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LIPOSOMES AS "TARGETED" DRUG CARRIERS: A PHYSICAL CHEMICAL PERSPECTIVE

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Abstract - Over the last several years liposomes have increasingly caught the imagination of physical chemists, biologists, and medical scientists. In the last-mentioned context, they are being studied as carriers for selective drug delivery. In this lecture I will first give a brief overview of the possibilities and problems of liposomal carriers. Then I will discuss several instances in which physical chemical ideas are contributing directly to the medical objectives. Finally, I will indicate remaining problem areas in need of additional physical chemical insight.

INTRODUCTION

To the colloid chemist a liposome is smectic mesophase -- or an association colloid formed by amphipathic lipid molecules of more or less cylindrical shape. To the pharmacologist interested in drug carriers, the liposome is a discrete structure of finite size into whose closed aqueous spaces can be put hydrophilic drugs and into whose lipid bilayers can be put hydrophobic ones. These perspectives diverged rather quickly after Alec Bangham and his colleagues discovered in the mid-1960's that liposomes do, indeed, form closed structures (reviewed in 1). As indicated in Table 1, there are now three broad arenas in which liposomes are under intensive study. Among them, the paper production comes to several hundred per year.

TABLE 1. Areas of interest in liposomes

PHYSICAL CHEMISTRY
BIOLOGY
(as models)
- - - - - - - - - -
(as vectors)
MEDICINE

Given that there are an indefinitely large number of systems on which a physical chemist could choose to spend his or her life, liposomes have received more than their fair share of physical chemical attention because they are made of biological molecules and can serve as models for biological membranes. Cell biologists also see liposomes as models but, in another context, see them as vectors for insinuating hydrophilic molecules into the cell cytoplasm or hydrophobic proteins and lipids into the cell membrane. Finally, there are the medical possibilities -- that liposomes can be used as carriers of drugs in vivo. The dashed line in Table 1 roughly divides the scientific from the engineering -- those disciplines in which the "bottom line" question is "How does it work?" from those in which it is "How can I make it work?"

When writing the abstract for this meeting, I was principally impressed by the maturation of medical interest in liposomes. Drawing upon a number of other disciplines, as indicated in Fig. 1, the field seemed to me to be developing an appropriate scientific base. However, as I was preparing the lecture, the feeling grew that the input from other areas of biology and from organic chemistry has been more intense than that from physical chemistry.

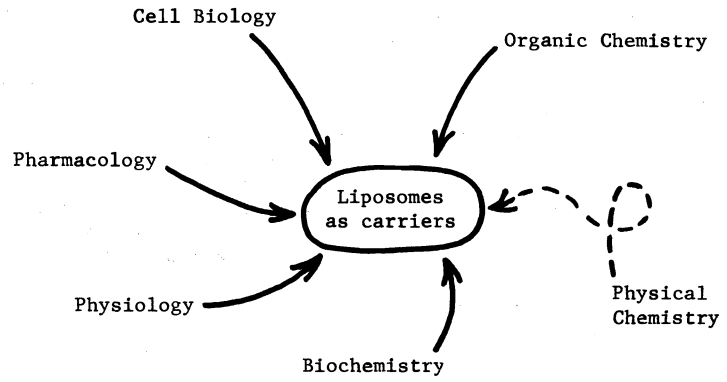


Fig. 1. Contribution of various fields to growth of a scientific base for work on liposomal drug carriers.

Physical insights have penetrated the carrier field only slowly. Such communication as has taken place has been largely the work of a few individuals with a foot in each camp, most notably Demetrios Papahadjopoulos. Given that state of affairs, I plan to do the following in this lecture: First, to give a capsule summary of the field of drug delivery with liposomes; second, in the last few minutes, to introduce several clear instances in which physical chemical insights are advancing the field. Third, to suggest a number of specific problems for which more physical chemical input will be necessary in the future. Given the brevity of this treatment and the personal nature of the perspective, I will not be able to give credit to all of those on whose work I touch. Several recent reviews (2-5) are available for further information and entry into the literature.

In summarizing the field of liposomal drug carriers for a physical chemical audience, I am reminded of what the geneticist S.E. Luria asked when physicist Leo Szilard visited his laboratory (6): "Dr. Szilard, I don't know how much to explain. I don't know what to assume..." Replied Szilard: "You may assume absolute ignorance and unlimited intelligence." I will not assume quite absolute ignorance on the physical chemistry of liposomes; presumably, anyone who has accidentally wandered into this lecture also wandered accidentally into Donald Small's elegant presentation at this meeting on the physical characteristics of lipid systems. I will also assume a modicum of biological background.

TYPES OF LIPOSOMES

Liposomes are discrete structures composed of one or more concentric lipid bilayers enclosing an equal number of aqueous spaces. (The term "vesicle" will be used synonymously.) Fig. 2 shows the three essentially different types.
The multilamellar forms (sometimes called "Bangosomes," after Alec Bangham) form spontaneously when the appropriate bilayer-forming lipids or lipid mixtures are hydrated in water. The most often used lipids are phosphatidyl cholines (synonym: lecithins), phosphatidyl ethanolamines, phosphatidyl serines, and sphingomyelins, each of which has a polar headgroup and two fatty acid chains. Cholesterol will not by itself form bilayers but can be incorporated to about 50 mole percent to "toughen" liposomes made of fluid lipids. The multilayers can be of any size up to tens of microns, and probably represent the equilibrium state for most or all bilayer-forming lipids.

