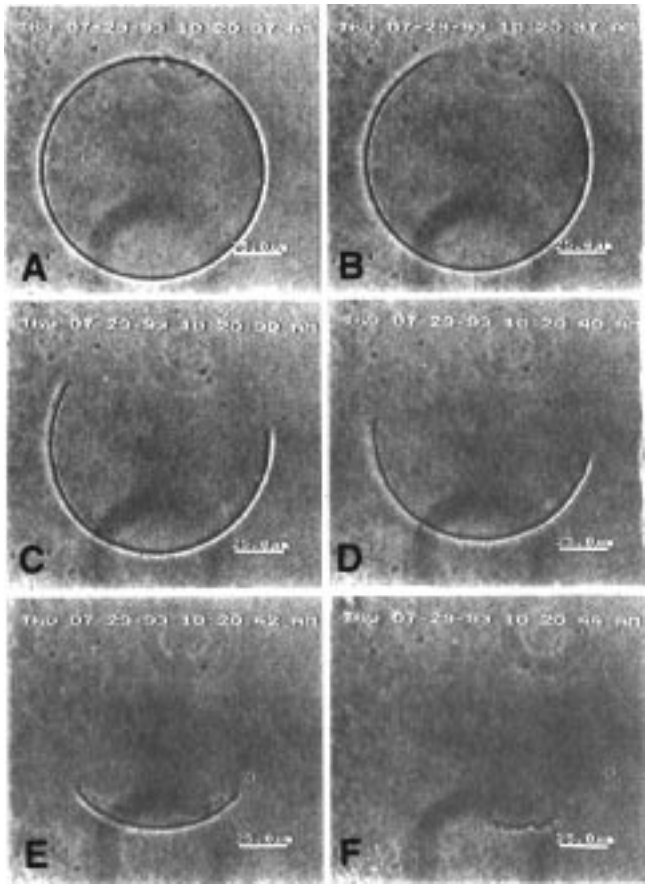


FIGURE 11. Decay of GUV immediately following foraging seen in Figure 10. Dissolution takes only 6 s.

GUV systems composed of phospholipids can also be perturbed by external or internal microinjection of phospholipase-A_2, an enzyme that hydrolyzes off one of the two lipid chains from phospholipids. As with most GUV research, the resulting transformations were monitored in real time by light microscopy and recorded by video analysis.[30] Addition of the enzyme to the outside of the vesicle caused it to burst, whereas injection of the enzyme inside the vesicle resulted in a slow and constant decrease in size until the structure became submicroscopic. The marked difference between external and internal injection of the enzyme is not understood. Perhaps the difference is related to the ability of the lysolipid or lysolipid/phospholipid complexes to diffuse away from the GUV only when they are generated externally; internally formed enzyme products are confined within the GUV.

One final property will be mentioned in this section: membrane viscosity. This parameter has recently been estimated in an experiment that allows a polystyrene sphere to attach to a 50 μm GUV.[31] A polystyrene sphere of 1−3 μm diameter, which usually embeds itself across

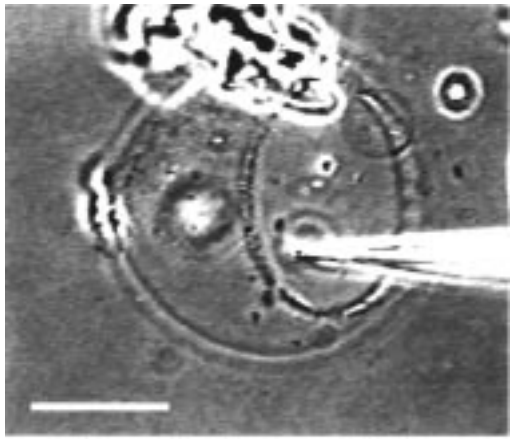


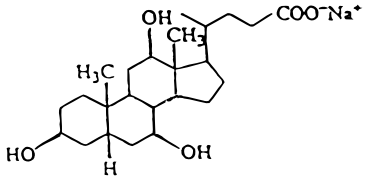
FIGURE 12. A hole formed in a DDAB GUV by external injection of KI to create a "nanocup".

the vesicle contour, is small enough to undergo visible Brownian motion over the GUV surface. Without going into a difficult mathematical analysis, we can simply mention the possibility of extracting the membrane viscosity from the random walk excursions of the particle. The surface shear viscosity of SOPC bilayers in the fluid state was determined to be $(3-8) \times 10^{-6}$ surface poise.

Selected Experiments

The title of this Account implies that giant vesicles can undergo membrane transformations that mimic those of living cells, and this is indeed the case. Figure 9 shows schematically a few of the cytomimetic phenomena observed in the course of our initial GUV studies.[4,8] These studies, incidentally, involved mostly GUVs made of DDAB because they lent themselves well to preparation by the solid hydration method. As mentioned previously, this method has now largely been supplanted by electroformation of phospholipid vesicles. In this section, we cite three experiments to illustrate typical GUV phenomena.

A. Vesicle Foraging.[4] A cluster of small vesicles was exposed to 50 μL of 5 mM cholate (a member of the bile salt family of steroids that solubilizes fatty material in the gut).



As seen in the phase-contrast photomicrographs in Figure 10, a GUV in the upper right-hand corner then begins to consume its neighbors. With each small vesicle that is consumed, the foraging GUV grows in diameter. When vesicular "food" is no longer available, the GUV undergoes a surprising transformation: It disintegrates in a few seconds by continuous erosion of the vesicle periphery (Figure 11). The "foraging, growth, and death" sequence likely involves defect formation in the bilayer upon adsorption of the amphiphilic bile salt. Small vesicles can fuse with the GUV at defect sites, and the GUV grows accordingly. After all the small vesicles are gone, a defect allows quick destruction of the membrane as bile salt micelles in the water remove lipid from the edge of the defect where hydrocarbon chains are exposed.

