Liposomes: Research Applications Grow

Liposomes have been a useful biochemical tool for some 13 years now. The structures, which are a product of the laboratory and not of the cell, are microscopic sacs composed of membrane-like lipid layers surrounding aqueous compartments. At first, biochemists were interested in liposomes primarily because they provided good models for studying the properties and functions of cellular membranes. More recently, however, attention has focused on liposomes because of their potential—and the investigators doing the work stress the word "potential"—applications in drug-delivery systems.

During the preparation of liposomes various substances, including enzymes, nucleic acids, and even viruses, can be incorporated into the aqueous compartments. Researchers have already shown that under the proper conditions the enclosed materials may be induced to enter cultured cells. Thus, they think that one day it may be possible, for example, to use liposomes as vehicles for delivering antitumor drugs to cancer cells, but not to normal ones, or for introducing enzymes or even genes into the cells of individuals with certain genetic diseases, such as Tay-Sachs disease, in which particular enzymes are missing or defective. With one possible exception, however, the biomedical applications of liposomes belong only—if at all—to the distant future.

The possible exception is the use of liposomes to treat respiratory distress syndrome (RDS, formerly called hyaline membrane disease) in premature infants. Every year more than 100,000 infants in the United States suffer some degree of RDS, and 10,000 of them die of the condition. The cause of RDS is a lack of "surfactant" production by the premature lung. The surfactant forms a coating on the inner lining of the small sacs (alveoli) that form the oxygen- and carbon dioxide-exchanging surfaces of the lungs. When production of this surfactant is inadequate, the surface tension of the alveoli increases. As a result, portions of the lung may collapse and produce respiratory distress.

Since the major components of the surfactant are the phospholipids phosphatidyl choline and phosphatidyl glycerol, providing a mist containing the phospholipids for the infants to inhale would appear to be the obvious way to treat RDS. However, early attempts to do this failed. But recently, Hallam Ivey and John Kattwinkel of the University of Virginia Medical Center and Stephen Roth of Johns Hopkins University have obtained preliminary evidence that administration of the phospholipids as liposomes might help infants afflicted with extremely severe RDS. They find that the oxygen concentration of the babies' blood increases during inhalation of a liposome aerosol but not during inhalation of a dilute salt solution.

The investigators hypothesize that the liposomal treatment is more successful than previous phospholipid therapies because liposomes may adhere more readily to the cells of the lungs than phospholipids administered as simple dispersions in water. Other researchers have shown that liposomes adhere to cells, at least in culture.

Roth says, however, that he and his colleagues are still cautious about the meaning of their results. To date, only 25 infants have received the experimental treatment. Although their survival rate was better than expected for infants with severe RDS, this might have been partially due to the special attention they received as participants in an experimental program rather than to the therapy itself. Moreover, it is still too early to tell whether the treated infants will suffer any long-term damage. Nevertheless, the investigators are hopeful that inhalation of phospholipids in the form of liposomes may help to reduce the mortality and morbidity of RDS.

Thus far, the RDS trial is the only clinical study of liposomes being carried out in this country. In Europe, a few attempts have been made to use liposomes as vehicles for enzyme replacement therapy, but investigators here have limited their efforts to work with cultured cells and laboratory animals. Several investigators have shown that liposomes can be used as vehicles for introducing into cultured cells substances that would not normally enter the cells. For example, Gerald Weissmann and his colleagues at New York University School of Medicine introduced hexosaminidase A (the enzyme missing in the cells of patients with Tay-Sachs disease) into white blood cells obtained from human Tay-Sachs patients. These cells do not take up the free enzyme but did take up the enzyme when it was encapsulated in appropriately constituted liposomes.

Liposomes can also be used for introducing nucleic acids into cultured cells. Mark Ostrow and his colleagues at the University of Illinois Medical Center in Chicago prepared liposomes carrying messenger RNA (mRNA) for rabbit globin and incubated them with cultured human cells. The cells took up the mRNA and began synthesizing rabbit globin. Even viruses can now be encapsulated in liposomes and transmitted to cells that would not normally be infected, according to Demetrios Papahadjopoulos and his colleagues at Roswell Park Memorial Institute.

