review

Phospholipid spherules (liposomes) as a model for biological membranes

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ABSTRACT This review describes the properties of artificial spherules composed of phospholipids and various long-chain anions or cations. The lipids, which are in the liquid-crystal state, trap aqueous solutes such as cations, anions, glucose, or glycine in aqueous compartments between a series of lipid bilayers. The diffusion of these solutes from the spherules can be studied in the same way that diffusion across biological membranes is studied.

The spherules exhibit many of the properties of natural membrane-bounded structures: they are capable of ion-discrimination, osmotic swelling, and response to a variety of physiologic and pharmacologic agents. These agents (steroids, drugs, toxins, antibiotics) accelerate or retard diffusion of ions or molecules from the spherules in a way that qualitatively mimics their action on erythrocytes, lysosomes, or mitochondria.

Thus the spherules constitute a valuable model system with which to study the properties of biological membranes that may be dependent on their lipid components.

KEY WORDS smectic mesophase · liposomes · lipid spherules · model membranes · phospholipids · cholesterol · steroids · drugs · narcotics · toxins · antibiotics

ALTHOUGH THE MOLECULAR CONFIGURATION of membranes which bound cells and organelles is by no means established, it appears likely that many of their biological properties can be interpreted in terms of the chemistry and physics of lipids. As a result of the lipid bilayer proposal of Danielli and Davson (1), later modified into the "unit membrane" hypothesis of Robertson (2), several model systems have been devised, the purpose of which was to correlate one or another property of natural membranes with the behavior of pure lipids. The earlier experiments with monolayer films of lipid have recently been augmented by studies of model bilayers (3), of nonbiological membranes impregnated with purified lipids (4), and of artificial lipid–protein complexes (5). Such studies are not within the scope of this review, which will deal with properties of artificial lipid spherules.[1] Arranged as multicentric, lamellar structures in aqueous media, these constitute a model system that offers some advantages for the study of membrane behavior (6).

At appropriate temperatures, the preferred phase structure of many biological lipids in water or salt solution is that of liquid crystals. The precise structure of such liquid crystals depends upon the relative concentration of lipid constituents, the temperature at which the liquid crystals are permitted to form, the species of lipid considered, and the ionic strength of the bulk liquid phase (7–9). The most common structures observed are spherules or myelin figures, which most commonly consist of several bimolecular layers of lipid separated by aqueous compartments, although hexagonal or tubular structures may also be seen (9). Since it is improbable, for thermodynamic reasons (7), that any exposed hydrocarbon–water interfaces remain free, spherules and myelin figures probably consist of

Abbreviations: DOC, deoxycorticosterone; DMSO, dimethylsulfoxide.

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[1] Throughout this review, the artificial structures will, for convenience, be referred to as "spherules." A. D. Bangham has used the term "smectic mesophases," and colloquially we have called them "liposomes" or "Bangasomes." As the literature dealing with these structures accumulates, the term "liposome" is gaining favor, and should win general acceptance.

bilayers, each of which forms an unbroken membrane separating discrete aqueous compartments (8).

Several years ago, A. D. Bangham observed that the strongly positive birefringence of lecithin dispersions in water was lost, or even reversed in sign, upon the incorporation into the lipid lamellae of increasing amounts of long-chain anions or cations. From this observation alone he deduced what was subsequently confirmed by electron microscopy, namely that the aqueous channels between lamellar structures were widened by introduction of charged, mutually repulsive molecules into the previously uncharged lecithin layers. The lipid spherules (for that was their form when viewed by negative staining techniques in the electron microscope) were found to trap whatever ionic species was dissolved in the aqueous phase at the time of their formation. After removal of any untrapped ions or markers from the spherules by dialysis or gel filtration, the rate of leakage of sequestered ions formed a good measure of the over-all permeability of the structures, each containing several lamellae (6, 10). By measurements of this kind, which are entirely analogous to determinations of ion efflux from cells or organelles, these models have been used to determine the modes of action of toxins, drugs, hormones, and anesthetics, as well for studies of the permeability of lipid membranes under defined conditions.

