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The hydration of phospholipids and its biological significance

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The difference between the physical properties of water near the surface of certain materials and the same properties of solution water has been known for some time. The idea that this difference may also apply to the surfaces of biomolecules, cells, and tissues and, thus, may be involved in the structure/function relationships of these biological entities has only recently been appreciated. The purpose of this review is to discuss the nature of this water-surface interaction as it relates to biology and medicine, to illustrate some of the methods by which this water is studied and, perhaps most importantly, to inspire the reader to consider how this "bound water" concept might be applied to his or her research. Such research would include transport across membranes, lateral movement along the cell surface, and lipid-protein interactions, as well as many metabolic processes, both normal and pathological. Although the emphasis in this review is on the interaction of water with phospholipids, it is the author's experience that bound water is rapidly being accepted as a useful concept in the study of other macromolecular processes, such as radiation damage in DNA and bioenergetic mechanisms in proteins. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:599–609, 1996.)

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If there is magic on this planet,
it is contained in water.
Loren Eiseley

Although this aphorism was written by a renowned anthropologist and is meant to apply to the evolution of living organisms,[1] we believe it also applies, although in a more restricted sense, to the subject of this monograph. At first glance, the hydration of the ubiquitous phospholipids may seem to be a rather esoteric subject, of interest only to physical biochemists. We hope here, however, to present the topic in a manner so as to help the reader become more aware of the many biologic phenomena involving the interaction of phospholipids with water.

Bound water

It has long been known that the water near certain surfaces may have different physical properties as opposed to that of "free" (i.e., solution) water. For example, this water may freeze at temperatures well below 0°C, the freezing point of bulk or free water. The electrical properties of this "bound" water may also be different as compared with that of the surrounding water. A variety of measurements indicate that the water near surfaces has different molecular translational and rotational properties, as well as density as contrasted with these same properties in free water. Because many biological, including metabolic, processes occur at cellular surfaces, the involvement of this surface water in these processes is of considerable interest. This surface water is depicted in a somewhat sketchy fashion in *Figure 1*. The water near surfaces has been referred to as bound, ice-like, structured as well as by a number of other terms. Universal agreement on describing this water does not yet seem to have been attained and, indeed, from a semantic standpoint, the descriptive noun chosen may well influence the investigator's interpretation of the experimental results. This ambiguity perhaps arises from the fact that the water interacting with the surface being studied is not "permanently" bound, but rather is in exchange with the "bulk" water surrounding the surface, as is suggested in *Figure 1*. It, thus, becomes important to be cognizant of the time scale on

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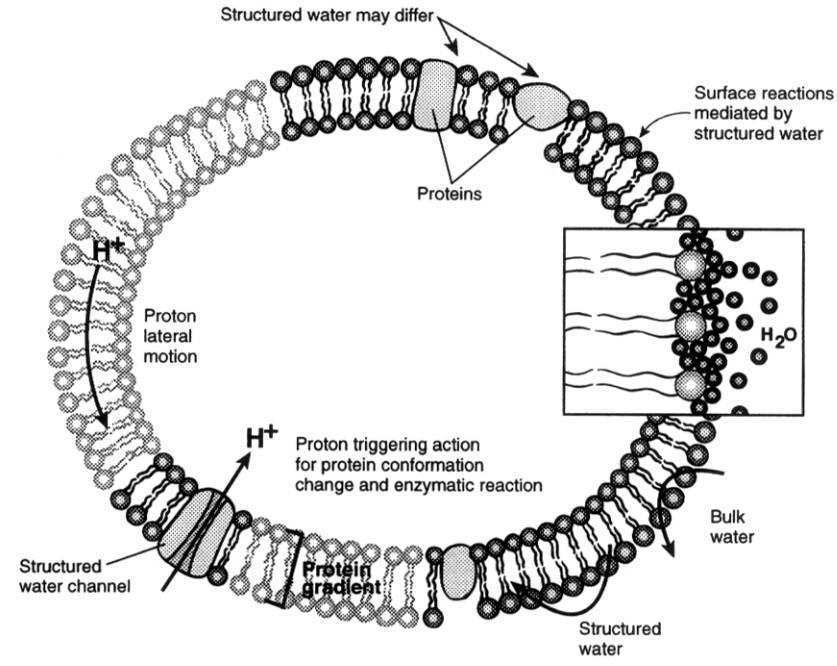
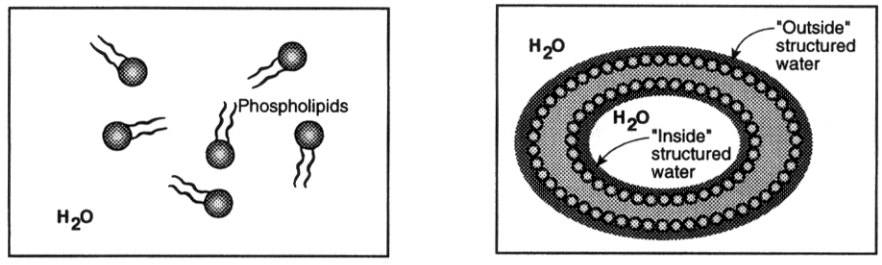


Cell membrane with exterior and interior structured water: possible role of the structured water in membrane morphology and biological function.



Interaction of phospholipids with water resulting in phospholipid aggregate formation and the appearance of structured water on the inside and outside lipid vesical surfaces. Inside and outside structured water may or may not have the same arrangement.

Figure 1 Representation of water interacting with the surface formed by phospholipid head-groups. This illustration is not intended to be an accurate picture of what actually occurs at such a surface.

which the various physical measurements are conducted, relative to that in which exchange of the surface water with the bulk water occurs. In terms of the physical properties of the water interacting with the surface, there does seem to be agreement that the effect of the surface on the water diminishes as the distance from the surface increases; there does not, however, seem to be a distinct demarcation between the water interacting with the surface and the bulk water itself. In this monograph, we will discuss the interaction of water with phospholipids in terms of the method of measurement and we will use one or the other descriptive words with no preferred model, unless specified. Although there exists much experimental data, a complete theoretical treatment of this data awaits further work. A brief, but excellent, general review on the state of water in biological systems is given in Ref. 2, though this work is not specifically concerned with phospholipid water binding.

Experimental methods

Various experimental approaches to the study of phospholipid hydration will be discussed in the following sections. These approaches include gravimetric,[3–9] electrical,[6,7,10,11] x-ray diffraction,[12,13] force measurements,[14] nuclear magnetic resonance (NMR),[15,16] and several other methodologies.[13] This list is not, however, intended to be all-inclusive. For the details of the various experimental approaches, the reader is referred to the references cited.

