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LIPOSOMES, DIMITRI PAPAHADJOPOULOS, AND US

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INTRODUCTION

Dimitri Papahadjopoulos has played a leading role in developing the science and technology of liposomal systems. As a result, he has influenced the careers of most scientists in the field, ourselves amongst them. It is a real pleasure to acknowledge this influence in research areas we have pursued. These include procedures for making liposomes, the use of liposomes as model systems to examine membrane fusion procedures as well as our development of liposomes as carriers in drug delivery applications.

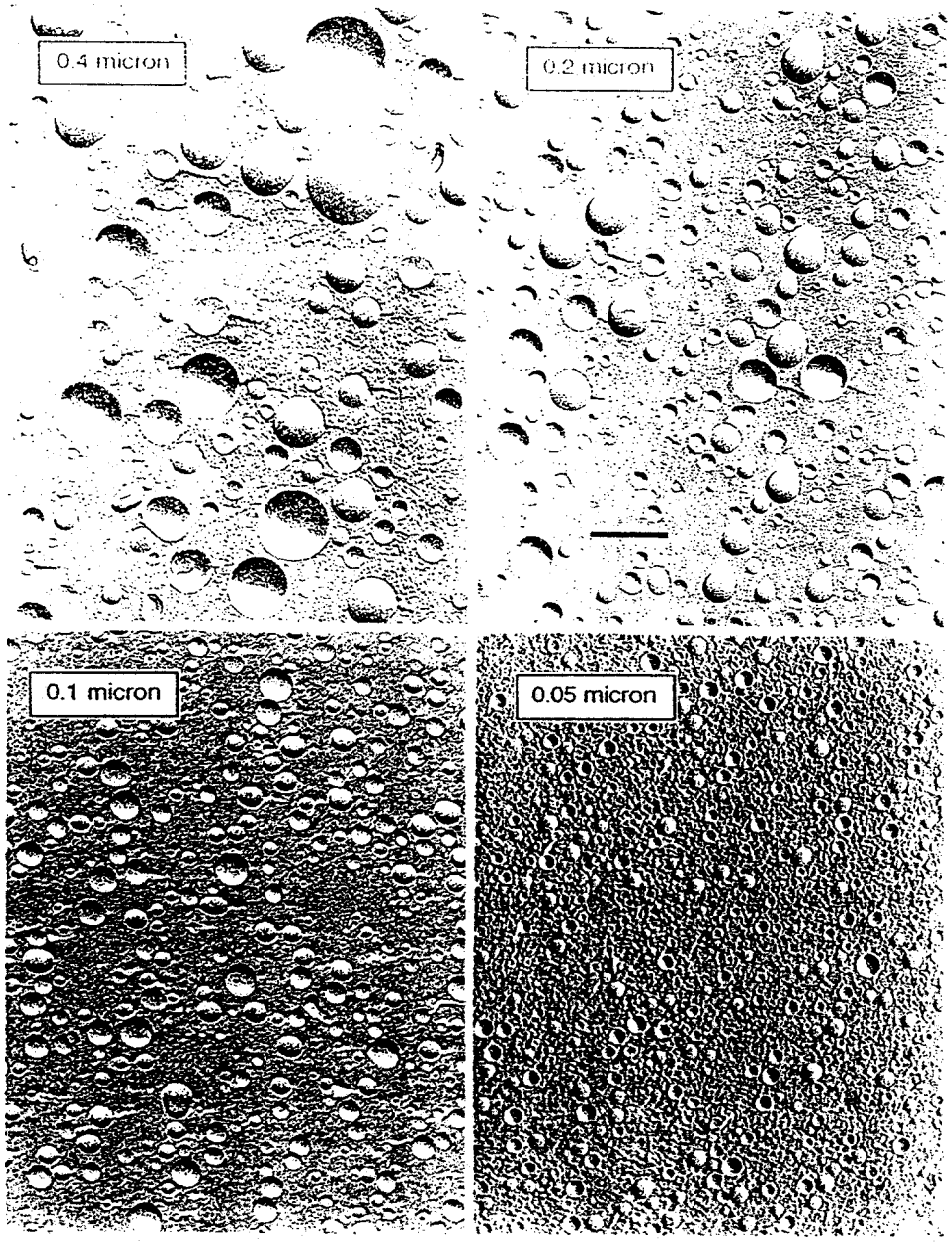

FIG 1. Freeze fracture micrographs of LUV prepared from egg phosphatidylcholine MLV by extrusion through filters with pore sizes of 0.4 μm, 0.2 μm, 0.1 μm and 0.05 μm. Vesicles extruded through pore sizes of 0.1 μm or less are unilamellar. Bar 200 nm.