Small unilamellar vesicles (sometimes called "Huangosomes" after C. Huang, who did some of the early work on their characterization (7)) are formed from the multilayers by an input of energy, generally ultrasound. They can be as small as about 200 Å in diameter. Presumably, strains in packing of the lipid molecules become prohibitive and prevent formation of structures with even lower radii of curvature than those of these "limit vesicles." Vesicles with high cholesterol content appear to be at least 400 Å in diameter.

The third type of liposome, the large unilamellar vesicle, is a relative newcomer. The last several years have seen publication of a number of recipes for their formation (8), one of which will be discussed at some length later. They can be anything from several hundred angstroms to 100 microns in diameter, depending on conditions of formation. For drug carriage they have the clear advantage of a large trapped volume/lipid ratio. Their physical chemistry and their characteristics in biological systems are just now being worked out. You can expect a great expansion of the literature on them over the next few years.

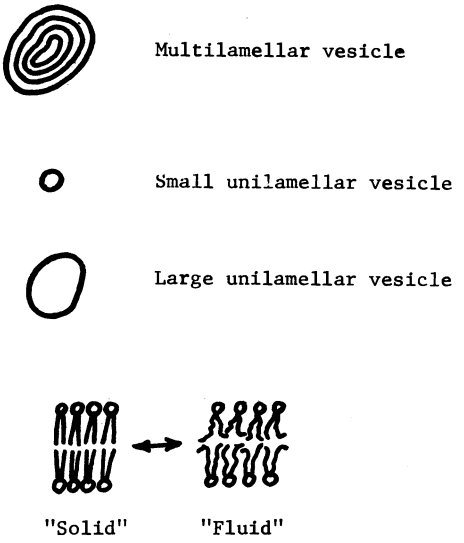


Fig. 2. The three principal types of liposomes. Also shown in highly schematic form is the liquid crystalline phase transition.

The most striking physical chemical feature of all of these liposome types when made with saturated lipids is the phase transition from a quasi-crystalline rigidity of the fatty acid chains to a rather fluid organization.

LIPOSOME-CELL INTERACTIONS

Liposomes can interact with cells in a number of ways, as indicated in Fig. 3.

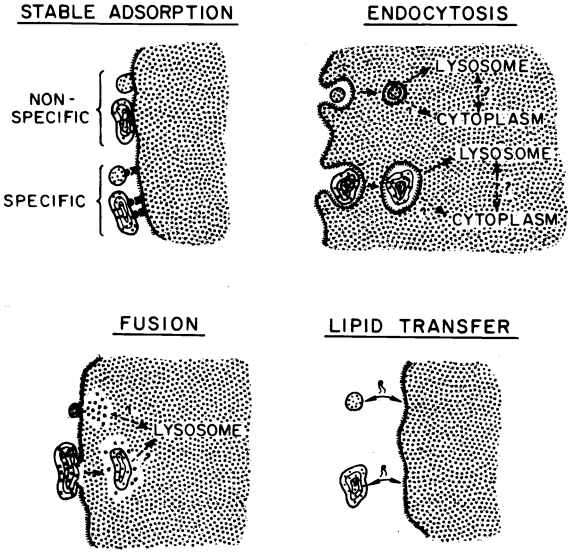


Fig. 3. Mechanisms of interaction between liposomes and cells.

Fusion is the Holy Grail of liposome engineering -- for introduction of materials into cytoplasm or membrane. Unfortunately, spontaneous fusion has proved almost as elusive an object of quest as the Grail, being a low level process under most circumstances. That makes good physiological sense, since cells do not fuse with other cells either except under specialized and carefully regulated circumstances. Given this problem, there is evolving a new generation of techniques in which fusion or endocytosis is enhanced by perturbing the cells and/or associated vesicles. The agents used include polyethylene glycol (9), glycerol(10), and viral coat proteins which promote entry of virus particles into cells(11). These approaches are proving effective in vitro but will probably not be possible in vivo.

At one time it was thought that most spontaneous association of liposomal markers with cells other than specialized phagocytes was due to fusion. It has since become clear that binding to the cell surface, exchange of lipid, and/or endocytosis predominate. When liposomes are injected into the blood stream, a large proportion of them are taken up by specialized phagocytes of liver, spleen, and other tissues. That is a major barrier to many projected uses of liposomes for drug delivery but, as will be discussed later, has also led to the most promising application to date.

RATIONALES FOR USE OF LIPOSOMES

Liposomes can be regarded as one class of vehicle in the broader field of drug carriers. Other types include microspheres, lipid emulsions of various types, and covalent conjugates of drug with polymers, beads, toxins, antibodies, or other proteins. The following reasons have been given for use of liposomes as opposed to free drug in various therapeutic situations:

Timed release. Liposomes remain in the circulation much longer than do most free drugs, so dosage can be spread out in time by encapsulation in liposomes. This may be useful, for example, with "cell-cycle specific" anti-tumor agents, which must be present as different cells in the tumor pass through the particular stage of their cycle.

Sequestration as particles. In therapy of tumors and infection, and in the diagnosis of myocardial infarction, it has been hoped that liposomes would preferentially escape from the vascular system through leaky capillary walls and localize their contents in areas of disease.