It is important to note here that all of the techniques do not use the phospholipids in the same macroscopic physical configuration: for example, the gravimetric technique, which is a direct measure of adsorbed water, uses the phospholipid in the solid state. A number of other measurement techniques involve the phospholipid particles in water solution, with the phospholipid in the vesicular or liposome

configuration. These nongravimetric measurements, moreover, are usually indirect measurements of the water adsorbed by the phospholipids. The gravimetric measurements, on the other hand, are not only direct, but provide information on phospholipid water adsorption over a large range of partial vapor pressures of water corresponding to a large range of number of adsorbed water molecules per phospholipid molecule. These water adsorption isotherms obtained gravimetrically specify the activity of the adsorbed water and, thus, the work required to transfer water from the bulk solution to the region between the phospholipid bilayers. In several of the other techniques, because the measurements are made in lipid solutions the results apply only to the highest water vapor pressure, i.e., one (1). There is also some question as to the relationship between measurements made in solution versus those made at vapor pressures approaching 1. For example, in the case of certain electrically charged phospholipids, it is found that phospholipid vesicles in solution take up large quantities of water (swell), whereas in the solid state configuration, rather modest amounts of water, even at vapor pressures approaching 1, are taken up. This has led to what has been described by some as a "paradox"[13] and interesting theoretical work is now being done on this phenomenon.*

Because phospholipids in a hydrated environment may also take on several morphological forms at the molecular level, depending on the degree of hydration and temperature, one must again use discretion when comparing the results obtained using these various experimental techniques. This multiplicity of morphologies is related to the fact that the phospholipids are amphipathic because they usually have two long hydrocarbon chains as well as a short polar group attached to the glycerol moiety; the result is that the polar groups tend to interact with the solution water, whereas the hydrocarbon chains "avoid" the water environment. Depending on the water available, the temperature and the properties of the phospholipid in question, the lipids may thus form a variety of structures. Additionally, intermolecular interactions in these various structures impose restrictions on the allowed conformations of the polar headgroups and the hydrocarbon chains. Perhaps the most common configuration, especially at the higher hydration levels and at temperatures near that of the human body, is the bilayer configuration where the phospholipids arrange themselves in bilayer leaflets with the hydrocarbon chains interior to the leaflet and the polar headgroups exterior and exposed to the water. This, of course, is reminiscent of the cellular membrane structure in living systems. In this bilayer arrangement, phospholipids can further exist in two different states, i.e., the gel and the liquid crystalline state. In the gel state, the motion of the head-group and the hydrocarbon chains are much more restricted and "crystal like" than is the liquid-crystalline state, where the hydrocarbon chains are in a much more fluid-like condition. The transition from gel to liquid-crystalline state can be characterized, for a given set of environmental conditions and a particular phospholipid, by a phase transition temperature T_C. This temperature depends on the electrical charge of the polar head-group, the length and amount of unsaturation of the hydrocarbon chains, the degree of hydration, as well as other environmental factors. This variety of available molecular conformations for the phospholipids can be seen to complicate any discussion of phospholipid hydration. The hydration of a particular phospholipid at a given water activity will thus depend on the lipid molecular character as well as on the aggregate conformation in which it exists. Additionally, as the thermodynamic activity of the available water changes, so may the aggregate conformation. From a biological standpoint, cellular membrane lipids of several types are usually present (along with other non-lipid moieties) and, thus, the lipids may exist in regions of different conformation over the cell surface. This lyotropic mesomorphism is very important, however, its physical chemistry is too involved for a detailed discussion in this review; excellent treatments of this topic are found in the literature.[17,18] The above complexity notwithstanding, the hydration results obtained using the various experimental techniques often agree remarkably well. What is not yet clear, however, is how these results obtained in simple model systems containing the particular phospholipids, apply to biological systems containing the same phospholipids, but in a much more complex setting; this is obviously of great significance in biology. In any case, it is likely that the most complete picture of phospholipid hydration will be obtained by using the results of several experimental approaches in a complementary manner.

Inasmuch as phospholipids are usually present at cellular and, therefore, tissue surfaces and also at the surfaces of such important entities as lipoproteins, the interaction of water with phospholipids is of rather "global" interest because many biological processes occur at the surfaces of cells as well as at the surfaces of the structures internal to cells and these surfaces are intimately involved in metabolic function. In what follows, it should be kept in mind that because of the formation of aggregates by phospholipids, the interaction of water with phospholipids is really the interaction of water with the surfaces of these aggregates. The properties of these extended phospholipid surfaces, although arising from the molecular properties of the phospholipids themselves, nevertheless, should be considered as a collective phenomenon.

Experimental results

Elworthy and coworkers,[3,4] in pioneering work using gravimetric techniques, were perhaps the earliest workers to directly study phospholipid hydration. They not only measured the hydration of phospholipids but attempted to apply BET theory[19] analysis to this phospholipid hydration; BET theory, where applicable, enables such quantities as monolayer coverage and binding energies of the water adsorbed to a surface to be determined. In this early work, they hypothesized that the polar moieties of the phospholipids were primarily responsible for the water adsorption. Both phosphatidylcholines (PCs) and cephalin were studied and the PCs were found to adsorb significantly more water than did the cephalins. These workers also discussed their results in terms of various "layers" of water adsorption as the basis of their application of BET theory to the adsorption process.

*A. Parsegian, private communication.

Extending this analysis, they found endothermic differential heats of adsorption and positive differential entropies of adsorption for the phospholipid hydration processes. Interestingly, for certain phospholipids, hysteresis in the hydration process was found at the very lowest partial vapor pressures of water, i.e., for the first few water molecules adsorbed. This hysteresis may be indicative of a rearrangement of the phospholipids in the "dry" state as they adsorb the first few water molecules and agrees with later work as will be discussed.

More recently, other workers[5,6,7] applying a microbalance technique to thin films of phospholipids, obtained water adsorption plots for a variety of phospholipid systems. These workers interpreted their results in terms of levels of the binding strength of the adsorbed water. In *Figure 2* are shown adsorption isotherms obtained for a number of these phospholipid systems containing a variety of unsaturation levels in their hydrocarbon chains. As can be seen, for a given lipid headgroup, in this case choline, the amount of water adsorbed increases as the number of double bonds in the hydrocarbon chains increases. This is interpreted to mean that as the unsaturation of the hydrocarbon chain regions of the lipid bilayers increases, the volume containing the lipid headgroup available for the water adsorption also increases, perhaps as a result of increasing disorder in the hydrophobic chain region. In support of this interpretation is the fact that cholesterol, complexed to phospholipids, significantly increases the water adsorbed by these phospholipids[5] even though the cholesterol itself adsorbs a negligible amount of water; because cholesterol "sits" in the hydrophobic region of phospholipid bilayers and, therefore, acts as a "spacer molecule," it might be expected to separate the phospholipid molecules from each other, thus increasing the volume available for water adsorption. On the basis of their shapes, all of the adsorption isotherms shown for PC in *Figure 2* as well as those for phospholipid-cholesterol complexes indicate "strong" water adsorption by the phosphatidylcholines; this determination is made using the conceptual framework of BET theory.[19] From the isotherms shown in *Figure 2,* we qualitatively describe the water bound to the PCs at vapor pressures of 0 to 0.3 as the most tightly bound water; for the PCs this corresponds to 1 to 3 water molecules bound per phospholipid molecule. Less tightly bound water would be adsorbed over the partial vapor pressure range of 0.3 to 0.7, whereas the most weakly bound (although still not free) water is adsorbed over the higher partial vapor pressure range approaching 1. An example of an adsorption isotherm indicating weak water binding is that for egg phosphatidylethanolamine (EPE), also shown in *Figure 2.*

On the basis of the isotherm shapes and amounts of adsorbed water illustrated in *Figure 2,* it can be said that for these zwitterionic PCs, the presence of methyl groups on the N^+ headgroup moiety seems to determine the binding strength and the amount of water adsorbed. EPE, which is zwitterionic, but contains no methyl groups on the N^+ element is a weak water adsorber, whereas monomethyl egg phosphatidylethanolamine (MMEPE), which is also zwitterionic, but has one methyl group attached to its N^+ element is a strong water adsorber and, moreover, adsorbs more water than does EPE; egg phosphatidylcholine (EPC)