PROPERTIES OF ARTIFICIAL SPHERULES (LIPOSOMES)

Structure

Pure ovolecithin, containing a mixture of saturated and unsaturated fatty acids, swells in aqueous media to form an opalescent suspension, and neither the addition of cholesterol nor of long-chain anions or cations changes the quality (i.e., appearance and response to shaking), of the dispersions. The electron-microscopic appearance of spherules, prepared for the usual type of diffusion studies, may be seen in Fig. 1. Viewed by negative staining techniques in the electron microscope, the dispersions present a variety of sizes and shapes, with interlamellar distances of approximately 55 A and a lamellar width of approximately 40 A (in the dried state).

In the light microscope with crossed polaroids, the spherules present positive, negative, or zero birefringence, depending upon their zeta potential (surface charge) (6). Their apparent birefringence is the sum of a *form* component and an *intrinsic* component. The form component is negative and its size varies with the thickness of the lipid bilayer and differences between the refractive indexes of the aqueous compartment and the bilayers (lamellar stacking), while the intrinsic component is positive and depends upon the species and orientation of the individual molecules. Since the intrinsic component is due to the finer structure of the crystal lattice, it is usually stronger. When lecithin (which has no net surface charge) swells in salt, it gives rise to spherules of positive birefringence. However, as the charge of the lipid lamellae is increased by the addition of either long-chain anions or long-chain cations, the repulsive forces between the now charged lamellae increases the size of the aqueous compartments and therefore the negative birefringence (form component). Consequently, loss and subsequent reversal of the sign of apparent birefringence is observed. This behavior was predictable on the basis of the Verwey–Overbeek theory (11) which states that the equilibrium separation of two charged surfaces in ionic media is a function of the surface charge and the ionic strength of the aqueous phase.

Ion Diffusion

Indeed, these physical properties may explain the behavior of captured cations. Bangham, Standish, and Watkins (6) demonstrated that the amount of K^+ that remained associated with the spherules (even after prolonged dialysis) increased as a direct function of the surface charge, and was much higher than could be expected by simple ion-pairing or binding. Since increased trapping of cations was observed as the molar ratio of charged membrane components was increased, the results confirmed views arrived at from examination of the spherules by optical methods: the aqueous channels (and space available for ion trapping) increased directly with the surface charge.

The spherules, whatever the net charge, are several orders of magnitude less permeable to cations than to anions, while water is freely diffusible. Leakage of cations from negatively-charged spherules at room temperature is continuous for 24 hr. However, at 60°C the kinetics of diffusion follow Fick's diffusion law for a two-compartment system only for one-sixth of the total loss (the first 30 min). This finding can be explained by the assumption that diffusion across the first (outer) layer is essentially complete by 30 min; subsequent diffusion across remaining lamellae would resemble kinetics of diffusion through a sphere of high resistance. As surface charge density increases, especially with negatively charged spherules, there is concomitant increase in the permeability of the spherules to cations, for which two possible explanations have been offered. Firstly, cations would accumulate in the immediate vicinity of highly charged (anionic) membranes and their diffusion rate would increase as this relative concentration gradient was increased.