Protection of contents or host. Liposomal encapsulation could protect the contents against the ravages of host enzymes and immunological system, or conversely, protect the host against action of the drug until it had reached its target.

Confinement to an anatomical compartment. Liposomes can be used to confine an agent to a particular anatomical compartment, for example, a joint space to treat arthritis.

Targeting. The possibility of directing liposomes to particular target cell types or sites will be discussed presently.

Prayer. I harbor an image from classical pharmaceutical chemistry of an infinite number of chemists sitting at an infinite number of laboratory benches turning out variations on successful drug molecules -- guided in part by logical principles, in part simply by the hope that something good will happen. With liposomes, the same thought operates, particularly in cancer chemotherapy, in which therapeutic and toxic effects are delicately balanced. Any change in the pharmacokinetic rules could open up new possibilities. So far, such prayer has not been answered in any system of which I am aware.

PROBLEMS IN THERAPY WITH LIPOSOMES

Uptake in liver and spleen. As already mentioned, much of an intravenous load of liposomes is taken up by phagocytes in liver and spleen, a problem if the target of therapy is elsewhere.

Endothelial barrier. Except for the sinusoidal endothelia of liver, spleen, and bone marrow, liposomes cannot easily leave the circulation to reach extra-vascular targets.

Disruption by physiological fluids. Serum components, principally but not exclusively the lipoproteins, disrupt liposomes. Much of the early literature on in vivo distribution of radiotracers of the liposomal lipids or contents was really not following the fate of liposomes as intact structures at all. Three ways to toughen liposomes against physiological disruption are the inclusion of cholesterol (about 33 - 50 mole %), use of gel state lipids, and inclusion of sphingomyelin.

Antigenicity and immunogenicity. Paradoxically, liposomes have been claimed to sequester antigenic contents from the immune system and, on the other hand, to serve as effective adjuvants or carriers for immune sensitization. It remains to be seen how much of which really happens when.

Toxicity of drug or liposome. Liposomes made of phosphatidylcholine and cholesterol appear not to be toxic, even in quite large amounts. The toxicity of other, more exotic components is under investigation. It must be remembered that, just as the pharmacokinetic rules for therapeutic effect are changed by encapsulation of a drug in liposomes, so are the rules for toxicity.

Stability, shelf life. It has been said that drug companies like to make small molecules in large quantities to put in small bottles to sell for large profits. Whether liposomes can be fitted into that paradigm remains to be seen. By freeze-drying or other techniques of preservation, it may be possible to design preparations of suitably long shelf-life and ease of administration. But for some proposed uses, these mundane problems will be formidable.

TARGETING LIPOSOMES

The verb "to target" still sounds to me like some monstrosity from a bureaucratic memorandum. It can perhaps be justified by usage or because no one has found a suitably compact alternative. I am to a degree mollified by finding that some editions of Webster's dictionary include "to target" as a transitive verb, albeit with a definition somewhat different from that current in this field. I think we can distinguish four fundamentally different types of targeting, as listed in Table 2.

TABLE 2. Type of targeting

1.	NATURAL TARGETING (i.e., to phagocytic cells)
2.	COMPARTMENTAL TARGETING (e.g., in lung, joint, peritoneal cavity)
3.	LIGAND-MEDIATED TARGETING (by antibody, hormone, lectin, carbohydrate, etc.)
4.	LOCAL RELEASE OR ACTIVATION (e.g., temperature-sensitive, pH-sensitive, photosensitive liposomes)

Natural targeting makes use of the inherent tendency of liposomes to be taken up by phagocytes. Shortly, I will have more to say about one instance of natural targeting, the treatment of parasites residing in phagocytes. In our hurry to move on to more sophisticated forms of targeting, there is a tendency to forget what the liposome does best.

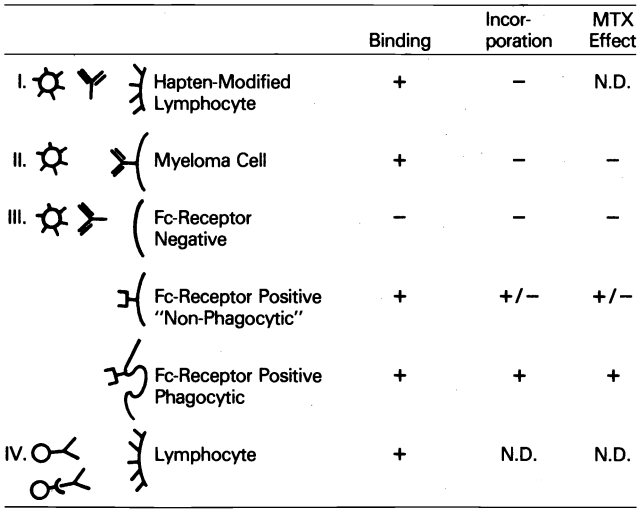
In compartmental targeting, the site of administration determines the site of therapeutic effect. In ligand-mediated targeting, recognition molecules placed on the liposome surface, covalently or otherwise, are intended to recognize complementary molecules on the surface of the target cell type. Fig. 4 summarizes a series of our own studies over the last several years on the use of antibody as a targeting agent (12-15). The clear lesson is that vesicles and their contents can be bound to cells, at least in vitro, but that uptake into the cell and effect of an encapsulated drug depend on endocytosis. In vitro it may be possible to bind liposomes to cells via ligand and then to obtain fusion by one of the second generation techniques mentioned earlier, but it is not clear that the same will be possible in vivo. Other limitations of ligand-mediated targeting include the paucity of usable cell surface targets (for example, in cancer), the endothelial barrier for extravascular targets, the immunogenicity of most ligands, and the possibility of rapid removal from the circulation.