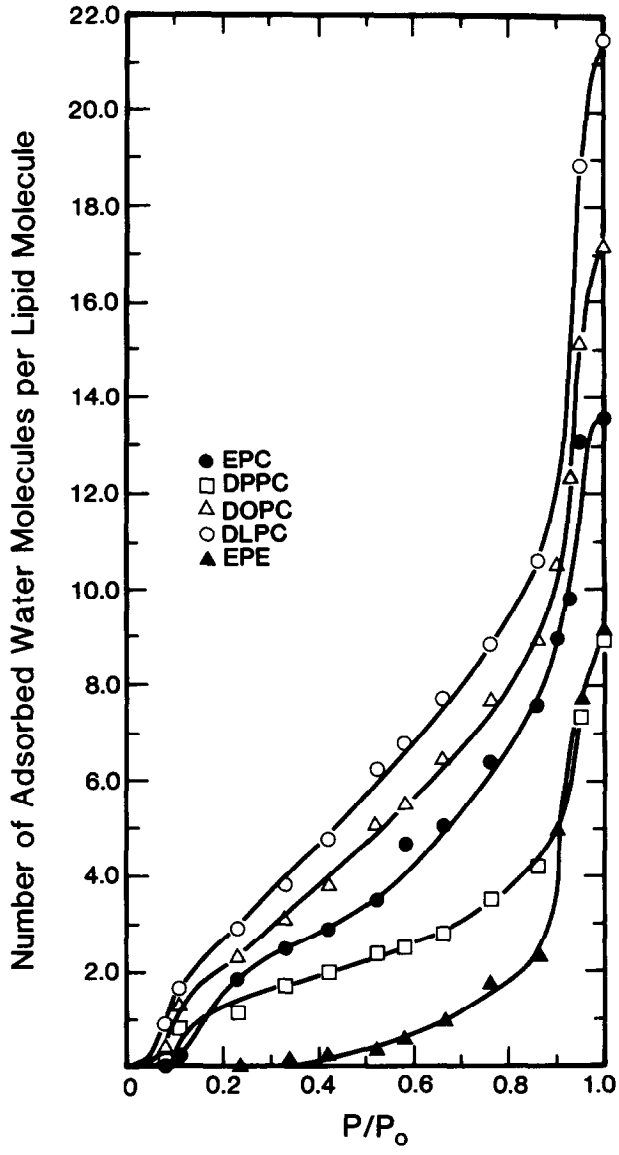


Figure 2 Water adsorption isotherms of various phosphatidylcholines. These adsorption isotherms show the number of water molecules adsorbed per phospholipid molecule at a given partial vapor pressure (p/p_o) of water. This figure illustrates the effect of the phospholipid, hydrocarbon chain unsaturation on the water adsorption of the lipids, as well as the effect of the N^+, CH_3 groups on the adsorption. For purposes of comparison, the adsorption isotherm for EPE is also shown.

has three methyl groups on the N^+ element, is a strong water adsorber, and adsorbs significantly more water in general than does MMEPE. It is thus apparent that, at least for the PCs, the choline methyl groups control the strength and amount of bound water, whereas the unsaturation of the hydrocarbon chains modulates the amount, but not necessarily the nature, of the bound water. Very recent work on sphingomyelin (SM), however, suggests that this methyl group effect may not apply to all phospholipids; SM has three (3) methyls in its headgroup and, moreover, is quite similar to PC in its total molecular structure. SM, however, adsorbs a rather small amount of water and, furthermore, the

adsorption isotherm suggests weak water binding.* The presence of a net electrical charge on the phospholipid headgroup results in a somewhat more complex situation[20] and is still under investigation. The weak water binding by EPE vis-à-vis EPC is often ascribed[21] to intra-intermolecular, hydrogen bonding in EPE; this bonding is apparently reduced by the presence of methyl groups on N^+. For the phospholipids common to both the work of Elworthy[3,4] and the later work,[5,6] the gravimetric results are in quite good agreement quantitatively as well as interpretatively.

In an interesting approach, later workers[8] combined gravimetric measurements with differential thermal analysis (DTA). On the basis of their measurements these workers postulated that EPC, dipalmitoyl phospatidylcholine (DPPC), and dilinoleoyl phosphatidylcholine (DLPC) tightly bind about nine water molecules per phospholipid molecule. This number is not necessarily in disagreement with that found in the previously mentioned work,[5] however, because in that work, further differentiation of the bound water for these same phospholipids was done using BET theory, and each EPC, DPPC, and DLPC molecule was postulated to bind 2.7, 1.3, and 3 water molecules respectively, in the most tightly bound water layer; the next most tightly bound water layer for these same phospholipids resulted in 7, 3, and 6 water molecules respectively bound per molecule of phospholipid. There is thus general agreement between the two experimental approaches and the difference in number of the most tightly bound water molecules is apparently an operational one in that differential scanning calorimetry techniques were used in the work of Ref. 8, to propose the "mostly tightly bound" water where, as described in Ref. 5, this corresponding water was further subdivided into two classes based on the nature of the adsorption isotherms and the accompanying BET analysis. This comparison illustrates the quantitative differences one may obtain depending on what model of water adsorption one assumes. The two different sets of experimental results are in agreement, at least qualitatively, in that cholesterol was found to increase the hydration of PC in complexes of cholesterol with PC and they also agree in the distinctly different adsorption isotherms obtained for EPC and EPE (see *Figure 2*). Again, in this latter study, the hydration was discussed in terms of the organization of the bound water around the phospholipid polar headgroup, as it was by those workers who used gravimetric methods.

In a somewhat different approach,[11] dielectric measurements along with gravimetric ones were made in a complementary fashion. This work, although done with DMPC, should be applicable to PCs in general. The dielectric results were obtained with a rather elegant technique[22] called thermally stimulated depolarization currents (TSDS). Over the temperature range of 77° to 320°C, these workers hypothesized three types of water: loosely bound water, the water associated with the rotation of the polar headgroups, and water associated with space charge polarization. Additionally they postulated[11] that water in excess of three molecules of water per molecule of DMPC forms "clusters." The formation of these hydrogen-bonded clusters may involve percolative proton transport and would also be consistent with the direct current electrical conductivity increase found for fully hydrated phospholipids.[10] It should be noted here that the so called "loosely bound water" postulated on the basis of dielectric measurements would seem to be equivalent to the third class of bound water put forth in the earlier work[5] however, this comparison of the dielectric and gravimetric work again underscores the difficulty of correlating classes of bound water defined on the basis of results obtained with different measurement techniques.