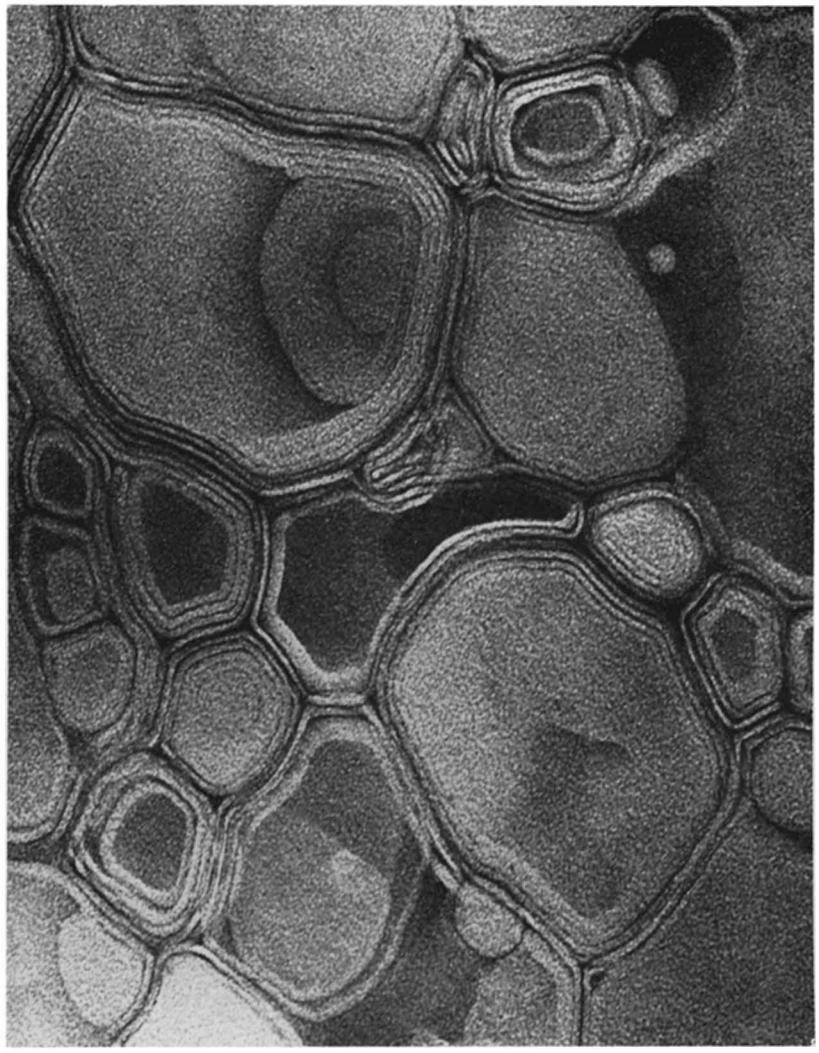

FIG. 1. Electron micrograph of artificial lipid spherules (ovolecithin–dicetyl phosphate–cholesterol 70:20:10 by weight), showing concentric lipid bilayers separated by electron-opaque aqueous layers. The lipid bilayers measure 40 A across. Negatively stained with ammonium molybdate, ×300,000; courtesy of Dr. J. Freer.

Secondly, time-average "pores" of greater diameter should form as increases in surface charge density augment the intermolecular electrostatic energy of repulsion within the lamellae. Finally, it should be noted that increases in surface area would, by increasing total surface charge, lead to increases of *absolute* leakage rate, without any change in the rate of diffusion *per unit area*.

Positively-charged membranes are even more impermeable to cations. Thus the presence of as little as 5% octadecylamine markedly retards diffusion of cations. Indeed, while increases of temperature promptly increase the diffusion of K^+ from negative spherules, positively-charged spherules remain impermeable to the cation even at 60°C. Such differences between positive and negative membranes in cation and anion discrimination clearly indicate that ion diffusion takes place *across* lipid layers, and cannot be accounted for by the physical disruption of whole spherules. Again, if *open* channels were to exist, both anions and cations would be released at equal rates.

Anions diffuse at much greater rates than do cations, in the order $I^- > Cl^- > NO_3^- > SO_4^= > HPO_4^=$ (12). Anions are also captured to a greater extent by spherules possessing higher charge densities due either to positively or negatively charged membrane components. Graded permeabilities are found with other solutes: ethylene glycol, methylurea, and ethylurea penetrated as rapidly as Cl^- or H_2O; glycerol and urea somewhat more slowly; and malonamide and erythritol more slowly still. Sodium acetate and sucrose are poor penetrants (12).

Water Diffusion

Since water diffused through the spherules as fast as, if not faster than, Cl^-, it was suggested that the spherules might be susceptible to osmotic lysis (12). Rendi (13) had studied the osmotic properties of phospholipid liquid crystals. After relating the absorbance of smectic dispersions to lipid concentrations, he demonstrated that the absorbance at a given concentration of lipid decreased with decreasing sucrose concentrations. The pellet volumes obtained upon centrifugation of the lipids were inversely proportional to the concentration of sucrose, their absolute values depending upon extrusion of water during packing of the pellets.

Bangham and coworkers have also investigated pellet volumes of spherules after centrifugation (12). Spherules prepared with charged membranes became packed into pellets whose volumes were reciprocally related to the ionic strength of the suspending medium, but uncharged spherules prepared with phosphatidyl choline formed pellets of a constant size over a wide range of ionic strength. Conclusions from these studies therefore agree both with those from birefringence data and those from studies on ion trapping: only charged membranes create aqueous compartments in which solutes are trapped and which vary in size with the surface charge.