With these limitations in mind, other principles for targeting have been sought. Local activation or release of liposome contents triggered by a physical change in the environment of the liposome does not have these problems. Later, I will be discussing the physical chemical background to one such targeting strategy, the use of temperature-sensitive liposomes.

DISEASES FOR WHICH LIPOSOMAL THERAPY HAS BEEN STUDIED

Liposomes have been called a treatment in search of a disease. The following is a brief list of diseases for which therapy has been envisioned. See (2-4) for references.

Enzyme deficiencies. For genetic deficiencies of lysosomal enzymes, the idea has been to encapsulate the required enzyme in liposomes and inject them for delivery to phagocytic cells in vivo.



	Binding	Incorporation	MTX Effect
I. Hapten-Modified Lymphocyte	+	−	N.D.
II. Myeloma Cell	+	−	−
III. Fc-Receptor Negative	−	−	−
Fc-Receptor Positive "Non-Phagocytic"	+	+/−	+/−
Fc-Receptor Positive Phagocytic	+	+	+
IV. Lymphocyte	+	N.D.	N.D.

N.D. — Not Done.

Fig. 4. Summary of experiments on antibody-mediated targeting of liposomes. Circles at left represent small unilamellar vesicles. I. liposomes modified with dinitrophenyl (DNP) hapten are bound to trinitrophenyl-modified lymphocytes by anti-nitrophenyl immunoglobulin G (IgG) (12). II. DNP-liposomes are bound to mouse myeloma cells which bear on their surface an anti-nitrophenyl IgA (13). III. DNP-liposomes are bound to cells having receptors for the Fc portion of an anti-nitrophenyl IgG (14). IV. Naturally occurring surface antigens on lymphocytes are targets for IgG linked to liposomes covalently or through covalently linked protein A (15). In each case, binding leads to incorporation of the vesicle and its contents only in so far as endocytosis is possible.

Heavy metal poisoning. In cases of iron overload resulting from hematologic disease (e.g., thalassemia) or poisoning by lead, mercury, and possibly plutonium, liposomes containing appropriate chelators may be injected to obtain clearance of the metal from affected cells.

Respiratory distress syndrome. In respiratory distress syndrome of newborn, the surfactant required to keep pulmonary alveoli open seems to be deficient. Liposomes made of the lipids found in lung surfactant (dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidyl ethanolamine) have been administered to the respiratory tree in an attempt to combat the problem.

Arthritis. Steroids are often injected into inflamed joints for local anti-inflammatory effect. Appropriate steroids have been incorporated into the bilayers of liposomes for local injection. Since one of the main culprits in the inflammatory process is the macrophage, the liposomes in the joint space may be phagocytosed by the very cells at which therapy is aimed. In concept, this strategy combines "natural" and "compartmental" targeting.

Diabetes. Liposomes have been considered for oral delivery of agents, including insulin. In spite of one encouraging early report, this enterprise seem unlikely to work out.

Myocardial infarction. Liposomes have been reported to concentrate in areas whose vascular lining has been made leaky by myocardial infarction. If so, liposomes with gamma emitting radionuclides could be used to diagnose areas of injury.

Cancer. Inevitably, much of the therapeutic interest has been focused on cancer, with a number of hopes in mind: a time-capsule effect for cell cycle specific drugs (that is, achieving nearly the equivalent of a continuous infusion from a single injection); targeting to cell surface markers; compartmental targeting (for example, in intraperitoneal disease); and natural targeting to hepatoma. There is no time now even to list the major approaches taken. However, it is worth pointing out one dichotomy: intravenously or intralymphatically administered liposomes may prove useful against tumor in those compartments, but liposomes so injected are unlikely to be effective against extravascular solid tumors if the liposomes must cross the endothelial barrier to work. A different approach with considerable

potential is the use of liposomes as carriers for molecules that can activate cytotoxic cells locally in a tumor (e.g., in the lung) or otherwise modulate immune function.

After this whirlwind survey, it must be emphasized that we are discussing applications in the future tense. And even very good ideas are only occasionally translatable into a useful function in the real world. I am not sure that any of the above will turn out to be useful. But let us now to turn to a probable success story -- the use of liposomes to treat a parasitic disease.

Leishmaniasis. Though not well known in most industrialized countries, Leishmaniasis is very widespread, especially in the tropics (Fig. 5), and is

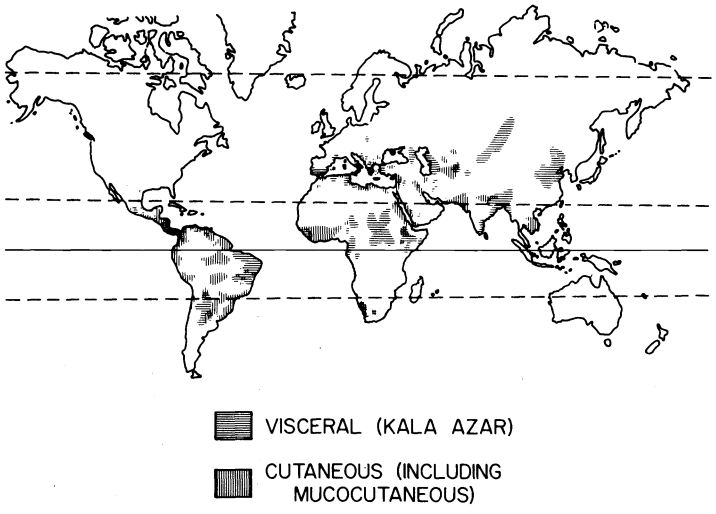


Fig. 5. Distribution of leishmaniasis. Reproduced with permission from (19).

