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The Paramagnetic Hydrophilic Probing of Colloidal Systems by NMR Spectroscopy

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The principal results of the use of a hydrophilic paramagnetic probe in ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy to study the structure, stability, and local density of packing of colloidal systems have been examined. The magnetic properties of the ions of *f*- and *d*-metals used as shift and relaxation probes have been discussed. Methods for estimating the stability of micelles and the conformation of the polar fragments of the molecules of surface active substances (SAS) on the surface of micelles and vesicles have been described. Particular attention has been paid to the study of dynamics, the compositional asymmetry of lipid membranes, the analysis of their interaction with physiologically active compounds, and the structure of biocolloidal systems.
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I. INTRODUCTION

NMR spectroscopy is one of the most effective methods for determining the structure of organic compounds and also supermolecular formations such as micelles, liposomes, and other complex colloidal aggregates. The informative character of NMR spectra, particularly proton NMR spectra, however, is lowered considerably because the nuclei of aliphatic groups not adjoining electronegative atoms give signals with similar chemical shifts. In addition, there are a number of factors leading to the broadening and the mutual overlap of the signals in the NMR spectra of colloidal systems. The most important of these are the presence of spin multiplets, dipole–dipole relaxation in the interaction of directly coupled nuclei, intermolecular association, the low fragmentary mobility of aliphatic chains, microheterogeneity, and the appreciable viscosity of dispersions.[1]

To overcome these difficulties and increase the informative character of the NMR spectra of colloidal systems, Soviet authors[2-9] proposed the method of hydrophilic shift and relaxation paramagnetic probes—the ions of *f*- and *d*-metals having an unpaired electron.

The influence of paramagnetism on the characteristics of nuclear resonance was detected by Bloembergen and Dickinson.[10] The characteristics of NMR in paramagnetic systems have been summarised in a number of reviews and monographs.[11-16] The interaction between the paramagnetic centre and the nucleus whose magnetic resonance is being observed is revealed in the isotropic hyperfine shift,[17] the change in the nuclear relaxation times T_1 and T_2,[18] and the nuclear polarisation of the spins as a consequence of the Overhauser effect.[19] These phenomena give information on the electronic and geometric structures of the ligands joined to the paramagnetic centres.

1. The Paramagnetic Shift

The isotropic shift of the signals in the NMR spectra of paramagnetic substances is the sum of two types of interaction: contact and pseudo-contact.

The Fermi contact shift takes place if the spin density of the unpaired electron is delocalised, and transferred to the nucleus through a system of chemical bonds. The contact shift obeys the Curie law, and for a one-spin state with an isotropic *g*-tensor it is described by the equation:[10]

$$\frac{\Delta H}{H_0} = -\frac{Ag\beta^2}{g_N\beta_N}\frac{s(s+1)}{3kT}$$

where A is the hyperfine interaction constant, g_N the nuclear *g*-factor, β_N the nuclear magneton, H_0 the intensity of the external magnetic field, β the Bohr magneton, g the Lande factor, k the Boltzmann constant, T the absolute temperature, and s the the spin-quantum number of the electron.

The contact shift of the signal is observed with the condition $T_{1e}^{-1} \gg A$, where T_{1e} is the electronic spin–lattice relaxation time. The contact shifts are large for the nuclei of oxygen and nitrogen directly coordinated to a paramagnetic centre, and also for carbon chemically bound to these atoms. For atoms at a greater distance and, commonly, for all protons, the contact contribution is small, and the observed paramagnetic shifts are pseudo-contact in character.[11-16]

The pseudo-contact shift of a signal is determined by the dipole–dipole interaction of the spins of the unpaired electron and the nucleus. It depends on the electron configuration of the paramagnetic ion, the magnetic anisotropy of the ligand field, and temperature. For axial symmetry of the complex, the pseudo-contact shift is given by the McConnell–Robertson equation:[17]

$$\frac{\Delta H}{H_0} = -\frac{\beta^2 s(s+1)}{9kT}(g_\parallel^2 - g_\perp^2)\frac{3\cos^2\theta - 1}{r^3} \qquad (1)$$

where $g_\parallel$ and $g_\perp$ are the components of the *g*-tensor of the magnetic anisotropy parallel to and perpendicular to the principal axis of the complex, r the distance from the paramagnetic centre to the nucleus, and θ the angle between the radius vector $\vec{r}$ and the principal axis of the complex.