LIPOSOME GENERATION

One of the most practical contributions made by Dimitri Papahadjopoulos has concerned making liposomal systems. Following the initial characterization of multilamellar vesicles (MLV) by Bangham in 1965 (I), Papahadjopoulos was one of the first to employ small unilamellar vesicles (SUV) as model membrane systems, using sonicated dispersions, for example, to study the ion permeability properties of lipid bilayers (2). This work prompted the development of other procedures to produce well characterized, single bilayer model membrane vesicles many of which originated from his group. Techniques such as reverse phase hydration, where lipids are hydrated directly from an organic phase (3) and detergent dialysis (4), have become common laboratory practice in model membrane research. But all of these protocols suffer limitations, particularly when applied to produce potential drug delivery systems. These range from the low (drug) encapsulation volumes common to small unilamellar vesicles (SW) with diameters ≤ 50 nm in diameter to difficulties in removing residual organic solvents or detergent (5).

Characteristically, Dimitri Papahadjopoulos and colleagues published a paper (6) which indicated a way to overcome some of these limitations. They showed that multilamellar vesicles produced by reverse phase hydration could be sequentially passed through polycarbonate filters of decreasing pore size, resulting in the formation of homogeneous vesicle populations with diameters that reflected the size of the filter pore. This stimulated us to build a specialized device, now known as the **Extruder™** which was designed to enable high pressures to be applied during extrusion of MLVs through the polycarbonate filter. Using this technique we found that MLV could be forced directly through pore sizes of 100 nm, resulting in the generation of large unilamellar vesicles (LUV) in 5 min or less (7). This procedure has now become the most commonly employed laboratory technique for making LUVs, both as models of biological membranes and as drug delivery vehicles. Representative freeze-fracture electron micrographs are shown in Figure 1.

MEMBRANE FUSION

Membrane fusion was and remains, a fundamental problem in understanding membrane structure and function Again, Dimitri Papahadjopoulos played a pivotal role in attracting

attention to the area. He determined how divalent cations can alter the phase behaviour of phospholipids and destabilize membrane structure (8,9), work \vhich led to early mechanistic models of the fusion event (IO). These studies stimulated us to investigate the potential rote of lipid polymorphism in membrane fusion, leading to our proposal (1 1) that non-bilayer structures, such as inverted micellcs. play an important intermediate role in membrane fusion events In turn this has led to the sophisticated analysis of Siegei and co-workers (12) pointing to the formation of transitory interbilaycr stalks as an initial event in the fusion process (See Figure 2)

DRUG DELIVERY

Dimitri Papahadjopoulos has made fundamental contributions to the liposomal drug delivery field, and in the process has stimulated many researchers, ourselves included, to enter this area. Particular contributions include the development of protocols for the attachment of targeting information to liposomes (I 3,14) and, in conjunction with Terry Allen, the development of "pegylated" liposomes which exhibit long circulation lifetimes (15). A particular requirement for liposomal delivery systems was the need for techniques capable of achieving efficient encapsulation of conventional drugs at high drug-to-lipid ratios. This was addressed by the "remote loading" procedure developed in our laboratory for drugs such as doxorubicin (16). This technique, illustrated in Figure 3, relies on the weak base properties of many drugs which results in their accumulation into liposomes with an acidic interior, where the permeating species is the neutral form of the drug. A variant of this procedure, which relies on $(NH_4)_2SO_4$ gradients, has also been developed (17). These procedures, in combination with extrusion and pegylation protocols, have now resulted in a variety of pharmaceutical products which are in the late stages of clinical testing.

GENE THERAPY

Again, Dimitri, was an early proponent of the potential utility of liposomes as gene delivery systems. This included the development of reverse phase procedures to encapsulate