one of the six diseases targeted by the World Health Organization for particular attention. There are three types: visceral (affecting principally the liver and spleen), cutaneous, and mucocutaneous. The first is almost uniformly fatal if untreated, the last two can be terribly disfiguring and are often difficult to treat. In each case, the parasite lives inside phagocytic cells and thus might be a natural target for liposomes. This possibility has been studied in several laboratories (16-19), most extensively in that of Alving. Work on the visceral form is well advanced. In the animal study whose results are shown in Fig. 6 an antimonial drug trapped in liposomes was

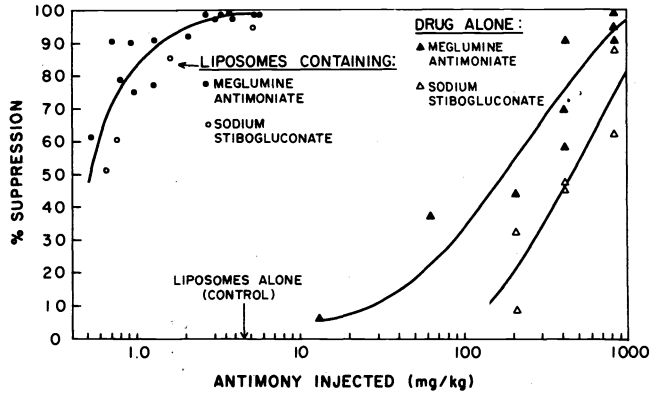


Fig. 6. Comparison of liposomal and free antimonial compound in treatment of visceral leishmaniasis in hamsters. See (19) for protocol.

effective at a 700-fold lower dose than the free drug. Since other studies showed the toxicity in this system to be increase about 10-fold, the improvement in therapeutic index was perhaps 70-fold. Although a great deal remains to be understood about this system, clinical trials can be expected to begin soon. From a humane point of view, if liposomes succeeded as a treatment for leishmaniasis, that success would justify all of the enthusiasm and money thus far invested in therapeutic efforts with liposomes. Though the work is less advanced, liposomes (containing glycolipids) are also under study for treatment of a second major parasitic disease, malaria (20).

In this brief survey of the medical uses of liposomes, the following two general categories of effort demand attention, though not themselves diseases:

Liposome immunology. In the mid-1970's liposomes were found to serve as adjuvants in immunization (21). Since that time there has been an explosion of interests in immunologic uses of liposomes -- as adjuvants and carriers for haptens, as targets for cellular and humoral immune attack, and as antibody-targeted particles (22). Several meetings on liposome immunology have taken place, and Alving counted 94 articles on the subject published in the single year 1980 (23).

Genetic manipulation. Fusion of liposomes with cells has been envisioned as an efficient way of transferring genetic material in vitro (and, very speculatively, in vivo). The technology is still in it infancy. Using glycerol, a perturbant mentioned earlier, Papahadjopoulos and coworkers have achieved efficiencies of transfer comparable to those of other available techniques but applicable to a wider range of cell types (10). In particular, they have recently reported success with plant protoplast cells, for which other approaches have not worked. If this finding can be translated into a practical method, the implications for agriculture will be enormous.

CONTRIBUTIONS FROM PHYSICAL CHEMISTRY

So much for the hopes and dreams. What role can the physical chemist play? Let me approach that question by discussing briefly three instances in which physical chemical insights have been crucial.

Reverse phase evaporation vesicles. Fig. 7 shows schematically

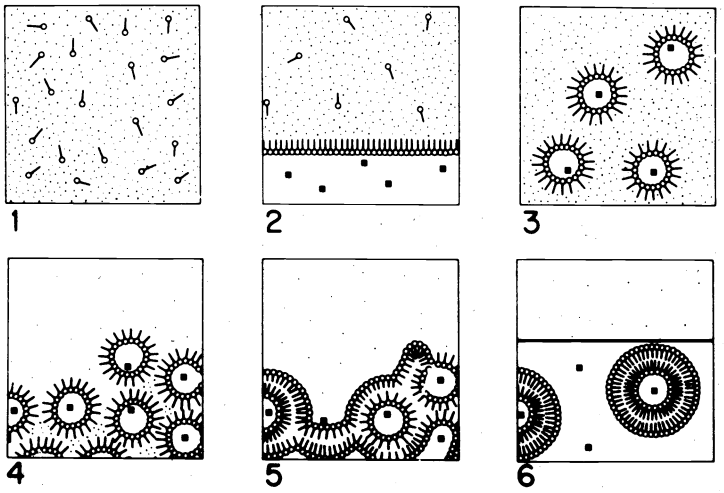


Fig. 7. Schematic view of the formation of large unilamellar vesicles by the reverse phase evaporation technique. See text for explanation. Reproduced with permission from (24).

how the most widely used preparation of large unilamellar vesicles is thought to form. The lipid is dissolved in organic solvent, usually ether. Aqueous medium, containing the

material to be encapsulated, is added and sonicated to form a water in oil emulsion. The organic solvent is then removed under vacuum until a gel forms and collapses to leave large, mostly unilamellar liposomes. This approach, developed by Szoka and Papahadopoulos (24), arose from their thinking about the formation of water in oil emulsions. There is a direct analogy with double emulsions of water in oil in water. Substitution of bilayer-forming lipids in the liposomal system for the single-chain surfactants often used in double emulsions allows the oil phase to be removed entirely.