The dependence of the pseudo-contact shift on the geometry of a complex, described by Eqn.(1), makes it possible to assign the signals in the spectrum of a ligand of known structure and to solve the inverse problem, that is from the known assignment to obtain information on the geometry of a molecule.[5,11-17]

2. Paramagnetic Broadening

In the presence of a paramagnetic centre, broadening of the NMR signals is observed, due to the increased rate of relaxation of the nuclear spin and the influence of exchange between the bonded and free molecules of the substrate. The spin–lattice T_1 and spin–spin T_2 relaxation times for nuclei with a spin of 1/2 in the presence of a paramagnetic centre are given by the Solomon–Bloembergen equations (2) and (3):[18]

$$\frac{1}{T_1}=\frac{2}{15}\frac{(\beta_N g_N \beta g)^2 s(s+1)}{r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2}+\frac{7\tau_c}{1+\omega_s^2\tau_c^2}\right)+\frac{2}{3}s(s+1)\left(\frac{A}{\hbar}\right)^2\frac{\tau_e}{1+\omega_s^2\tau_e^2} \quad (2)$$

$$\frac{1}{T_2}=\frac{1}{15}\frac{(\beta_N g_N \beta g)^2 s(s+1)}{r^6}\left(4\tau_c+\frac{3\tau_c}{1+\omega_I^2\tau_c^2}+\frac{13\tau_c}{1+\omega_s^2\tau_c^2}\right)+ +\frac{1}{3}s(s+1)\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_s^2\tau_e^2}+\tau_e\right) \quad (3)$$

where ω_I and ω_s are the Larmor precession frequencies of the nucleus and the electron, and $\hbar = h/2\pi$ is Planck's constant.

The first terms of these equations are due to dipole–dipole interaction and depend on the distance r between the nucleus being observed and the paramagnetic centre. This interaction is determined by the correlation time of the electron–nucleus dipole interaction τ_c. The second terms, which are independent of r, are characterised by the correlation time of the contact interaction τ_e. The correlation times are defined as

$$\tau_c^{-1}=\tau_R^{-1}+\tau_s^{-1}+\tau_M^{-1}+\tau_D^{-1}$$
$$\tau_e^{-1}=\tau_s^{-1}+\tau_M^{-1}$$

where τ_R, τ_s, τ_M, and τ_D are the correlation times corresponding to the rotational motion, the relaxation of the electron spin, chemical exchange, and diffusion exchange respectively.

The relaxation times are influenced only by variable effects for which the correlation time has the same order of magnitude as the period of the Larmor precession of the nuclear spins.

II. THE HYDROPHILIC PROBING OF MICELLAR DISPERSIONS

The rapid decrease in the contribution of dipole–dipole interaction to the relaxation rates T_1^{-1} (2) and T_2^{-1} (3) with distance to the paramagnetic centre (r^{-6}) and the absence of its angular dependence make ions with high values of A and T_e (Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Gd^{3+}, etc.) valuable probes for structural studies. These ions are used as hydrophilic relaxation probes both to characterise the stability and local denisty of packing of micelles formed by different surface-active substances (SAS) and also to estimate the spatial orientation of the polar fragments in their structure.

1. Estimation of the Stability of Micelles

The stability of micellar aggregates is of fundamental interest both for solving the problems of micellar catalysis and in cell biochemistry. The estimation of the stability of micelles by the hydrophilic relaxation paramagnetic probe method[2–9] is based on the Dill–Flory statistical lattice model of the micelle.[20]

Paramagnetic cations can form dynamic complexes with the polar groups of SAS, oriented in micelles into the aqueous phase. The relaxation of the protons of definite fragments of the SAS molecule takes place only on the collision of the hydrated ion and the fragment of the molecule. It is therefore possible to determine whether the hydrophilic paramagnetic ions are in contact with definite parts of the molecule. An analysis[7,21–23] of the ^1H NMR spectra of micelles of sodium, copper, cobalt, and vanadyl dodecyl sulphate showed that not only the protons of the CH_2–SO_4^- groups but also the protons of the $(CH_2)_{10}$ chains and CH_3 groups collide effectively with Cu^{2+}, Co^{2+}, VO^{2+}, and lanthanide ions. These collisions may take place either as a result of the penetration of the ions into the micelles or as a result of the diffusional movement of the hydrocarbon chains into the Stern layer.[20,23,24]