From the volumes of pellets obtained by centrifugation, both the interlamellar volume and the external surface area could be calculated. Since the hydrophobic cores of phospholipid bilayers are probably liquid at room temperatures and above, the deformation of centrifuged spherules should be almost perfect. Thus, in spun-down pellets, the spacing between *adjacent* spherules should depend upon the same repulsive forces that determine the spacings between two lamellae in any *one* spherule. Indeed, it would follow that the interstitial volume *between* spherules in a perfectly deformed array would be proportional to the total surface area of the outermost lamellae. This volume was determined by the distribution of sucrose-^{14}C between pellets and supernatant fractions of centrifuged, frozen dispersions and was found to constitute 10% of the pellet volume. This measurement therefore provides (when divided by the interlamellar spacing) a value for the actual surface area, which was also arrived at by the following independent means. At a given surface potential, the difference was measured of the amount of uranyl nitrate required for the titration of a closely packed phospholipid monolayer in the presence or absence of a known amount of spherules prepared with phospholipids identical to those in the monolayer. By this method, the actual surface area was equal to 303 cm^2/μmole of phospholipid while the total area of a hypothetical monolayer of equivalent amounts of phospholipid (assuming 60 A^2/molecule) would have been 3600 cm^2/μmole of phospholipid. Sonicated preparations yielded surface area values which were three to five times as large, which suggests that such preparations contained a larger number of spherules, each surrounded by fewer, perhaps only one or two, membranes. Such sonicated "sacs" also trapped fewer ions, as might be expected from the diminished water space within them. After an estimate of the outer surface area of such spherule dispersions had been made, it was possible to calculate an osmotic water permeability coefficient: 0.8 μsec^{-1}. This figure agrees well with coefficients derived from bilayer studies made with deuterated water, but less well with measurements made on such membranes by osmotic means (12, 14).

LIPOSOMES PREPARED WITH VARIOUS PHOSPHOLIPIDS

The degree of swelling and the configuration of liquid crystals formed by various phospholipids depend upon the particular phospholipid as well as on the ionic strength, ion valency, and the pH of the medium (12). Similarly, the exact size and shape of the resultant spherules will depend upon the method of dispersion: mechanical agitation promotes fragmentation and produces spherules 5–50 μ in diameter, while ultrasonication induces the formation of smaller sacs, averaging 0.5 μ across (15, 16).

Papahadjopoulos, Miller, and Watkins related the structure to the permeability of spherules formed with various phospholipids (15, 16). Most phospholipids formed suspensions of spherules and myelin figures in which the size and shape of the particles, the degree of birefringence, and the extent to which negative stains penetrated between lamellae during electron microscopy depended on the phospholipid species used. Phosphatidyl ethanolamine was exceptional in its tendency to form aggregates and tubular myelinics (as opposed to spherules) in media which were isotonic (0.145 M KCl) or of low ionic strength. Low angle X-ray diffraction studies confirmed the presence of multilamellar structures with repeating distances (one lamella plus one water space) of 66 A (wet) and 52 A (dry) for phosphatidyl choline, 54 A (wet) and 42 A (dry) for phosphatidyl ethanolamine, and 75 A (wet) for phosphatidyl serine. Permeability studies were consistent with the view that each of these phospholipids yielded spherules that were closed, i.e., capable of clearly discriminating between K^+ and Cl^-, but that phosphatidyl ethanolamine formed incompletely sealed particles. Admixture of other phospholipids with phosphatidyl ethanolamine yielded the usual closed structures. Chloride diffusion was much faster than that of K^+ or Na^+ (as above) through phosphatidyl

choline, phosphatidyl serine, and mixtures of phosphatidyl choline and phosphatidyl ethanolamine or cholesterol. In contrast, cations diffused faster through phosphatidic acid and phosphatidyl inositol, and these differences were explained in terms of the possible orientation of permanent dipoles and of charged groups (16).

Fast (17), examining a similar system which had been composed of phosphatidyl choline and various amounts of phosphatidyl ethanolamine, observed that the amount of glucose retained by spherules was roughly proportional to the amount of phosphatidyl ethanolamine present.