Multilayer spacings and the problem of fusion. Parsegian, Rand, and their coworkers (25,26) have been studying the forces that determine the spacing of bilayers in multilamellar vesicles. As depicted in Fig. 8, the

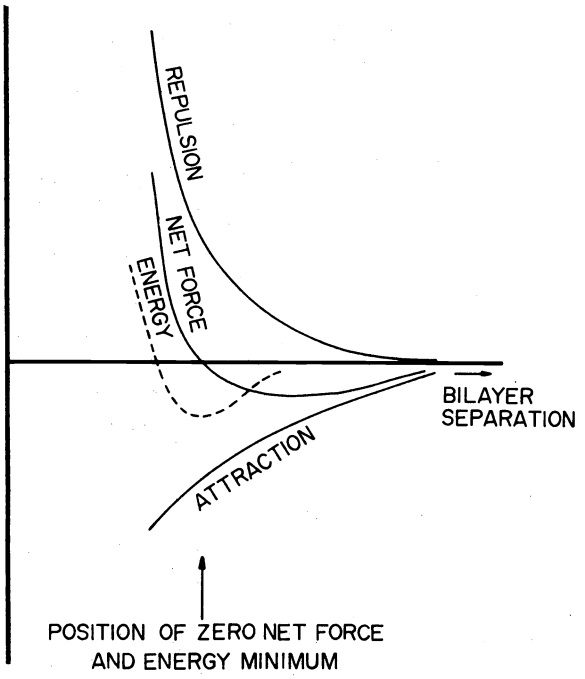


Fig. 8. Schematic representation of forces between bilayers. (reproduced with permission from (26).

system can be modelled in terms of an equilibrium between van der Waals forces of attraction on the one hand and hydration and electrostatic forces of repulsion on the other. To perturb the bilayer spacing, a force is applied by one of the three means shown in Fig. 9. The results can be expressed in terms of the force required to produce a given spacing, as shown in Fig. 10 for egg yolk phosphatidyl choline.

Similar experiments have been done for a number of lipid types in the presence of media of various ionic compositions. Among the generalizations possible from the work:

1. The repulsive hydration force is very similar for all bilayers, and differences in equilibrium separation probably relate to differences in the van der Waals forces.
2. The repulsive hydration force becomes an enormous barrier to approach of the bilayers within about 15 to 20 Å of each other. This observation suggests why most types of liposomes interact with each other only quite slowly -- and why, apart from the presence of a shaggy coat of protein and carbohydrate, cells may fuse so little with vesicles.
3. Equilibrium spacings are less for solid phase lipids and for phosphatidylethanolamine (small hydrophilic head group) than for phosphatidylcholines (larger hydrophilic head group).
4. If there is little electrostatic shielding, bilayers made of charged lipids separate to an effectively infinite distance.
5. Compression of bilayers may lead to phase segregation of the component lipids, and calcium may do the same to mixed multilayers containing negatively charged and neutral lipids.
6. Calcium, but not magnesium, can collapse negatively charged bilayers into a more or less dehydrated state.

It is possible to extrapolate mathematically from these observations on multilayers to the problem in which two spheres of specified radius of curvature approach each other. As an

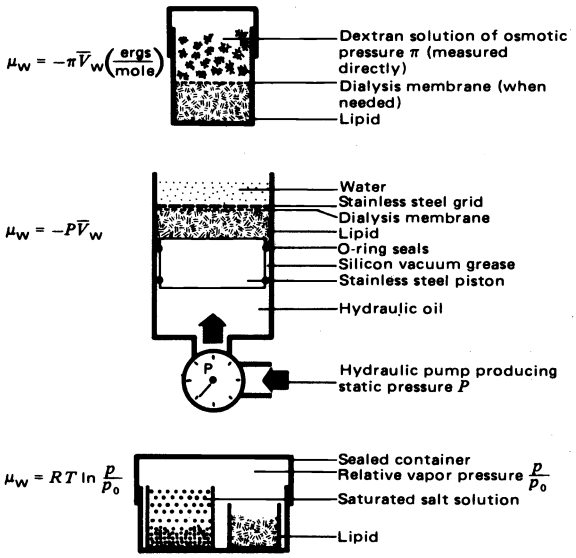


Figure 9. Schematic view of three methods used to apply (equivalent) osmotic or hydrostatic pressures to multilamellar systems. Reproduced with permission from (25). See original article for discussion of symbols.