To further monitor the water adsorbed by the dry solid state phospholipid films, concurrent dc electrical conductivity measurements were performed as the adsorption isotherms were being obtained.[6,7,10] Interestingly, all phospholipids showed a very large increase in the dc electrical conductivity as the samples were hydrated over the relative vapor pressure range from an operationally defined 0 to approximately 1. This conductivity increase was some eight to twelve orders of magnitude and did not seem to exhibit specificity for the nature of the phospholipid. An example of such conductivity behavior is shown in *Figure 3,* where the electrical conductivity of EPC (a strong water adsorber) is shown along with that for EPE (a weak water adsorber). The electrical conductivity has proven to be a most sensitive indicator of the phospholipid "dry state." Note that the most rapid increase in conductivity occurs for the first one to three water molecules adsorbed per phospholipid molecule that, in turn, corresponds to the first monolayer coverage of the phospholipid by the water molecules, as ob-

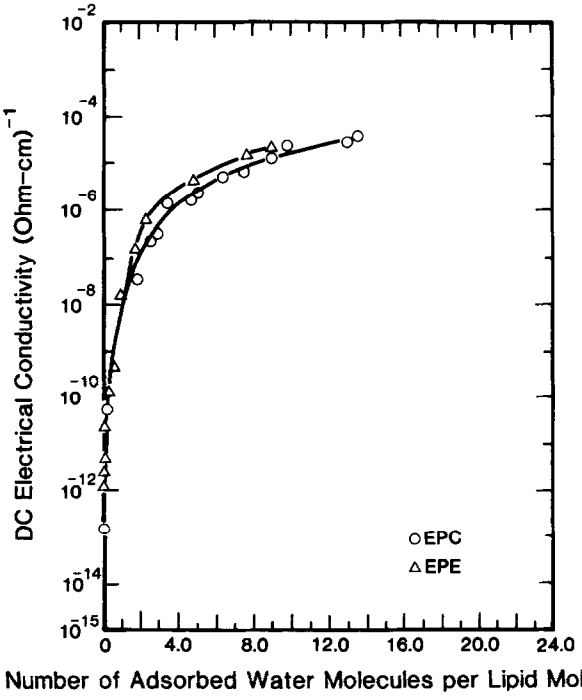


Figure 3 The D.C. electrical conductivity versus number of water molecules adsorbed per phospholipid molecule for EPC and EPE, respectively. Note the very rapid increase in electrical conductivity for the first few water molecules adsorbed; the ordinate scale is a logarithmic one. The electrical data is obtained concurrently with the adsorption data shown in *Figure 2.*

*G.L. Jendrasiak and R.L. Smith, work in progress.

tained in Ref. 5 using BET theory. This rapid increase of the electrical conductivity, followed by a leveling off, is ascribed to the completion of hydrogen bonded conductivity paths[20] and protonic conduction is proposed as the dominant charge carrier mechanism for this hydrogen bonded, ice-like matrix. The initial increase of the dc electrical conductivity of the hydrated phospholipid films would also seem to correspond with the first three non-clustered moles of water per mole of DMPC, proposed in the dielectric studies[11]; beyond this amount of water, it is speculated that clusters form. The relationship between the nature of water bound to phospholipids and the electrical results are of some biological interest: the transport of protons across cell membranes, is a central tenet of the chemiosmotic theory of Mitchell.[23] Tiessie[24–26] and others[27] have done elegant work on the transport of protons laterally along the headgroup surface of monolayers and bilayers of phospholipids and attempt to relate their results to the chemiosmotic theory. If such lateral proton transport does occur along the surface of cellular membranes, the path may well be dependent on the structure of the water at the cell-water interface. This is a very interesting yet difficult area of study and needs further development, especially as it may be related to those biological transport processes driven by protonic pumps.

Nuclear magnetic resonance

NMR has played a prominent role in the study of phospholipid hydration. Finer and Darke[15] were pioneers in these studies. These investigators studied the hydration of EPC, EPE, and ox brain sodium phosphatidylserine (PS) and found that the three phospholipids exhibited different hydration behavior on the NMR time scale. These investigators postulated that PS binds about one molecule of water per phospholipid molecule in an inner hydration shell that they ascribed to the phosphate group; an additional 10 molecules of water are believed to bind in the headgroup region and another 12 molecules with the sodium ion in the PS salt. Finally, up to 120 molecules per lipid molecule were believed to be trapped between the phospholipid lamelae. All of these water molecules were postulated to undergo rapid exchange with each other (greater than 10^4 sec^{-1}) but exchange only slowly ($<10^2$ sec^{-1}) with bulk or solution water. EPC, on the other hand differed in that it was found to bind 11 water molecules in the polar headgroup region and another 11 molecules trapped between the lamelae. EPE was postulated to resemble EPC in its hydration behavior except that no water is trapped between lamelae. The strength of binding of the most tightly bound water was found to increase in the order PS < EPC < EPE. This NMR work is a rather striking example of comparing hydration values obtained by using experimental techniques that measure hydration on different time scales, e.g., 10^{-8} sec for NMR and hours for gravimetric measurements. Consider the NMR results just mentioned and compare them with those obtained by gravimetric techniques.[3,4,5] The NMR studies were performed with the lipid suspended in water, whereas the gravimetric measurements were made with solid state samples of lipid on a substrate with the samples exposed to an atmosphere at various relative humidities. The gravimetric studies showed that both EPE and PS displayed adsorption isotherms indicative of weak water binding; this implies that the binding energy of the first monolayer of water to the phospholipid surface is less than the liquefaction energy of water. Using BET calculations, it is found that fewer than 0.5 molecules of water per lipid molecule bind to these phospholipids and this is only an upper estimate. EPC, on the other hand, exhibits the water adsorption isotherm characteristic of strong water binding. The binding energy of the first monolayer of water as well as a monolayer value of 1.5 waters per EPC molecule, can be determined by using the BET approach. If the NMR and gravimetric results are carefully studied, it would thus seem that they are in qualitative agreement, but the meaning of the terms (tightly bound water) and binding energy differ because, as mentioned, the experimental results differ substantially and are obtained on quite different time scales.

Other examples of the NMR approach are given in the studies of Seelig and coworkers.[16] This group has done elegant work on the influence of hydration on the orientation of the phospholipid headgroup. They interpret their results in terms of the alignment of the phosphocholine dipole in the bilayer membranes, as this alignment is affected by hydration. Using 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with deuterium substituted for hydrogen at the two methylene segments of the phosphocholine headgroup, they studied the alignment of the headgroups as a function of hydration in the range of 10 to 70% H_2O. Interestingly, they found that with decreasing hydration, the N^+ end of the headgroup dipole moves closer to the hydrocarbon layer of the phospholipid bilayer. These workers, thus, conclude that the hydration of a lipid bilayer not only results in an ordering of the water molecules (ice-like) at the bilayer surface, along with immobilization of the headgroups but concomitantly is accompanied by a reorientation of the $P^- - N^+$ dipole toward the membrane interior. If one combines this explanation with that for the gravimetric results interpreted in the light of BET theory, we can begin to obtain a molecular picture of the energetics and confirmational changes in the phospholipid molecule as the first few water molecules are adsorbed: we hypothesize[20] that the first few water molecules adsorbed by the dry phospholipid result in a surface that, in turn, adsorbs water in a manner consistent with the measured BET isotherm. The BET interpretation of the number of waters adsorbed in the first monolayer of water and the energy of this binding would thus apply to this "slightly" hydrated new surface. This new surface, at least in some cases, could result from a phase transition of the phospholipid and thus may be accompanied by a reorientation of the phosphocholine bilayer as seen in the NMR studies.[16] Such an interpretation also seems to be in agreement with the conclusions of Gawrisch and coworkers.[28]

Further significant hydration results were obtained by other deuterium NMR studies.[29,30] Again, these studies indicate a concerted change in headgroup confirmation upon lipid hydration together with an increase in headgroup mobility. Interestingly, these workers present their NMR results in a manner that they consider equivalent to the water adsorption isotherms for the same lipids. Furthermore, they

propose a model wherein the effective accessible headgroup volume expands with increasing hydration.[30]

These examples illustrate some of the strengths of the NMR technique in that it can be very useful in determining changes in molecular structure, as a function of some environmental factor such as hydration. When the experimental results are combined with those obtained with other methodologies such as x-ray diffraction or gravimetry, the potential scientific usefulness of the results can be greatly enhanced as compared to that based on results obtained with a single experimental technique alone.