All of the work involving the encapsulation of large molecules in liposomes has depended on the development of new techniques for producing the vesicles. The early methods, which were developed largely as a result of the pioneering work of Alec Bangham and his colleagues at the Institute of Animal Physiology in Cambridge, England, produced multilamellar liposomes. These are onion-like structures consisting of several lipid layers surrounding an equal number of aqueous compartments. The compartments do not have enough room for macromolecules. Now, investigators, including Bangham and Papahadjopoulos, have learned how to prepare unilamellar vesicles, some of which are large enough to accommodate proteins, nucleic acids, and viruses. In fact, the Roswell Park workers have recently prepared liposomes large enough to encapsulate bacteria.

If liposomes can be used to introduce RNA or viruses into cells, they might also be used for introducing DNA, which might thus effect a permanent change in the genetic composition of the recipient cells. Introduction of an enzyme or mRNA into cells will produce only temporary changes since proteins and RNA's are readily broken down by cellular enzymes. Although the goal of gene insertion is the cure, not just the palliation, of genetic diseases, research aimed at application of liposomes for genetic engineering is almost certain to be controversial.

Not so likely to be controversial, however, is research directed at designing drug delivery systems for cancer chemotherapy. Oncologists have long wanted a way of killing tumor cells without damaging normal cells. The hope is that chemotherapeutic drugs can be enclosed in liposomes that can be designed to deliver the drugs only—or at least primari-

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ly—to cancer cells. Another potential use for liposomes in cancer chemotherapy is the circumvention of the drug resistance that may be developed by tumor cells. Often the resistance results from cell surface changes that block the entry of a formerly effective agent. The drug actinomycin D, when encapsulated in liposomes, enters cultured cells that are otherwise impermeable and resistant to the agent, according to Papahadjopoulos and George Poste, also of Roswell Park Memorial Institute. Encapsulation of antitumor or other drugs in liposomes might also help to prevent premature breakdown of the agents in the blood stream before they reach their targets.

There is at least one example in which liposome encapsulation of a drug appears to have improved the survival of treated animals. Papahadjopoulos and Eric Mayhew of Roswell Park found that leukemic mice live longer when they are treated with the antitumor drug Ara-C (1-β-D-arabinofuranosylcytosine) in liposomes than when they are given the drug only. Since these workers did not observe any differences in the way liposome-encapsulated Ara-C affects cultured cells, they think it unlikely that entrapment in liposomes affected transport of the agent into the cells. Thus they hypothesize that the liposomes may protect the drug from breakdown in the animals. Alternatively, the liposomes may have acted as a time-release system and prolonged the activity of the Ara-C, or they may have altered the tissue distribution of the drug with the result that more of it was available to the leukemic blood cells.

Problems with Medical Applications

Despite the promising results with cultured cells and some suggestive studies with experimental animals, many investigators are pessimistic about the possibility that liposomes will ever be effective in drug delivery systems. A number of formidable problems will have to be solved before most of the suggested applications can be achieved. The most difficult involves targeting the liposomes, that is, finding a way of directing them to the cells where they are needed.

For RDS therapy, the liposomes are forced into the lungs by a respirator, but most other applications require that they first travel through the bloodstream. Several investigators have shown that when liposomes are injected into animals by a variety of routes, most of them may end up in the liver and spleen. Removal of particles from the blood is one of the functions of these organs and getting around this obstacle will not be easy. As Papahadjopoulos points out, however, the design of liposomes for medical applications is still in its infancy. Recent research from a number of laboratories indicates that the survival times of liposomes in the blood and their ultimate distribution in the body may be influenced by altering their size. Thus, appropriate tinkering with liposome structure may help to minimize their uptake by liver and spleen.

Moreover, there may be some circumstances in which preferential localization of liposomes in these organs is advantageous. For example, the parasite *Leishmania donovani*, which causes a serious disease that is endemic in many parts of the world, often invades the liver and spleen. The toxicity of the antimony-containing drugs used to treat leishmaniasis limits the doses that can be given to patients. But Carl Alving of Walter Reed Army Institute of Research has evidence that encapsulation of the drugs in liposomes greatly potentiates their activity in hamsters infected with the parasite and thus permits a reduction in the doses required to treat the disease.

Some investigators have suggested that tagging liposomes with antibodies may help to direct them to the appropriate target cells. Such antibody-labeling may facilitate the uptake of liposome-encapsulated materials by cultured cells. Weissmann found this to be true for the uptake of hexosaminidase A by Tay-Sachs cells. But this is not necessarily always the case. John Weinstein and his colleagues at the National Cancer Institute have shown that antibody can facilitate the binding of vesicles to some target cells without having any effect on the actual transfer of the liposome contents into the cells.