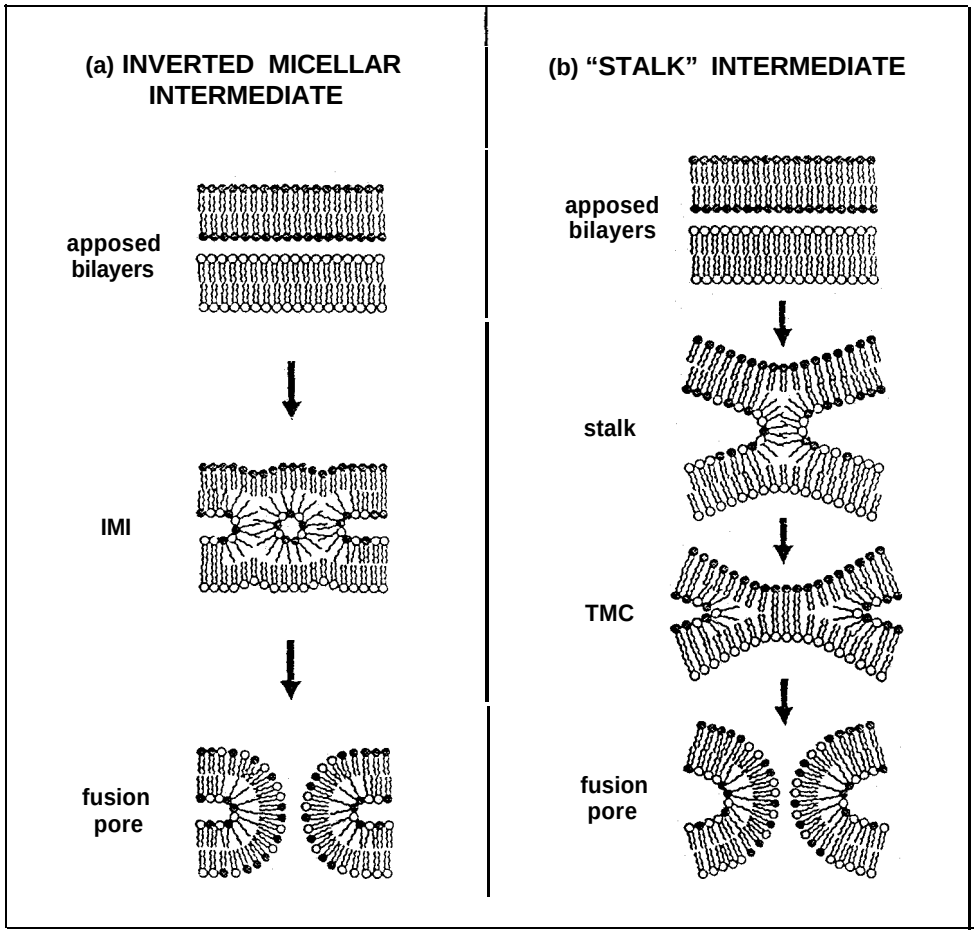

FIG 2. influence of a pH gradient on the transbilayer distribution of drugs which can be classified as lipophilic amines. If the dissociation constant for the amine moiety (K_d) < < the pH either side of the bilayer, then at equilibrium $[drug]_{IN}/[drug]_{OUT}$ is approximately equal to the proton gradient. For example, for a three-unit pH gradient (ΔpH = 3, inside acidic) this corresponds to an equilibrium concentration of drug inside the vesicle system which is 1000 times higher than outside.

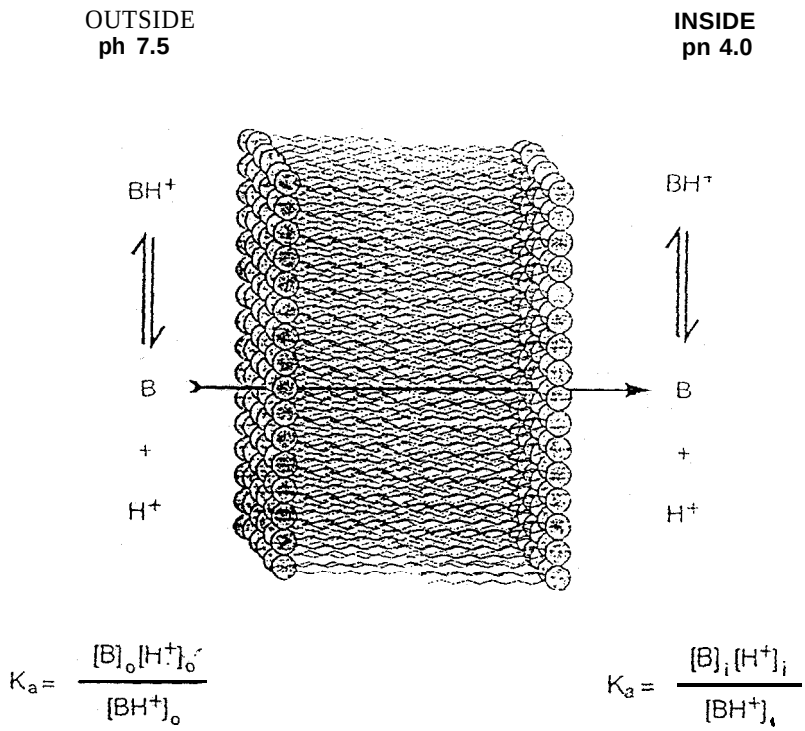

FIG 3. Two possible mechanisms of membrane fusion, involving (A) inverted micellar intermediates (IMI) and (B) an interbilayer stalk. (Redrawn from ref. 12).

DNA (3) and conducting early research into the use of liposomes to deliver nucleic acids to cells (18, 19). This application of lipid based drug delivery systems has rapidly expanded since the pivotal studies of Feigner et al (20), demonstrating that liposomes containing cationic lipids could indeed result in the efficient intracellular delivery and expression of plasmid DNA. In many ways, the development of injectable liposomal gene delivery systems is the next stage of Dimitri's legacy. Such systems must include efficient entrapment protocols, long circulation lifetimes and fusogenic qualities, and may be expected to play a fundamental role in gene therapy protocols in the future

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