Example of integration and applications of physical techniques to a biological system

Some very significant work on bound water and its biological significance has been done by Clegg and coworkers on Artemia cysts.[31] This work, although not specifically directed at the water interacting with lipid surfaces almost certainly involves lipid hydration; the work, moreover, is noteworthy in its integration of a number of the physical techniques, mentioned previously, in studying water interacting with cellular surfaces.[32] The conclusion drawn from this work is that at least a very large percentage of water in this biological system has molecular translational and rotational properties that are markedly different from those of water molecules in dilute aqueous solutions. From a conceptual standpoint, the author points out that a number of basic processes are shared by almost all cells and it is reasonable to include the interactions of water with intracellular architecture, in this list. The role of this restricted water in the cyst metabolism is alluded to by the author. A very interesting aspect of this work is the use of water vapor adsorption isotherms and BET theory for studying the water binding to these biological systems.[31] Interestingly, a value of close to 6gm H_2O per 100gm dried cysts is found for the monolayer coverage of water (as defined in BET theory). In our own work we find, on the average about 4.5 gm H_2O per 100 gm lipid as the monolayer coverage value for strong, water-binding phospholipids.[20] Although the lipid compositional data of the cysts is not given in the paper,[32] nevertheless, the similarity in monolayer values is provocative.

Hydration forces

It seems quite reasonable in light of what has been said so far that the water associated with the surfaces of particles containing phospholipids should have different properties than does that of the water in the surrounding bulk phase. One important effect of this difference is the development of water-mediated repulsive forces between the hydrated phospholipid bilayers.[13,14,33] These hydration forces are thought to arise from the structuring of the water molecules at the phospholipid, polar surface and the forces are found to decay approximately exponentially with increasing separation between the bilayers. At small distances between the bilayers, this repulsive force becomes stronger than the van de Waals attraction and is apparently responsible, at least in part, for the resistance of certain model and biological membranes to spontaneously coalesce and fuse. The hydration forces thus have obvious biological implications. It should be mentioned here that the presence of hydration forces between surfaces is not confined to neutral lipid bilayer membranes, but these forces have also been observed in minerals and glasses, DNA helices, polysaccharides, and proteins. An excellent discussion of these forces is contained in a recent review article.[34] Because the hydration force between lipid bilayers exhibits an exponential decay length of between 1–3 Å, one might expect it to play a prominent role in the most "intimate" interactions such as those between liposomes, between cells, and between liposomes and cells as well as other lipid particle interactions in biology. Interestingly, it has been found[13] that the work needed for bringing EPC bilayers to "contact" gives an energy of approximately 5 kcal per mole; 5 kcal per mole is the energy involved in forming a single H-bond[35] and this number is in good agreement with that found for phospholipids using the BET theory to calculate the energy of the first "monolayer" of the water adsorbed by phospholipid films.[20] It would also argue for an "ice-like" layer of water at the phospholipid-bulk water interface, in agreement with the electrical measurement results mentioned earlier.[7,10] Thus, from a very simplistic standpoint, as one phospholipid particle approaches another, one can speculate that the repulsive and attractive forces compete as the distance between the particles decreases. When the two particles attain a certain separation, the various types of water bound to each particle may begin to interact. For the two particles to coalesce and fuse, dehydration or rearrangement of at least some of this bound water must occur for the fusion to become effective. Such a scheme seems to be applicable to model membranes: PE containing liposomes, PE being a weak water binder fuse, whereas PC liposomes, PC being a strong water binder, do not fuse. Dehydration would thus appear to be a necessary, albeit insufficient step, for liposome fusion. Such a water restructuring event may well occur in the fusion of cellular particles, also. As put so aptly in Ref. 34, "the interaction of water-soluble particles is best seen as their interaction with water itself. In clinging to the solvent, they consequently repel each other except in peculiar and intentional circumstances where the match between molecular surfaces is stronger than with the host solvent itself."

In an important paper[36] connecting the water adsorption characteristics of phospholipids with the hydration force, Marsh has calculated the repulsive hydration forces on the basis of water adsorption isotherms themselves for a variety of diacyl and monoacyl phospholipid systems; he found that for diacyl phosphatidylcholines, the hydration force and its characteristic decay length are in good agreement with the values previously obtained by x-ray diffraction techniques.[37,38] Importantly, the repulsive force was found to be a continuous function of the number of water molecules associated with the lipids, i.e., of the energetics of hydration and, therefore, the adsorption isotherms are a valid reflection of the hydration force, when extrapolated to high water content; good agreement was obtained between the parameters of hydration for EPC, DOPC, and DPPC, deduced from the adsorption isotherms, and those parameters obtained directly from measurements with x-ray diffraction, at considerably higher water concentrations.[38] This agreement

is provocative because it connects the energetics of the first few water molecules adsorbed to phospholipids with the hydration forces between bilayers of these same phospholipids.

In another interesting study,[39] the author attempts to relate this exponentially decaying hydration force to the interfacial structure of phospholipid membranes. A generalized non-local electrostatic model of hydration is discussed in this work. The author concludes that if the effective interfacial width significantly exceeds the water structure decay length, the structural aspects of the adsorbing surface may become more important than the solvent characteristics themselves. It is further concluded that for a relatively thick interface, such as that at the phospholipid bilayer surface, the hydration force may act as a "messenger" of the surface interfacial structure. This, of course, could lead to biological "signalling" by the hydration force and, moreover, would relate this to the nature of the water adsorbed at the phospholipid, polar headgroup.

Certainly these hydration forces, although still not well explained, would be expected to play an important role in the study of a number of biological phenomena in the future.

Biological applications

Although the relevance of phospholipid-bound-water to various biological processes has been discussed throughout this review, a few more specific examples are in order: these examples involve such diverse applications as the use of phospholipids in drug delivery systems and the role of water binding to phospholipids in clinical magnetic resonance imaging (MRI). These applications are not only contemporary, but also are proving to be quite useful in both biochemistry and medicine.

Liposomes are vesicular particles consisting of phospholipid bilayers surrounding a central aqueous core. There may be a single bilayer or multibilayers surrounding this central water core. In the multibilayer case, additional water would be entrapped between the bilayers. Various hydrophilic substances can be contained in the entrapped water as well as in the water in the core; hydrophobic material can be contained in the phospholipid bilayers. Because the phospholipids are often those present in the organism being studied, the lipids, themselves, are usually considered harmless. The capacity of these benign particles to transport hydrophilic and/or hydrophobic drugs thus makes their use in diagnostic and therapeutic medicine worthy of consideration.