Incorporation of Protein

Spherules have been formed in the presence of cytochrome *c* and of albumin (15, 16). When suspensions of phosphatidyl choline, phosphatidyl serine, or phosphatidyl choline and phosphatidyl ethanolamine were shaken with small quantities of these proteins, small (0.5 μ) sacs with only two or three lamellae were formed, the repeat distances of which were as great as 125 A (wet). Although considerable amounts of protein became associated with the spherules (up to 80% of total protein in the case of phosphatidyl serine and cytochrome *c*) the proteins did not affect diffusion rates, a finding which was thought to demonstrate lack of penetration of the phospholipid layers by protein chains. Capture of ions was, however, appreciably decreased when protein was added to phosphatidyl serine–cytochrome *c* preparations, presumably because of complex formation by the basic protein and the acidic lipid.

LABILIZATION AND STABILIZATION OF LIPOSOMES

Steroids

The spherules have proved to be most useful in exploring the mechanisms whereby various steroids, drugs, detergents, and toxins act on lipid-bounded structures (10). These agents are listed in Table 1. Indeed, almost as soon as the spherules were devised as a model for measuring the release of captured ions, they were employed for studies of steroid-induced membrane changes. At least one theory of the general mechanism of the pharmacologic action of steroids is that these agents regulate the exchange of materials across the membrane of cells or organelles (see, for example, the discussion of cortisol action in reference 18). Therefore Bangham and coworkers studied the capacity of various steroids to induce release of cations from spherules prepared with ovolecithin, dicetyl phosphate, and cholesterol (10). They found that diethylstilbestrol, DOC, and

TABLE 1 LABILIZATION AND STABILIZATION OF LIPOSOMES: AGENTS THAT AFFECT DIFFUSION OF MARKER IONS OR MOLECULES

Increase	Decrease
Triton X-100	Cortisone
Lysolecithin	Cortisol
Streptolysin S	Chloroquine
Staphylococcal α-toxin	17β-Estradiol (when preincorporated)
Melittin	
Filipin	Cholesterol (when preincorporated)
Nystatin	
Amphotericin B	Nupercaine (and congeners)
Etruscomycin	
Organic solvents (narcotics)	
Ca^{++}	
Diethylstilbestrol	
Deoxycorticosterone	
Testosterone	
Etiocholanolone (other 5β-H steroids)	
Progesterone	
Gramicidin	
Valinomycin	
Streptolysin O (weakly)	
Cereolysin (weakly)	

various 5β-H steroids such as etiocholanolone caused prompt diffusion of cations; in contrast, the 5α-H isomers of active steroids, e.g. androsterone, were without effect. This property could be directly related to the capacity of steroids to induce release of hemoglobin from erythrocytes, or acid phosphatase from lysosomes. In sum, the correlation between the capacity of 14 steroids to disrupt both natural membranes and artificial spherules was extremely close. Furthermore, cortisone, cortisol, their acetates, and chloroquine, all *reduced* the release of cations from the spherules, an effect in keeping with their action upon erythrocytes and lysosomes (18). When cortisone was added to the spherules *before* diethylstilbestrol or etiocholanolone, the structures were partially protected against further steroid-induced release of ions (10).

The effect of steroids upon the spherules was not limited to release of cations. Glucose (19), glycine, $Cr_2O_4^{=}$, and $H_2PO_4^{-}$ (20) could be sequestered by the spherules and each of these marker molecules was subsequently released upon the addition of diethylstilbestrol, DOC, and 5β-H steroids.

It is difficult to extrapolate these actions to biological systems because the concentrations of steroids required to disrupt artificial spherules (10^{-3} M) are clearly beyond the physiologic or even pharmacologic range. Indeed the steroids cannot properly be considered in solution at all since these concentrations are also above their limits of solubility; thus they must be regarded as suspended in a mixture of solvent (DMSO) and salt solution. Yet, when one compares the lipid concentrations in the artificial system (15 μmoles/ml) with the lipid

concentrations of natural systems in which steroids disrupt lysosomes and erythrocytes (approx. 0.3 μmole/ml) (unpublished data of the authors), it becomes clear that the ratio of steroid to membrane lipid is not dissimilar in the two systems, since steroid effects on natural systems can be obtained at concentrations between 10^{-6} and 10^{-4} M. However, the advantage of using artificial spherules is that one can incorporate membrane-active agents into spherule lipids at the onset of swelling and thus create a number of artificial lipid structures containing various amounts of steroids as part of their membrane structure, so that the effect of the steroid can be amplified for study.

