# Chemically-Induced Birthing and Foraging in Vesicle Systems

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Unraveling the mysteries of molecular organization is now becoming one of the major endeavors of organic chemistry.[1] Recently, we viewed molecular assemblies by light microscopy in a study of chemically-induced aggregation, budding, and fusion in giant vesicles.[2] Superficially, the processes resemble those of living cells.[3] In the ensuing paper, we first describe a new and efficient way to prepare giant unilamellar vesicles (GUVs) from a synthetic lipid. These vesicles, 10–100 μm in diameter and observable under the light microscope, were exposed to various surfactants with remarkable results. Thus, octyl glucoside induces the "birth" of a vesicle in which a small vesicle, inside a much larger one, forces its way to the outside; the resulting injury to the "parent vesicle" then heals spontaneously. A bile salt, on the other hand, causes a "feeding frenzy" in which a vesicle gobbles up its neighbors until it ultimately bursts.

Previously published methods for GUV production[4] created an excessive number of multilamellar structures with our particular lipid, didodecyldimethylammonium bromide (DDAB). We therefore devised a more suitable procedure: DDAB (0.1–0.2 mg) was placed within a 14-mm-i.d. rubber washer cemented onto a glass slide. The powder was then immersed in 450 μL of water and allowed to hydrate. (The amounts of DDAB and water are important; too much DDAB gives multilamellar vesicles; too little results in only a few vesicles visible by light microscopy.) Hydration begins instantly as seen by worm-like tubules forming at the surface of the solid particles. After about 5 min, one observes many grape-like clusters, some of which degrade into small vesicles (5–10 μm). Remaining clusters develop (*via* a poorly understood fusion process requiring about 2 h) into giant unilamellar vesicles. These were easily distinguishable, by phase-contrast microscopy, from the small population of more opaque multilamellar vesicles. The low-energy input of our method is critical to its success.[5]

Vesicles were examined at 100×, 200×, and 400× under phase-contrast illumination using a Nikon Diaphot-TMD inverted microscope connected, in sequence, to a Dage-MTI CCD-72 solid-state camera, Hamamatsu Argus-10 image processor, Panasonic AG-1960 SVHS videocassette recorder, Hitachi black and white monitor, and Mitsubishi CP100U printer.

Surfactant/GUV interaction attracted our attention owing to the widespread use of surfactants in solubilizing biomembrane components.[6] M. N. Jones[7] proposed that surfactants bind to phospholipid membranes by means of hydrophobic forces. When saturation levels are reached, the bilayer begins to expel mixed micelles composed of lipid plus surfactant. Ueno and Akechi,[8] who observed 200-nm vesicles by freeze fracture electron microscopy, found that octyl glucoside transforms a vesicle into a cluster of 20–30-nm spheres (resembling a raspberry). These tiny particles depart one-by-one from the surface to become, soon thereafter, solubilized in the water as mixed micelles. We know of no example in which vesicle destruction by a surfactant has been observed by light microscopy.[9]

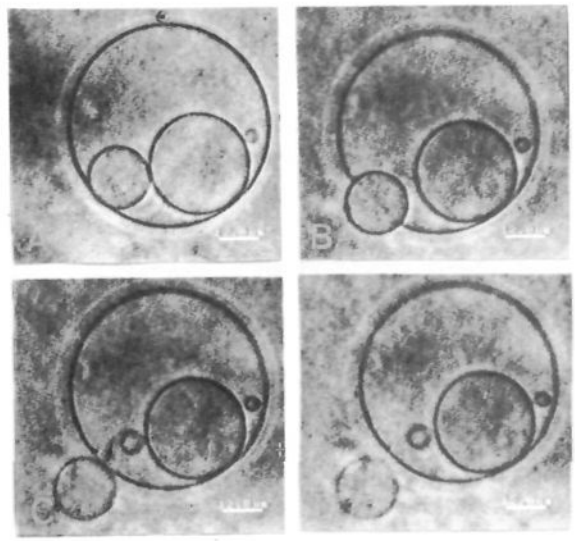

**Figure 1.** "Birthing" of a vesicle (800×, phase-contrast) induced by octyl glucoside. Elapsed time: A to B, 11 s; B to D, less than 1 s. The arrow in C points to the gap in the "parent vesicle" that forms upon the exit of the smaller vesicle. The wound is healed in D.

Adding 25 μL of 5 mM octyl glucoside[10] to a standard GUV preparation (20–21 °C), giving a surfactant concentration well below its cmc of 25 mM, caused minimal vesicle destruction in the first hour. The most striking observation was a "birthing process" involving the occasional GUV that possessed a smaller vesicle within it. Under the influence of octyl glucoside, the entrapped vesicle can force its way through the bilayer wall of the "parent vesicle" (Figure 1). A gap is created in the parent vesicle that heals instantly (Figure 1C,D). The sequence of events was observed repeatedly with every preparation. Light microscopy can obviously capture dynamic events that are difficult to detect by the more commonly used electron microscope. Moreover, artifacts from drying, metal coating, etc., a problem in electron microscopy, are avoided.