If these liposomal particles are to be effectively used, their targeting to the organ of interest is of paramount importance. The reticuloendothelial system (RES) of the human body, however, can "recognize" these liposomes as foreign bodies resulting in their accumulation in the liver and/or spleen as well as in other RES components. Although this RES concentration of the liposomes by the RES system can be exploited for medical and biological purposes,[40,41] this behavior severely limits the diagnostic/therapeutic usefulness of these liposomes as delivery vehicles. It is clear that the fate of liposomes introduced into the body is intimately related to their interaction with macromolecules and cellular surfaces. It is thus quite reasonable to postulate that such interactions might well involve the water interacting with the liposomal as well as with other macromolecular and membrane surfaces. A fuller understanding of the role of this water could lead to wider use of liposomes as drug delivery systems.

One way of lessening the scavenger action of the RES system is to include PEG-lipids as part of the liposome structure. Polyethylene glycol (PEG)-lipids consist of polyethylene glycol covalently attached to phospholipids. The presence of small amounts of these PEG-lipids in the liposome extends the circulation time of the particles in the blood, thereby increasing localized drug delivery, for example, to solid tumors. Because the interaction of these drug-containing liposomes with cancer cells must involve the range and magnitude of the forces between the cell membrane and liposome surface and, because the hydration force discussed previously is directly connected to the water bound to the liposome and/or cell surfaces, the influence of the PEG-lipids on phospholipid water binding is of obvious importance.

An example of this hydration relationship to PEG and PEG-lipids, is given by the results[42] of Arnold and coworkers, which can seemingly be explained by the influence of PEG, on the physicochemical properties of the surrounding aqueous phase. It should be noted that these experiments used liposomes along with PEG, but not PEG-lipids. More recently, some rather insightful work[43] has been published on the effects of PEG, on PC liposomal membranes. The authors find that one of the effects of PEG is an osmotic one due to the exclusion of the polymer from the liposome surface, resulting in a partial dehydration of the phospholipid headgroup. These same workers in an earlier paper[44] concluded that this water removal from the PC diminishes the effective size of the polar headgroup allowing for an enhanced lateral packing of the phospholipid acyl chains. It can be seen that these ideas are clearly related to the water adsorption measurements mentioned previously. Additionally, our preliminary results suggest that PEG-lipids are weak water adsorbers, but, nevertheless, adsorb a rather large amount of water at the higher partial vapor pressures of water.* The PEG-lipids, moreover, when combined with phospholipids significantly alter the water adsorption characteristics of the phospholipids. The usefulness of these various hydration studies for an understanding of the drug delivery process certainly indicates that further research is needed. This need is underscored by the great interest shown in cationic liposomes as delivery systems for DNA and the recently reported low water adsorption of cationic lipids.[20]

Another very interesting example of the relationship between bound water and biological function is provided by some recent work[45] on the enzymatic activity of phospholipase A_2 (PLA_2). As is pointed out, nearly all work that has been done on phospholipase activity focuses on the enzyme activity in the fully hydrated state. It has been found, however, that organisms that survive dehydration accumulate free fatty acids which, in turn, leads to cell fusion and leak-

*G.L. Jendrasiak and R.L. Smith, work in progress.

age of cell solutes. In these cases it is thought that there is not sufficient water present in the dehydrated organisms to allow enzymatic activity of the phospholipases. Using liposomes formed from phospholipids and drying those liposomes in the presence of a sugar such as trehalose results in dry liposomes that maintain their bilayer structure and the bilayer barrier properties. Moreover, it is known that in excess water the physical state of the lipid substrate is of importance for the enzymatic activity and thus studies with the physical state of the lipid substrate under low hydration conditions may provide more detailed information about the enzyme reactions. With this limited hydration, the more tightly bound water may well play a key role in the hydrolytic activity of PLA_2. The results of these investigations suggest that the physical state of the lipid is indeed important in determining the lipolytic activity of PLA_2 on phospholipid bilayers in the dehydrated or low water system state. Of interest in this work is the fact that it was found that membrane electrical surface charge, due to the presence of PS, affected the PLA_2 activity for PC. As has been found,[5] PS exhibits weak water adsorption, whereas PC is a strong water adsorber. There may well be a strong connection between water adsorbed to the phospholipids, phospholipid surface charge, and PLA_2 activity. What this exact connection is at the molecular level remains to be delineated, but the water intimately associated with the lipid substrate surface would be expected to play an important role.

A final example illustrating the relationship of phospholipid-bound-water to biology is actually a very clinical one. As is well-appreciated by clinicians, the value of MRI lies, in great part, in its superb soft-tissue contrast ability. High contrast in the images of soft tissue entities is, of course, necessary to study diseased tissue. The image contrast between diseased tissue and its neighboring, normal tissue depends on differences of the MRI imaging parameters between the abnormal and normal tissue. This difference in the MRI parameters between the two types of tissue, in turn, depends on differences in physiology and/or biochemistry of the two types of tissue. It is apparent that good image contrast is necessary both for accurate diagnosis of a disease process as well as for monitoring the progress of the disease, especially during and after therapy. The images obtained with the conventional clinical MRI instrument primarily reflect the presence of water as well as fat in an organ, diseased or normal. This being the case, it can readily be appreciated that the amount and state of water in a particular tissue system will play a major role in the nature of the MRI image of that organ. Furthermore, on the basis of what has been discussed previously in this review, it can be seen that both bound and free water can and will coexist in biological tissues, i.e., a two-compartment situation. The differences in the water properties in the two compartments will, of course, depend on the macromolecular surfaces involved, and because phospholipids are present in the cell membrane surfaces, the phospholipids often play a crucial role in determining the water distribution between the two compartments. Because the bound and free water are present in rather different environments, it is reasonable that their physical properties, including their MRI properties, will differ. Such differences in MRI properties have led to the development of an MRI imaging procedure called magnetization transfer contrast (MTC) imaging. The contrast in the MRI images so obtained is based on differences in the nature of the bound and free water, respectively. Tissues with high plasma membrane content, such as kidney and brain, usually exhibit significant MTC effects. This MTC procedure is already being used rather routinely in clinical MRI angiography and shows promise for other clinical MRI procedures as well.[46]

In view of the clinical potential of MTC, it becomes of importance to understand the biophysical mechanisms underlying the MTC phenomenon. Using lipid bilayers as models for cell membranes,[47] notable work on elucidating these biophysical mechanisms has been done.[48] It was found in these studies that neither EPC bilayers nor cholesterol (CHOL) by themselves exhibited significant MTC effects. When CHOL, however, was present in increasing concentration in the EPC bilayers, the pseudo-first-order magnetization exchange rate increased. The authors conclude that the cholesterol-induced magnetization exchange may be related to either longer correlation times of the lipid or to an increase in the number of water molecules associated with the bilayer. It should be noted here that the presence of CHOL in PC bilayers significantly increases the amount of water adsorbed by ECP[5] and DPPC[7]. For EPE, moreover, the CHOL not only increases the amount of water adsorbed, but also changes the nature of the adsorption process from "weak" adsorption by EPE[7] to "strong" adsorption by EPE-CHOL. This change in the nature of the water-binding process leads to an increase in the binding energy of the most intimately bound layer of water and, additionally, affects the nature of the more distantly bound water. These biophysical results agree well with the MTC results obtained in cat brain, in vivo, where higher MTC exchange rates were found for white matter than for gray matter.* White and gray matter are similar in composition except for the presence of myelin in white matter; myelin contains cholesterol-rich sheets of lipid bilayers.