In such preparations as little as 0.5% of cortisol or cortisone in the membranes of spherules formed with phosphatidyl choline, dicetyl phosphate, and cholesterol sufficed to render the structure less permeable to anions, cations, glucose, or phosphate (10, 19, 20). Indeed these amounts of cortisol, but not of its inert metabolite tetrahydrocortisol, were sufficient to inhibit the response of spherules to subsequent additions of etiocholanolone or diethylstilbestrol (20). Antagonism of this sort, between glucocorticoids and 5β-H steroids such as etiocholanolone, has been shown in their effect on human fever and may be related to the antagonism of their action upon the membranes of lysosomes (18).

Because males are more susceptible than females to fever induced by pharmacologic doses of etiocholanolone, we examined artificial spherules prepared in the presence of various amounts of either testosterone or 17β-estradiol (21). As little as 0.1% of estradiol in the membranes of spherules rendered them refractory to release of anions by etiocholanolone, but did not diminish the release of ions induced by other steroids. Thus it is clear that spherules respond to membrane-active agents in a fashion depending both on the nature of the labilizing additive and on the presence of minute amounts of extraneous steroids. These actions can sometimes be related to the biological effects of the labilizing and protective additives.

Steroids not only induce *functional* changes in the overall permeability of the spherules, but also provoke *structural* rearrangements of the lipid layers themselves. Upon the addition of DOC, for example, to spherules formed with phosphatidyl choline, dicetyl phosphate, and cholesterol, the ultrastructural appearance of spherules changed remarkably: many strand-like, tubular structures could be observed, many of which were continuous with unravelled spherules (19, 20). The means whereby steroids induce this rearrangement is by no means clear, although it is possible that changes from lamellar to micellar configuration, or from lamellar to hexagonal, may be involved (9, 22). Spherules prepared without cholesterol appear to respond to steroids in the same fashion as do spherules prepared with ovolecithin and cholesterol (both preparations require the presence of some charged species such as dicetyl phosphate), so that steroids may be presumed to interact with phospholipids (23). Finally, although it is possible that the action of steroids may simply reflect their capacity to enter into lipid layers, thereby expanding them until they become unstable, all available evidence is to the contrary. For example, Snart and Wilson (24) determined the distribution of isotopically labelled steroids between aqueous and lipid phases of spherule suspensions, and found that steroids such as DOC and cortisol did not differ much in their coefficients of distribution, despite their directly opposite effects upon ion release from spherules.

Bacterial Toxins

Many bacterial toxins (hemolysins) disrupt erythrocytes, other cell membranes, mitochondria, and lysosomes (25, 26). Since steroids were capable of inducing cell lysis, disruption of lysosomes, and release of ions from lipid spherules, we reasoned that the action of bacterial lysins on natural membranes might similarly reflect the capacity of lysins to react with a wide range of naturally occurring lipids.

Streptolysin S rapidly induced release of cations, anions, and other marker molecules from the spherules (10, 20). Its action was associated with definite changes in the morphological appearance of the structures: although many membranes remained intact, the spherules became fragmented and condensed, while many new channels appeared between the inner aqueous compartments and the bulk phase (10). Staphylococcal α-toxin was also shown to release marker molecules from spherules (27); its action was completely abolished if the lysin was exposed to its antitoxin before addition to the target structures.

The mode of action of α-toxin has been examined in detail (28, 29). The toxin may be subfractionated into two spherule-lytic components which differ in their avidity for erythrocytes and artificial spherules. Judged by extensive studies of their mode of action by negative-staining techniques, they produce morphologic alterations that differ considerably from those produced by steroids or streptolysin S. The toxin induces the formation of ring-like structures on the outer lamellae of lipid spherules, rings whose structure resembles that of the aggregated toxin, but evidence was obtained that ring formation was not a necessary feature for toxin-induced membrane damage. This morphologic pattern may, therefore, reflect late, perhaps secondary, interactions between molecules of toxin and lipids which had already been displaced from their usual, lamellar arrange-

ment (29). This interpretation is in keeping with the studies of Lucy and Glauert (30) on saponin-induced rearrangement of phospholipid–cholesterol structures: here too the lytic agent formed rings and new structures with displaced lipids. The studies with α-toxin and with streptolysin S are compatible with the hypothesis that some bacterial toxins possess amphipathic (hydrophobic–hydrophilic) configurations (27). Insertion of such molecules into either lamellae or globules of lipids in water would weaken the intermolecular (London–van der Waals) cohesive forces and ultimately lead to disruption of the structures.