B. Nanocups.[8] We have referred earlier to the fact that GUVs normally heal their small injuries in a matter of seconds or less. The healing of chemically induced pores

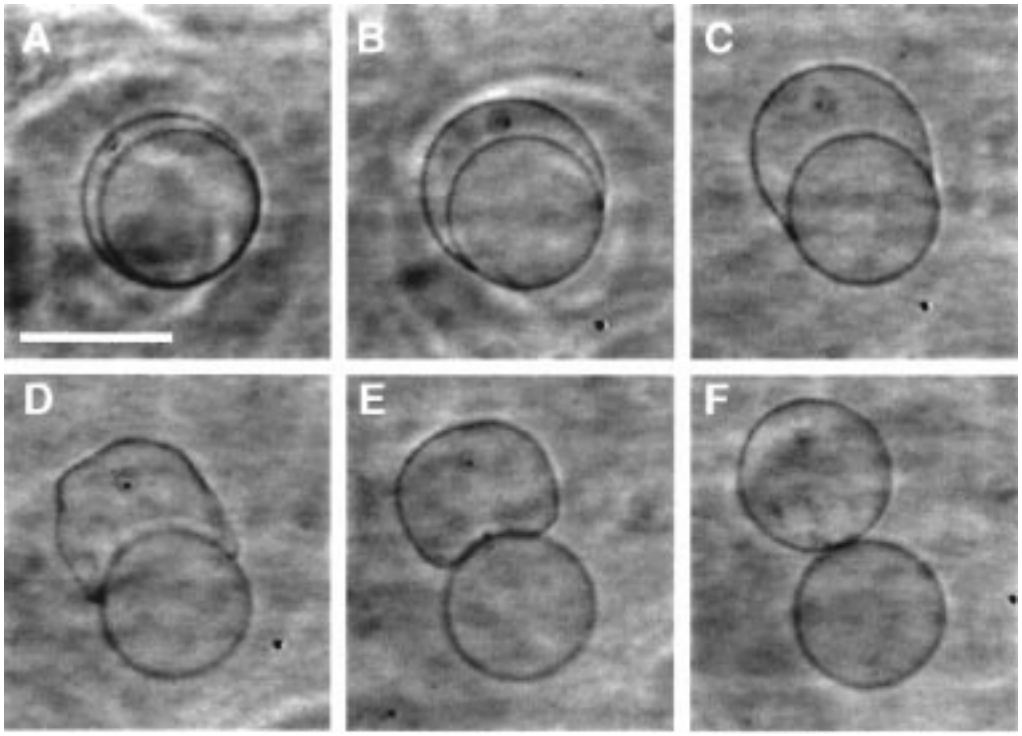
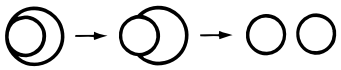


FIGURE 13. Separation of a binate vesicle, caused by osmotic stress, over the course of 3 min. Note undulation in lower left photo. Scale bar = 50 μm.

can be delayed if a precipitate is formed at the edge of the pore. A case in point is the hole formed on the surface of a DDAB/cholesterol GUV by external injection of KI, thereby creating a so-called "nanocup" (Figure 12). Insoluble particulate matter (presumably the iodide salt of the lipid) is visible at the lip of the circular defect, accounting in part for a retarded healing process that requires up to 1/2 h. Figure 12 portrays nicely the true spherical nature of the giant vesicles.

C. Induced Separation of a Binate Vesicle.[32] Two "binate" vesicles (consisting of one vesicle within another of slightly larger size) often have a common area of contact as shown below.



When a binate system was subjected to an osmotic pressure change by dilution with deionized water, the two vesicles separated from each other (Figure 13). Since the initial and final surface areas and volumes can be quantified, we had a prime opportunity to monitor the translocation of lipid and water during the morphological change. Thus, the volume of the outer vesicle in the initial binate system was 90.0 pL. The system then separated into two vesicles of 54.3 and 58.6 pL volume, for a total of 112.9 pL. Clearly, 22.9 pL of water must be supplied to effect the transformation. The need to provide external water is evident in Figure 13D, showing a flaccid and strongly undulating intermediate. Since the permeation rate of water through bilayers is generally much too slow to explain the observed influx, it is likely that water enters via a gap at the vesicle/vesicle juncture. Surface area data on the vesicles before and after the separation indicate that the two vesicles in the binate system share a common membrane (a fact that is difficult to discern from phase microscopy alone).

The Future

It is apparent from this Account that the biomembrane, with all its remarkable gymnastics vital to life, is a mystery largely because the relationship of self-assembly to the properties of lipid communities is not well understood. In the future, GUVs may provide important information on this topic owing to several attributes: (a) GUVs possess a membrane with a realistic, cell-like curvature. (b) As is not the case with complex biomembranes, the GUV components can be controlled and varied systematically to allow definitive experiments on properties vs composition. (c) As is not the case with submicroscopic vesicles (which have been the focus of most previous model-membrane research), one can directly visualize membrane transformations, an ability that is critical for assessing many membrane phenomena such as healing and budding. (d) GUVs can be readily manipulated, e.g., bestowed with different layers, injected with chemical and biological entities, pierced with holes, etc.

A major challenge in future GUV research, now that many technical problems have been solved with the advent of electroformation and other methods,[33] remains that of relating membrane transformations to molecular events. Progress here will surely continue as organic chemists, physical chemists, biochemists, biophysicists, and physicists each contribute their special talents.

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