More recent work has been published on the mechanism involved in the MTC phenomenon in white matter,[49] again using phospholipid bilayer systems as models for biological membranes. Suspensions of PC-CHOL or PC-SM exhibited greater magnetic transfer effects than did PC alone. Suspensions of PC-GC, however, exhibited magnetic transfer effects that were two to three times greater than those exhibited by either PC-CHOL or PC-SM suspensions. PC-CHOL, PC-SM, and PC-GC suspensions all showed greater magnetic transfer effects at acidic pH than they did at physiologic pH. The authors thus conclude that of all the major white matter lipids, GC has the strongest effect on magnetic transfer processes and, moreover, because the effect is pH dependent, chemical exchange of protons likely contributes to the magnetic transfer effects observed. The authors also believe that the large number of hydroxyl groups in GC are responsible for the observed effects. It should be recognized, of course, that this latter work[49] as well as the pre-

*Eng, J., Wolff, S.D., Berkowitz, B.A., and Balaban, R.S. (1989). In *Proceedings of the Society of Magnetic Resonance in Medicine,* 8th Annual Meeting, vol. I, p 213.

viously mentioned work[48] was done with model systems and, thus, the results observed may not directly apply to the more complex and heterogeneous white matter structures in vivo. The results, nevertheless, are intriguing and further work is indicated to optimize the application of MTC to brain imaging procedures.

Conclusion

Although we have presented the results of many studies (there are also many that could not be included here), and the usefulness of the bound water concept is becoming more recognized, nevertheless much basic science as well as biological and clinical application needs to be done.

Since the hydration forces are thought to be involved in "biological specificity," the physical origins of these short-range, repulsive forces need to be understood. Inasmuch as the repulsive force even exists between electrically uncharged surfaces, electrostatic repulsion is apparently not the origin of the force. Many workers believe that the force arises from the water interacting with the molecular aggregate surface, being partially polarized or reordered by the polar surface. Others* believe that the water molecules in the first hydration layer are ordered by hydrogen bonding with the phospholipid head groups and that this change in the water arrangement results in the hydration force; the dipole potential would then come from these oriented water molecules. This latter view would seem to be in line with the author's work on water adsorption.[20] Some workers,[50] on the other hand, concentrate their efforts on studying the more loosely bound water, arguing that this is more representative of the physiological situation where the phospholipid, particle surfaces exist in water solution. My own view is that the first few water molecules, interacting with "dry" phospholipid particle surfaces, results in a new surface on the particle. This new surface then directs the adsorption of further water as suggested in *Figure 2.* In any discussion of the biochemical processes occurring at the surface, all of the various strengths of bound water, since they are interdependent should be considered. Further work on this matter must be done, however, the experimental difficulties are formidable.

Another area that is in the forefront of surface water interaction studies is the so-called "Vapor Pressure Paradox." Many investigators have reported that multilamellar phases of electrically charged lipids in aqueous solution can swell, indicating very large amounts of water taken up by the phases. Those same lipids, however, take up much less water when attached to a solid substrate[20] or when bounded by an air/water interface. It seems to this author that the difference in the physical structure of the two phospholipid systems cold well provide the answer to the paradox and elegant theoretical treatments are now being developed to address this issue.**

It is also obvious from the previous discussion in this review that their is some discrepancy in the actual number of various bound water molecules measured gravimetrically and the number inferred from other types of measurements, such as NMR. It would be quite useful to bring the various measurements into agreement because a more quantitative idea of the relationship between bound water and its effects on the phospholipid surfaces could then be obtained. Finally, more investigations into the effects of this bound water on biological function are in order. As an example, consider the mechanism of action of volatile anesthetics. Although many investigators believe that this action results from the hydrophobic character of the anesthetic, interacting with the cell membrane, others[51] believe that the anesthetic effect results from the release or reordering of the water bound at the cell membrane surface; this idea would assign a paramount role to the phospholipids. Because an interaction with the phospholipid headgroup may influence the hydrophobic portion of the phospholipid and, vice versa, the problem is a difficult one. Because of the medical importance of anesthetics, however, the effort may well result in unsuspected medical benefits.

It can, thus, be seen that the bound water herein discussed not only plays an essential role in determining the structure of many physiological elements but also is intimately involved in their biochemical functions. A complete understanding of this relationship lies in the future. We hope, however, that we have increased the reader's awareness of the relationship of bound water to biology and in closing we would like to modify the maxim given at the beginning of this review:

If there is magic in the biochemical activity
of living organisms,
it results from the water associated with surfaces.

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References

1 Eiseley, L. (1946). *The Immense Journey,* p. 15 Random House, Inc. New York
2 Mathur-De Vré, R. (1984). Biomedical implications of the relaxation behavior of water related to NMR imaging. *British J. Radiol.* **57,** 955–976
3 Elworthy, P.H. (1961). The adsorption of water vapour by lecithin and lysolecithin, and the hydration of lysolecithin micelles. *Chem. Soc. J.,* 5305–5389
4 Elworthy, P.H. (1962). Sorption studies on phosphatides. Part II. Sorption of water vapour by a synthetic lecithin and cephalin. *Chem. Soc. J.,* 4897–4900
5 Jendrasiak, G.L. and Hasty, J.H. (1974). The hydration of phospholipids. *Biochim. Biophys. Acta* **337,** 79–91
6 Jendrasiak, G.L. and Mendible, J.C. (1976). The effect of the phase transition on the hydration and electrical conductivity of phospholipids. *Biochim. Biophys. Acta* **424,** 133–148
7 Jendrasiak, G.L. and Mendible, J.C (1976). The phospholipid headgroup orientation: Effect on hydration and electrical conductivity. *Biochim. Biophys. Acta* **424,** 149–159

*K. Gawrisch, J. Zimmerberg, A. Parsegian, R., and N. Fuller. (1990). *Biophys. J.* **57,** 35a
R. Podgornik, A. Parsegian, N. Fuller, and P. Rand. (1996). *Biophys. J* **70, p A441

8 Lundberg, B., Svens, E., and Ekman, S. (1978). The hydration of phospholipids and phospholipid-cholesterol complexes. *Chem. Phys. Lipids* **22,** 285–292

9 Wakamatsu, K., Hasoda, K., Mitoma, H, Ohya, M., Okahata, Y., and Yasunoga, Y. (1995). Direct in situ measurement of phospholipid hydration in an aqueous environment using a quartz crystal microbalance. *Anal. Chem.* **67,** 3336–3341.

10 Jendrasiak, G.L. and Hasty, J.H. (1974). The electrical conductivity of hydrated phospholipids. *Biochim. Biophys. Acta* **348,** 45–54

11 Pissis, P., Enders, A. and Nimtz, G. (1993). Hydration dependence of molecular mobility in phospholipid bilayers. *Chem. Phys.* **171,** 285–292

12 McIntosh, T.J. and Magid, A.D. (1992). Phospholipid hydration. In *Phospholipid Handbook* (ed. Cevc, G.), pp. 553–577, Marcel Dekker, Inc., New York

13 Rand, R.P. and Parsegian, V.A. (1989). Hydration forces between phospholipids bilayers. *Biochim. Biophys. Acta* **988,** 351–376

14 Israelachvili, J. (1987). Solvation forces and liquid structure as probed by direct force measurements. *Acc. Chem. Res.* **20,** 415–421

15 Finer, E.G. and Darke, A. (1974). Phospholipid hydration studied by deuteron magnetic resonance spectroscopy. *Chem. Phys. Lipids* **12,** 1–16

16 Bechinger, B. and Seelig, J. (1991). Conformational changes of the phosphatidylcholine headgroup due to membrane dehydration: A ^{2}H-NMR study. *Chem. Phys. Lipids* **58,** 1–5.