Other bacterial hemolysins, such as cereolysin (from *B. cereus*) and streptolysin O (from *S. hemolyticus*) were tested for their effects on artificial lipid spherules (31 and unpublished data of the authors). They induced only modest release of marker ions from the structures, and, compared to the lysins that are more effective in vitro (streptolysin S or α-toxin), showed a delayed onset of action.

Polyene Antibiotics

Perhaps the most intensively studied group of spherule-disruptive agents is that of the polyene antibiotics. These antifungal agents appear to exert their effects by interacting with specific lipids in the membranes of sensitive cells, and considerable evidence has suggested that sterols are the unique membrane component of target structures (32). It should therefore be expected, were the sterol hypothesis correct, that spherules with cholesterol in the membranes would be more sensitive to polyenes, while spherules with phospholipid alone should prove relatively resistant. Indeed this is so for amphotericin B and nystatin, the release of ions by which was enhanced as the cholesterol content of the target spherules was increased (23, 33). However, these polyenes also induced modest permeability changes when sterol was absent. Filipin was as disruptive to spherules prepared in the complete absence of sterol as to structures containing various amounts of cholesterol. These observations seemed in direct contradiction to studies on lipid bilayers (34) and on monolayers (35); in these systems filipin appeared to react preferentially with cholesterol-containing membranes.

It was established that this discrepancy could not have been due to the interaction of filipin with long-chain anions or cations present in the spherules (33) and recent experiments have contributed to the partial resolution of the conflict (23). Filipin, although isolated as a crystalline product, can be further resolved into four subfractions with rather different properties (M. E. Bergy and T. E. Eble, personal communication). Two of the subfractions (I and III), which constitute the majority of the crude material, appear to react preferentially with cholesterol-containing spherules, while the two others (II and IV) indiscriminately disrupt spherules whether or not sterol is present (unpublished data of the authors). Thus in biological systems, in monolayer studies, and in bilayer studies, where the effects of low concentrations of polyene (10^{-8} M) can be detected, the effect of the first two (cholesterol-reactive) fractions will be observed. In contrast, since the spherule system requires higher concentrations ($>10^{-5}$ M), all four fractions will exert effects and no sterol requirement will be evident. These studies are complicated not only by the heterogeneity of filipin, but by the ability of this polyene to react with many lipids in suspension, so that while sterols may be necessary for the disruption of *some* natural membranes, other lipids may also act as targets. Of the biological membrane-bounded systems, the polyenes effectively disrupt lysosomes, but have little effect upon mitochondria, a finding explicable, perhaps, by the relatively low cholesterol content of the latter.

The polyenes have also been shown to produce ultrastructural rearrangements of spherule lipids. Thus filipin has been reported to produce fraying and "honeycombing" of phospholipid structures prepared in the absence of sterol, while amphotericin has been shown to produce large aggregates with central "lakes"; these latter changes appear only in sterol-containing spherules (23). In suspensions of lipids that contain 50% cholesterol, rings and pits have been observed after addition of filipin (36) which resemble in form, but not dimensions, the circular structures observed after the addition of saponin (30).

Other Membrane-Active Agents

Agents such as lysolecithin (10), Triton X-100 [a nonionic detergent mixture of *p,t*-octyl poly(phenoxyethoxy)ethanols] (19, 20), and the ion-discriminating antibiotics gramicidin and valinomycin (37) have also been studied. Each of these induces ion release from the spherules: indeed both Triton X-100 and lysolecithin produce ultrastructural changes that are quite distinctive. But perhaps the most interesting of this miscellaneous group of compounds are the narcotic agents that have been studied by Bangham, Standish, and Miller (38). The model spherules showed measurable responses to these agents at concentrations similar to those used in vivo, but cation diffusion was different for two types of narcotics studied. Whereas organic solvents (ether, chloroform, *n*-alkyl alcohols) increased K^+ diffusion with decreasing chain length of the solvent, all four local anesthetics (nupercaine, tetracaine, cocaine, and procaine) reduced K^+ release, perhaps by reducing the surface charge density of negatively charged membranes.