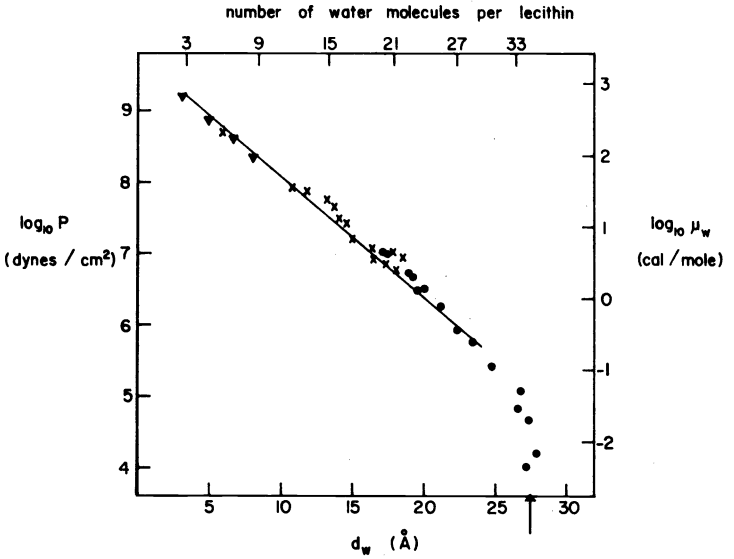


Figure 10. Experimentally measured repulsive pressure P between egg phosphatidyl choline bilayers as a function of the thickness of water layers (d) between lipid lamellae. The arrow indicates the separation at which pressure falls toward zero, i.e., the equilibrium separation of 28 Å. Reproduced with permission from (26).

initial principle, the difficulty of overcoming hydration forces in the last 15 to 20 Å suggests why promiscuous fusion doesn't take place in most artificial or biological systems. The further results correlate in an intriguing way with observations on vesicle-vesicle fusion by Papahadjopoulos and coworkers (27-29). They find that calcium dehydrates bilayer structures containing large amounts of negative lipid, leading to fusion. They also find that phosphatidyl ethanolamine in vesicles does not inhibit fusion as does phosphatidyl choline -- perhaps correlated with the possibility of closer approach of the vesicle surfaces. Fig. 11 indicates the further possibility of phase separation to place phosphatidyl ethanolamine in the area of incipient contact between vesicles. Since phosphatidyl ethanolamine has a tendency to form hexagonal inverted micellar structures, an inverted micelle such as that shown at the junction could play a role in the topological catastrophe of fusion.

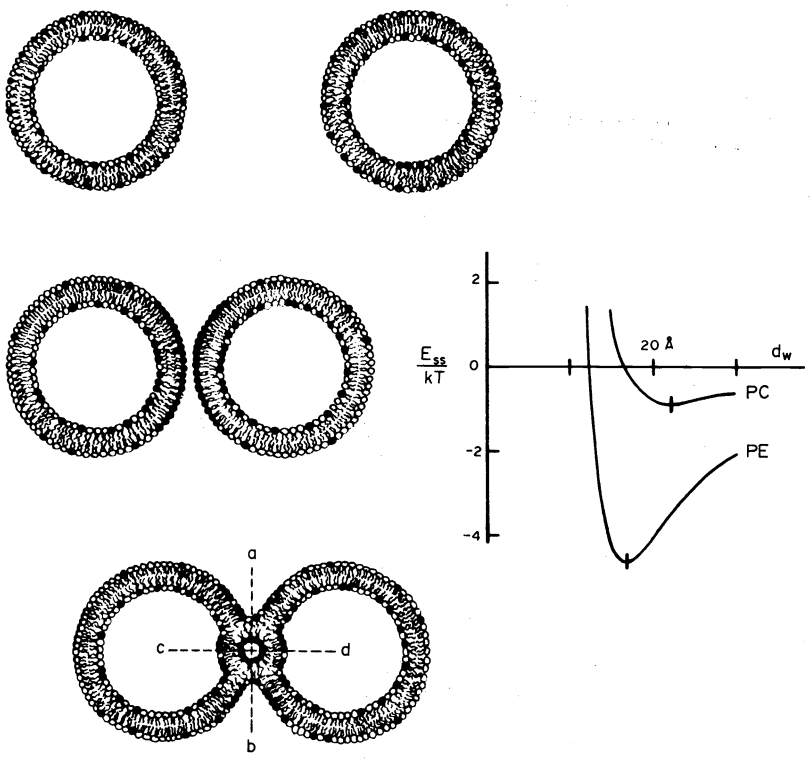


Figure 11. Hypothetical view of a fusion event. In the region of contact there is phase segregation of phosphatidyl ethanolamine from phosphatidyl choline and formation of a hexagonal inverted micellar structure at the junction. As indicated by the potential energy diagram, constructed in part from data such as those in Fig. 10, phosphatidyl ethanolamine permits closer approach, with greater stability. The 4 kT difference in free energy minimum provides the impetus to phase segregation. The ordinate is a measure of the interaction energy between liposomes, and the abscissa is the distance between approaching lipid surfaces. Reproduced with permission from (26).

The implication of these emerging principles and hypotheses for drug targeting become clear when we consider the very large parameter space in which liposome pharmacologists have been thrashing around empirically. To begin with, liposomes can be:

1. Large multilamellar, small unilamellar, or large unilamellar
2. Solid or fluid phase
3. Negatively charged, positively charged, or neutral
4. High cholesterol, low cholesterol, or no cholesterol
5. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin.