17 Jain, M.K. and Wagner, R.C. (1980). *Introduction to Biological Membranes,* John Wiley and Sons, New York, NY USA

18 Jones, M.N. and Chapman, D. (1995). *Micelles, Monolayers and Biomembranes,* John Wiley and Sons, New York, NY USA

19 Brunauer, S. (1943). *The Adsorption of Gases and Vapors,* Vol. 1. Princeton University Press, Princeton, NJ USA

20 Jendrasiak, G.L., Smith, R.L., and Shaw, W. (1996). The water adsorption characteristics of charged phospholipids. *Biochim. Biophys. Acta* **1279,** 63–69

21 Boggs, J.M. (1987). Lipid intermolecular hydrogen bonding: Influence on structural organization and membrane function. *Biochim. Biophys. Acta* **906,** 353–404

22 Pissis, P. (1989). Dielectric studies of protein hydration. *J. Molecular Liquids* **41,** 271–289

23 Boyer, P.D., Chance, B., Ernster, L., Mitchell, P., Racker, E., and Slater, E.C. (1977). Oxidative phosphorylation and photophosphorylation. *Annual Rev. Biochem.* **46,** 955–1026

24 Prats, M., Teissié, J., and Tocanne, J. (1986). Lateral proton conduction at lipid-water interfaces and its implications for the chemiosmotic-coupling hypotheses. *Nature* **322,** 756–758

25 Tocanne, J. and Teissié, J. (1990). Ionization of phospholipids and phospholipid-supported interfacial lateral diffusion of protons in membrane model systems. *Biochim. Biophys. Acta* **1031,** 111–142

26 Teissié, J., Gabriel, B., and Prats, M. (1993). Lateral communication by fast proton conduction: A model membrane study. *TIBS* **18,** 243–246

27 Morgan, H., Taylor, D.M., and Oliveira, O., Jr. (1991). Proton transport at the monolayer-water interace. *Biochim. Biophys. Acta* **1062,** 149–145

28 Gawrisch, K., Ruston, D., Zimmerberg, J., Parsegian, V.A., Rand, R.P., and Fuller, N. (1992). Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. *Biophys. J.* **61,** 1213–1223

29 Ulrich, A.S. and Watts, A. (1994). Lipid headgroup hydration studies by ^{2}H-NMR: A link between spectroscopy and thermodynamics. *Biophys. Chem.* **49,** 39–50

30 Ulrich, A.S. and Watts, A. (1994). Molecular response of the lipid headgroup to bilayer hydration monitored by ^{2}H-NMR. *Biophys. J.* **66,** 1441–1449

31 Clegg, J.S. (1974). Interrelationships Between Water and Metabolism in Artemia Salina Cysts; Hydration & Dehydration from the Liquid and Vapour Phases. *J. Exp. Biol* **61,** 291–308

32 Clegg, J.S. (1986). Artemia cysts as a model for the study of water in biological systems. *Methods in Enzymology* **127,** 230–239

33 Parsegian, V.A., Fuller, N., and Rand, R.P. (1979). Measured work of deformation and repulsion of lecithin bilayers. P.N.A.S. **76,** 2750–2754

34 Leikin, S., Parsegian, V.A., and Rau, D.C. (1993). Hydration forces. *Annu. Rev. Phys. Chem.* **44,** 369–395

35 Snell, F.M., Schulman, S., Spencer, R.P., and Moos, C. (1995). *Biophysical Principles of Structure and Function.* P. 49, Addison-Wesley Publishing Co., Reading, MA USA

36 Marsh, D. (1989). Water adsorption isotherms and hydration forces for lysolipids and diacyl phospholipids. *Biophys. J.* **55,** 1093–1100

37 Lis, L.J., McAlister, M., Fuller, N., Rand, R.P., and Parsegian, V.A. (1982). Interactions between neutral phospholipid bilayers. *Biophys.J.* **37,** 657–666

38 McIntosh, T.J., Magid, A.D., and Simon, S.A. (1987). Steric repulsion between phosphatidylcholine bilayers. *Biochemistry* **26,** 7325–7332

39 Cevc, G. (1991). Hydration force and the interfacial structure of the polar surface. *J. Chem. Soc., Faraday Trans.* **87,** 2733–2739

40 White, C., Slifkin, M., Seltzer, S.E., Blau, M., Adzamli, I.K., and Adams, D.F. (1991). Biodistribution and clearance of contrast-carrying MREV liposomes, *Invest. Radiol.* **25,** 1125–1129

41 Jendrasiak, G.L., Frey, S. Donald, and Heim, R.S., Jr. (1985). Liposomes as carriers of idololipid radiocontrast agents for CT scanning of the liver. *Invest. Radiology* **20,** 995–1002

42 Arnold, K. Zschoernig, O., Barthel, D., and Herold, W. (1990). Exclusion of poly (ethlyene glycol) from liposome surfaces. *Biochim. Biophys. Acta* **1022,** 303–310

43 Lehtonen, J.Y.A. and Kinnunen, P.K.J. (1994). Changes in the lipid dynamics of liposomal membranes induced by poly(ethylene glycol): Free volume alteration revealed by inter- and intramolecular excimer-forming phospholipid analogs. *Biophys. J.* **66,** 1981–1990

44 Lehtonen, J.Y.A. and Kinnunen, P.K.J. (1995). Poly(ethylene glycol)-induced and temperature-dependent phase separation in fluid binary phospholipid membranes. *Biophys. J.* **68,** 525–535

45 Oliver, A.E., Fisk, E., Crowe, L.M., de Araujo, P.S., and Crowe, J.H. (1995). Phospholipase A_2 activity in dehydrated systems: Effect of the physical state of the substrate. *Biochim. Biophys. Acta* **1267,** 92–100

46 Wolff, S.D. and Balaban, R.S. (1994). Magnetization transfer imaging: Practical aspects and clinical applications. *Radiology* **192,** 593–599

47 Szoka, R. and Papahodjopoulos, D. (1980). Comparative properties and methods of preparation of lipid vesicles (liposomes). *Annu. Rev. Biophys. Bioeng.* **9,** 467–508

48 Fralix, T.A., Ceckler, T.L., Wolff, S.D., Simon, S.A., and Balaban, R.S. (1991). Lipid bilayer and water proton magnetization transfer: Effect of cholesterol. *Mag. Res. Med.* **18,** 214–223

49 Kucharczyk, W., Macdonald, P.M., Stanisz, G.J., and Henkelman, B.M. (1994). Relaxivity and magnetization transfer of white matter lipids at MR Imaging: Importance of cerebrosides and pH. *Radiology* **192,** 521–529

50 Nagle, J.F., Zhang, R., Tristam-Nagle, S., Sun, W., Petrache, H.I., and Suter, R.M. (1996). X-ray structure determination of fully hydrated L_α phase dipalmitoylphosphatidylcholine bilayers, *Biophys. J.* **70,** 1419–1431

51 Tsai, Y., Ma, S., Nishimura, S., and Ueda, I. (1990). Infared spectra of phospholipid membranes: Interfacial dehydration by volatile anesthetics and phase transition. *Biochim. Biophys. Acta* **1022,** 245–250