We have recently found (39) that the amphipathic polypeptide melittin (the major protein component of bee venom) disrupts the lipid spherules. This action would support the hypothesis discussed above for bacterial lysins, i.e., that proteins of amphipathic tertiary structure can directly interact with lipid membranes.

CONCLUSION

How good a model system do the spherules provide? The spherules fulfill several desirable criteria. Their lamellar structure resembles that of at least one model of natural membranes. They are capable of ion discrimination: cations diffuse poorly from membranes which are permeable to monovalent anions and water. They swell osmotically, as do natural membrane-bounded structures. They are susceptible to labilization and stabilization by many agents which act similarly upon a wide variety of natural membranes. This group of agents includes detergents, hormones, steroids, antibiotics, narcotics, and drugs. It is possible to vary the membrane composition of the spherules either by incorporating trace amounts of test agents or by varying the lipid proportions. It is also a simple matter to vary the surface potential, and to measure diffusion of a wide variety of anions, cations, and other marker molecules. It may be possible to incorporate small amounts of protein into the lamellar structure, although this has not been extensively investigated. Finally, the spherules can be studied by a variety of physical means: their volumes, surface charges, birefringence, crystallinity, and ultrastructure in wet and dried states can be measured.

However, it is not possible to discuss model membranes without qualifying the term, since it is really unclear for which particular membrane these particular models stand. The wide variation in composition of natural membranes, ranging from myelin through cell membranes and intracellular membranes to plant and bacterial membranes, precludes any generally valid model. The spherules' composition can, however, be varied sufficiently so that their over-all composition (if not their structure) can resemble that of any given, well-defined natural membrane. Thus it has become possible to form structures with the gross lipid composition of mitochondria, erythrocytes, or lysosomes. Whatever compositional agreement can be attained, it must be appreciated that the three-dimensional structure of natural membranes is probably much more complex and polymorphous than a static lamellar model would suggest. Good evidence is available to suggest that at least some membranes contain structural proteins as major constituents. Indeed the surface properties of biomembranes may be determined by a mosaic structure, with globules or areas of amphipathic proteins alternating with areas of lipids (30). Until the interactions of purified structural proteins with lipid spherules have been extensively explored, many reservations must be held as to the relevance of pure lipid spherules to any actual biological membrane.

The spherules constitute perhaps a less suitable model for some kinds of study than monolayer and bilayer systems. The concentrations of various physiologic and pharmacologic agents that are required to act upon the spherules are much greater than those needed to influence monolayers or bilayers, and one cannot study adequately the mode of insertion of amphipathic molecules or measure their effect on small changes in surface area or pressure as in monolayer studies. Such differences may be due to differences in the surface areas under investigation; i.e., for a hand-shaken preparation of 15 μmoles of lipid, the target area would be 3000 cm^2 versus a bilayer preparation of 2–5 mm^2. The spherules do not lend themselves readily to studies of electrical properties, as do bilayer models. Their structure is by no means as rigorously established as that of the other models, which can be directly observed in the wet state and in which it is clear that diffusion does proceed across a defined membrane.

Finally, although a number of distinctive ultrastructural rearrangements following the addition of various agents to the lipid spherules (e.g. streptolysin S, lysolecithin, steroids) have been described, there is no assurance that their morphologic appearance in negatively stained specimens reflects structures that are present in the wet state. Indeed, from the studies with α-toxin, it seems that many of these dramatic configurations may reflect secondary events which occur after undetectable defects in the lamellae have released free lipids to interact with the lytic agent. Be that as it may, it is at least encouraging that structures similar to those observed in the artificial system can be seen when natural membranes (usually erythrocyte ghosts) interact with many of the disruptive agents.

In sum, the lipid spherules constitute a valuable tool with which to study many problems of membrane structure and function. Should it prove possible to incorporate proteins, enzymes, or nucleic acids into these models, this might constitute another step towards a true in vitro replica of membranes of living systems.

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