The permutations of these factors number 216 -- without taking into account all of the possible lipid mixtures, differences in technique of preparation, possible impurities, and orifices or compartments into which the liposomes can be injected. The task is further complicated by the predictable unpredictability of these colloidal systems. Multilamellar vesicle preparations, for example, contain a wide range of sizes and numbers of lamellae; their properties are averaged and for many purposes are irreproducible even within a given laboratory. In work such as that of Parsegian, Rand, and Papahadjopoulos, I think we see the elements of a rational physical basis for choices in the large space of possibilities.

Temperature-sensitive liposomes. The third example comes from our own work on temperature-sensitive liposomes (30-33). Multilamellar liposomes had been found to release their contents much more quickly near their liquid crystalline phase transition temperatures than at other temperatures. It seemed possible, then, that selective release of a drug could be obtained in a locally heated region of the body (see Fig. 12) by injecting liposomes designed to have a transition a few

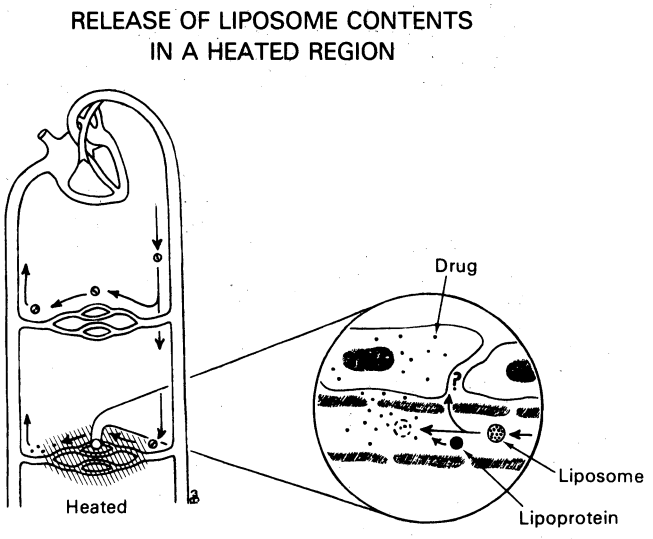


Fig. 12. Schematic view of the preferential release of contents from temperature-sensitive liposomes in a region of the body heated to the transition temperature. Serum components, principally the lipoproteins, induce rapid release and drug equilibrates with the extracellular medium. As indicated by the question mark, intact liposomes might also pass into the extracellular space through endothelia made leaky by heating. (reproduced with permission from (31)).

degrees above physiological temperatures. On the basis of their calorimetric behavior, we chose mixtures of dipalmitoyl and distearoyl phosphatidyl choline for the purpose. They are miscible in all proportions in both solid and fluid phases, and exhibit a single major endothermic transition at a temperature dependent on the molar ratio. However, when we tested for release at the transition, multilamellar vesicles leaked over too broad a temperature range to be useful, and most of the fast release observed was a function of osmotic imbalance. Small unilamellar vesicles leaked hardly at all. The prospects looked bleak, and we worried as well that the presence of serum would vitiate our efforts in vivo anyway, through broadening of the transition by cholesterol and possibly other serum components. To the contrary, we found that serum made the whole enterprise possible. Both small and large unilamellar vesicles are quite stable to its effects below transition but dramatically sensitive at that temperature (34). We have obtained a 14-fold difference in accumulation of the anti-tumor agent methotrexate in heated and non-heated tumors, along with a small effect on tumor growth. The future of temperature-sensitive liposomes will depend in large part on our ability to understand and control their interactions with serum components near the transition. We do not know enough at present about the molecular details of the interaction to do an intelligent job of optimizing the system.

FOR THE FUTURE

The following, inter alia, are areas in which I think major contributions can be made by those approaching liposomes from a physical chemical point of view:

1. Liposome formation. The millenium has not arrived. There is no type of liposome preparation adequate for all purposes. For example, those protocols involving organic solvent cannot be used if sensitive proteins are to be included. Those involving detergent leave a certain residue of the detergent or its impurities. The mechanisms of liposome morphogenesis during lipid hydration, sonication, detergent dialysis, and solvent evaporation remain to be explored.

2. Liposome-drug interactions. There is a large physical chemically oriented literature on the interaction of anaesthetics and a variety of other drugs with liposomal lipids. There is an empirically based literature on the encapsulation of various types of drugs (hydrophobic, hydrophilic, charged, zwitterionic) in liposomes. More interaction between these two perspectives would be helpful.

3. Liposome-serum interactions. Serum and other physiological fluids are exceedingly complex milieux, and their effects on the liposomal colloid are very poorly understood. What, for example, is the effective surface charge on negatively charged, positively charged, and neutral liposomes in serum?

4. Liposome-protein and liposome-nucleic acid interactions. How can these macromolecules be encapsulated efficiently in liposomes and what effects will they have on the structure?

5. Liposome fusion. Building on the insights discussed earlier, it will be important to understand what factors predispose to the topological catastrophe and how lipids reorganize during it.

Liposome pharmacology, in common with many biomedical fields, has experienced rather large swings in mood. The current phase might be characterized as cautiously optimistic about selected applications (for example, use in vaccines and treatment of leishmaniasis), but hardened against the concept of liposomes as a universal nostrum. Development of the scientific base has been, and clearly will be, critical. Since most of those studying liposomes as drug carriers come from the biological sciences, the relatively poor communication with physical chemistry is understandable. But unfortunate.

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