The penetration of the hydrophilic ions into the hydrophobic nucleus of a micelle might be assumed in a model[25] containing water in the hydrophobic nucleus. The untenability of this model of "aqueous channels and cavities" within the micelle was proved, however, by the correspondence between the mechanisms of the extinction of the fluorescence of pyrene on ions in solution and in a micellar dispersion of sodium dodecyl sulphate in water,[26] and also by the difference between the experimental ratios of the spin–lattice relaxation times T_1 for the ^{13}C nuclei and the ratios calculated using the model of "aqueous cavities".[27] It has been proved convincingly[28,29] that in the micelle, only three or four methylene groups of the hydrocarbon chain of the SAS, counting from the polar head, are hydrated. Thus water is capable of penetrating into the hydrophobic nucleus of a micelle to a depth of only 0.4–0.5 nm. The positions at which water is localised in micro-emulsions (Tween-81–n-hexadecane–H_2O) have been determined by ^1H, ^2H, and ^{13}C NMR spectroscopy using Tb^{3+} and $Fe(CN)_6^{3-}$ ions as paramagnetic probes.[30] It was shown that the water molecules do not penetrate into the hydrophobic regions of the micro-emulsions. In addition, EPR spectroscopy detected hydrated Mn^{2+} ions only in the Stern layer of aqueous micelles of manganese dodecyl sulphate.[31]

These experimental data provide a reliable basis for estimating the stability of micelles using a relaxation probe.[32–36] The difference in the broadening of the signals from different molecular fragments of SAS in the presence of hydrophilic relaxation probes characterises the degree of stability of the micelle. The magnitude of the increase in the difference between the half-widths of the signals from groups close to the Stern layer (for example α-CH_2) and the terminal CH_3 groups of SAS molecules when paramagnetic ions (Mn^{2+}) are introduced into the dispersion of micelles.[32,34] This is confirmed by the experimentally established increase by two orders of magnitude in the difference in the relaxation rates ΔT_1^{-1} for the ^{13}C nuclei of the α-CH_2 and CH_3 groups of sodium dodecyl sulphate with increase in its concentration in water above the critical concentration for micelle formation (CCM) in the presence of Mn^{2+} ions.[27] The difference in the relaxation mechanisms is attributed to the increase in the constant for complex formation by Mn^{2+} ions with sodium dodecyl sulphate molecules on going from the solution to the micellar system.

Table 1 gives the values of the stability criterion for a number of micelles formed by the potassium salts of aliphatic acids.[34] Table 1 shows that the most stable micelles are formed by potassium stearate, and also by a mixture of synthetic aliphatic acids and sodium alkyl sulphonate, used in industry as emulsifiers in polymerisation.[32] The stability of the micelles decreases with decrease in the length of the hydrocarbon chain and increase in its unsaturated character. The influence of leuconol on the stability of micelles formed by the potassium salts of alkyl-substituted benzylbenzoic and aliphatic acids has been studied.[33,34] It was shown using Mn^{2+} ions that leuconol itself does not form micelles in water, and breaks down the micelles formed by other SAS.

On the other hand, if SAS molecules are capable of forming aggregates in the micelle structure, the stability of the micelles is increased. Aggregates of this kind with an average lifetime of ~10 ms and a stoichiometry which depends on temperature were detected in mixed micelles of sodium alkyl sulphonate and potassium dehydroabietate.[32] The influence of the stability of a layer of SAS adsorbed by the polymeric and monomeric particles on the micro-block character of emulsion copolymers was demonstrated in Refs.34, 37, and 38, and its influence on the rate constant of copolymerisation in Ref.33.

Table 1. Difference in the half-widths of the ^1H NMR signals of the α-CH_2 and CH_3 groups of SAS dispersions (0.1 M in D_2O)[34] and the values of the stability criterion.

SAS	$(\Delta\nu_{1/2}^{\alpha\text{-}CH_2}-\Delta\nu_{1/2}^{CH_3})$, Hz		Increase in $(\Delta\nu_{1/2}^{\alpha\text{-}CH_2}-\Delta\nu_{1/2}^{CH_3})$ when Mn^{2+} is added, Hz
	without Mn^{2+}	10^{-5} M Mn^{2+}	
Potassium stearate	0.70	2.70	2.00
Potassium palmitate	0.23	0.71	0.48
Potassium myristate	0.65	0.86	0.21
Potassium oleate	0.50	1.70	1.20
Potassium linoleate	0.08	0.40	0.32
Potassium linolenate	0.07	0.32	0.25
Mixture of synthetic aliphatic acids	0.83	2.57	1.74
Sodium alkyl sulphonate	3.1	4.4	1.3

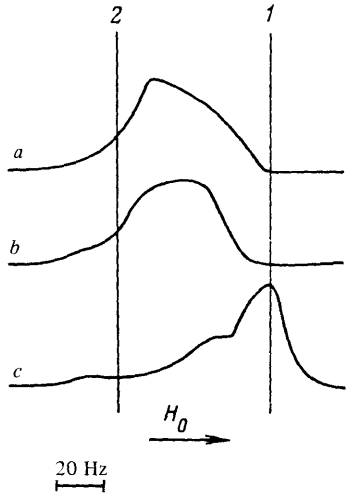


Figure 1. Form of the ^1H NMR signal of the oxyethyl protons in a 5% solution of Triton-X-100 in water in the presence of $TbCl_3$ (*a*), $Tb(NO_3)_3$ (*b*), and $Tb(ClO_4)_3$ (*c*); *1*) position of the signal of free polyhethylene glycol 1000 in the presence of these salts, *2*) position of the signal in the absence of the salts.[42]