Combining the results from both light microscopy and electron microscopy,[8] we surmise that octyl glucoside weakens the cohesion among the organized DDAB molecules, perhaps *via* the formation of weakly associated spherical particles. The membrane subunits are able to temporarily separate from one another and, thereby, allow the passage of an entrapped vesicle. Evidence for this model came from experiments using higher octyl glucoside concentrations (10–25 mM) that rapidly destroy the GUVs. During the process, 0.1–1.0-μm particles are seen to speckle the vesicle surface (not shown). These particles (which appear from bright-field microscopy to be either solid or multilamellar lipid) break away while, simultaneously, the diameter of the GUV diminishes at a rate dependent on the octyl glucoside concentration.

The dramatic effect of octyl glucoside on the giant vesicles led us to investigate a natural surfactant, sodium cholate. Cholate is a member of the bile salt family of steroids used to solubilize fatty material in the gut. Addition of cholate (50 μL of 5 mM)

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(7) Jones, M. N. *Chem. Soc. Rev.* **1992**, *21*, 127.
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(9) For previous publications on GUV systems, see: Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113. Fricke, K.; Sackmann, E. *Biochim. Biophys. Acta* **1984**, *803*, 145. Sackmann, E.; Duwe, H.-P.; Engelhardt, H. *Faraday Discuss. Chem. Soc.* **1986**, *81*, 281.
(10) The 25 μL was distributed evenly in tiny droplets over the surface of the GUV sample while trying to minimize agitation.

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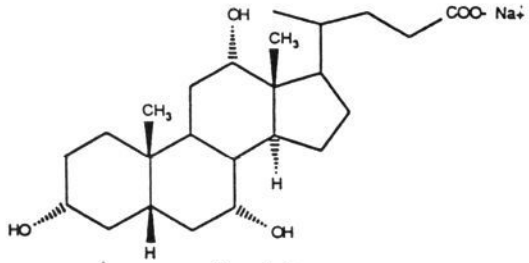


sodium cholate

to GUV preparations induced a rapid fusion process among the vesicles. As seen in Figure 2A,B, the GUV in the upper righthand corner "consumes" the smaller vesicles in the grape-like cluster beside it. The foraging vesicle grows in diameter with each small vesicle that is consumed. When small vesicles no longer exist in proximity of the foraging vesicle, an unexpected and previously unreported event occurs: The large vesicle disintegrates by a sequential ejection of lipid molecules (Figure 2C,D), a process that takes only a few seconds. It is as if a defect in the GUV created an "edge" of exposed lipid molecules. Lipid molecules are then continuously solubilized from this defect by the cholic acid until the vesicle bilayer disappears. Remarkably, the original spherical shape is retained throughout the process. The products of the membrane dissolution are submicroscopic and may well be the mixed micelles postulated by others.[7,8] Eventually, crystals of DDAB/cholate fall out of solution.

It should be noted that another steroid, cholesterol, has no such effect on the GUVs. In fact, 30% cholesterol incorporated into the DDAB bilayer actually stabilizes the vesicles.

Had the cholate induced foraging, growth, and fission (instead of foraging, growth, and decay), then a true self-replicating system would have been in hand. This remains one of our goals in the GUV research.

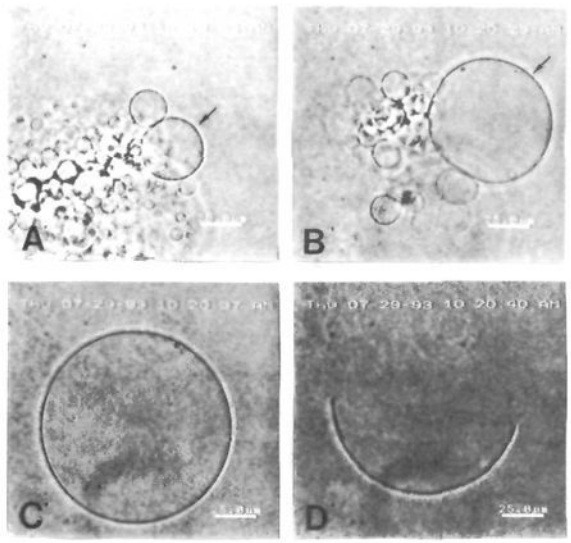


**Figure 2.** The cholate-induced "foraging" of a DDAB giant vesicle (arrow in photo A) to grow into a much larger vesicle (arrow in photo B). The vesicle continues to grow until the "food" supply is exhausted (photo C) whereupon the vesicle decays from the exposed edge to disappear. Photo D captures an instant in time where the vesicle is still half-intact. All photos are phase-contrast at 400×.

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