1 Preparation methods for giant unilamellar vesicles

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Lipids need your guidance for forming giant vesicles.

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1.1 GIANT UNILAMELLAR VESICLES

1.1.1 THE BIRTH OF A NEW BIOPHYSICAL PLATFORM

"A drop of water or sucrose solution was then placed on the glass slide, and seemed to seep in between the phospholipid lamellae. The swollen phospholipid membranes formed long fingerlike projections attached at one end to the glass slide. Eventually these membranous structures became detached and rounded up to form vesicles" (Figure 1.1).

By reporting their first observations of the gentle swelling of the strata of bilayers of phosphatidylcholine (PC) phospholipids in an aqueous solution, Reeves and Dowben deliberately initiated a new approach to the science of lipid membranes (Reeves and Dowben, 1969). The community working with giant liposomes largely shares the motivations for such an approach because it allows studying of the behavior of *single* lipid bilayer assemblies of a cell size, under conditions where membrane composition and the influence of the environment are under strict control. Indeed, as the bimolecular leaflet proposition of Danielli and Davson (1935) became largely accepted, methods started to be developed to study the fatty bilayer. By the 1960s, thin films (also known as black films) formed by anchoring a mixture of organic solvent and lipids to a small plastic diaphragm, thus separating two aqueous regions, were already used to study bilayer permeability to ions (Mueller et al., 1963). Moreover, the limits of the approach singled out by Reeves and Dowben are still recognized today: The bilayer thus formed is not only in direct contact with the remaining organic solvent but also under considerable tension. A different route was taken by Alec Bangham, the "father of liposomes" and his collaborators and by others (Bangham and Horne, 1964; Bangham et al., 1965; Papahadjopoulos and Miller, 1967), who studied the structure and permeability of "multilamellar lipid spherules" then recognized as "fragments of tubular myelin figures which form spontaneously when dried lipids swell in an aqueous medium." The tubular structures were called "myelin figures" given that such types of tubular molecular assemblies were originally found to form from myelin (Neubauer, 1867), a phospholipid-rich heterogeneous waxy material that surrounds the axon of most of the nerve cells of vertebrates (Deber and Reynolds, 1991) (for example images, see Sakurai and Kawamura, 1984). The key contribution of Reeves and Dowben was then to control the spontaneous swelling conditions of the dry lipids such that one obtains "phospholipid vesicles of pre-determined composition several microns in diameter bounded by walls one or a few bilayers thick" (Reeves and Dowben, 1969).

Interest in these single-walled vesicles that could be visualized under an optical microscope grew rapidly over the next decade (Papahadjopoulos and Kimelberg, 1974), and prompted studies such as those of Helfrich, who investigated for the first

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