Other problems include the possible toxicity of the liposome components. Although most studies indicate that liposomes are well tolerated by experimental animals, toxic effects have been reported. Moreover, either the liposomes themselves or the materials trapped within them, especially large molecules like proteins or nucleic acids, may elicit immunological reactions harmful to the host. Thus, although many investigators are optimistic that these problems can be solved, there are a number of other researchers who think that emphasis on the medical applications of liposomes is misplaced and that the real importance of the vesicles lies in their potential for helping to answer fundamental questions in cell biology.

A major reason why liposomes are so useful in basic research is that the composition of vesicle membranes, unlike that of natural membranes, can be controlled and systematically varied by the experimenter. This opens the door to a wide range of potential experiments—everything from classical studies of the effects of composition changes on the biophysical and chemical properties of membranes to investigtions of complex processes involving interactions between cells in which liposomes of known composition can be used as models for one of the interacting cell types.

Liposome Interaction with Cells

Investigators, including the Roswell Park group and Richard Pagano of the Carnegie Institution of Washington in Baltimore, have identified four ways in which liposomes may interact with cells: stable adsorption of the liposomes to the cells; endocytosis, in which whole liposomes are ingested by the cells; fusion, in which the outermost lipid layer of the liposomes merges with the cell membrane, with concomitant release of the liposomal contents into the cell cytoplasm; and lipid transfer from the liposomes to the cell membrane. The details of these interactions are a long way from being worked out, but the evidence indicates that the liposome composition is one of the factors determining which of these four mechanisms will predominate in any given interaction.

Although Pagano is one of the pessimists regarding the potential medical applications of liposomes, he points out that the studies of the liposome-cell interaction are providing the kind of information needed for rational design of drug delivery systems, whether in the living animal or in cultured cells. The manner of the liposome interaction with cells determines whether the liposomal contents will be transferred to the cells and where they will become localized. Fusion, for example, would be the desired interaction for introducing a drug or enzyme acting in the cell cytoplasm. In contrast, the lipid transfer reaction appears to provide a way for studying how composition changes affect natural membranes.

Another important application of liposomes is the investigation of immune reactions, many of which involve surface interactions between different cell types. As Harden McConnell of Stanford University puts it, "If you use liposomes as stand-ins for one of the interacting cell types, at least one of the entities is a known quantity." McConnell, and Stephen Kinsky of Washington University, have shown that liposomes can serve as targets for several types of immune attack.

Kinsky, who is now on sabbatical at the National Jewish Hospital and Research Center in Denver, initially ob-

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served that liposomes were lysed (ruptured) by the complement system. The complement system consists of nine proteins that act in concert to lyse cells (or liposomes in this case) that have been tagged by antibodies. One function of the complement system is the destruction of invading bacteria.

By varying the composition of the liposomal targets, McConnell and his colleagues are trying to determine what features of the model membrane surface are involved in triggering complement attack. In one series of experiments, they showed that increased exposure of the antigenic group projecting from the membrane favors antibody binding, a prerequisite for complement attack. In another, they found that increasing the cholesterol content of the liposomal membrane also favors the binding. They think that this effect may be at least partially due to high membrane cholesterol concentrations enhancing the accessibility of the antigenic group. The fluidity of the liposomal membrane appears to be another important determinant of whether antibody and complement attack the liposomes, with high fluidity favoring the attack, according to the Stanford workers.

Kinsky's work has helped to clarify what happens in the later stages of complement attack. The old theory was that complement activation resulted in the formation of an enzyme that broke down some membrane component. By using liposomes of known composition, Kinsky showed that this could not be the case. He now thinks that complement disrupts the lipid components of membranes much the way detergents disrupt grease on dishes.

McConnell and Kinsky find that properly constituted liposomes can elicit antibody production and serve as targets for cell-mediated immune attack, in addition to serving as targets for complement attack. They are currently trying to determine what features of liposomal membrane structure are involved in evoking these reactions.

Other investigations now in progress include the use of liposomes to study the functions of membrane-bound enzymes and the mechanism of membrane fusion, a fundamental process involved both in the uptake of substances during endocytosis and also in the secretion of proteins and other chemicals. All in all, liposomes appear applicable to research on a large variety of diverse membrane phenomena.—JEAN L. MARX

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