An analysis of the characteristics of the bonding of paramagnetic ions to sodium dodecyl sulphate micelles in water[23,39] made it possible to put forward a method for measuring CCM from the cobalt-induced shift of the ^1H NMR signals of the SAS.[40] With increase in the concentration of the SAS above the CCM in the presence of Co^{2+} ions, a sharp increase in the chemical shift of the signals from the alkyl chains is observed, relative to the signal of acetone added to the system as standard. This phenomenon can be attributed to the sorption of the Co^{2+} ions in the Stern layer on micelle formation.

Similar studies carried out for non-ionogenic SAS[41] make it possible to probe their aqueous micelles.[42] For example, the observed anisotropy of the shift indeced by Tb^{3+} ions in the ^1H NMR spectra of Triton-X-100 and Triton-X-305 in water (Fig.1) was related to the existence of a salt concentration gradient along the thickness of the oxyethyl layer, depending on the counter-ion. These results agree with fluorescence measurements[43] and indicate that the penetrating power of electrolytes into micelles of non-ionogenic SAS is determined by electrostatic interactions at the micelle–water boundary.

VO^{2+}, Cu^{2+}, and Co^{2+} ions have been used[21,22,44] to study the micellar catalysis of the exchange of water protons in the coordination sphere of cations. This micellar catalysis has been related to the existence of an electrostatic potential on the surface of a sodium dodecyl sulphate micelle.[21] Micro-emulsification of this system with toluene leads to an increase in the rate of exchange.[22,44]

As paramagnetic centres for the analysis of colloidal systems it is possible to use iminoxyl markers, as has been done for micelles of ionic SAS containing phenoxy-groups.[45] Finally, it is possible to carry out the NMR probing of micelles by making use of the shifts induced by magnetically anisotropic (usually aromatic) substances and solvents.[46,47] The efficiency of these methods, however, is much lower than that of the hydrophilic paramagnetic probe, and the methods have not been developed.

2. Determination of the Conformation of SAS Molecules in the Structure of Colloidal Systems

Since the rates of relaxation of the nuclei and the induced shift of their signals in the NMR spectra depend on the distance r to the paramagnetic centre [Eqns.(1)–(3)], it is possible to estimate the preferred conformation and spatial orientation of the polar fragment of an SAS molecule in the structure of a colloid.[5,35,36,48,49] For small SAS molecules it is also possible to determine the conformation of the alkyl chains.[50,51] The conformation of the polar fragment generally remains unchanged on going from the SAS monomers in water to the micelles.[35,49] Thus the introduction of Mn^{2+}, Pr^{3+}, or Eu^{3+} into a dispersion of N-carbalkoxymethylene-2-methyl-5-vinylpyridinium bromides when their concentrations in water were above and below the critical concentration of micelle formation (CCM_1) showed that the conformation of the SAS molecule relative to the CH_2–COO bond remains eclipsed. However, on going from spherical to asymmetric micelles above CCM_2, the torsional angle increases, the conformation is no longer eclipsed, and the pyridine ring moves out of the plane of the monolayer.[35]

Similar changes in conformation in polymorphic transformations were detected in aqueous dispersions of mono-, di-, and triallylammonium salts with the general formula $(CH_2=CH\text{–}CH_2)_nN(CH_3)_{3-n}\cdot CH_2COORBr^-$, where n = 1–3, and monomers basxed on methacryloyloxyethylammonium salts $CH_2=C(CH_3)COOC_2H_4\overset{+}{N}(CH_3)_2\cdot CH_2COORBr^-$, where R = $C_{10}H_{21}$–$C_{16}H_{33}$.[36]

The magnitude of the selective broadening of the signals in the ^1H NMR spectra of anisotropic micelles, and an analysis of the structure of SAS molecules using models, made it possible to use Eqn.(3) to estimate the preferential conformation of the polar fragment (Fig.2). The areas corresponding to the molecule, calculated for these conformations, show good agreement with those determined experimentally

at a water–air interface.[36] The stabilisation of the monolayer on going from asymmetric micelles to a lamellar structure is accompanied by the movement of the methacrylate and N-methyl groups into the aqueous phase.[36,48] This is indicated by the increased broadening of the signals from the corresponding fragments when Mn^{2+} ions are introduced (Table 2). The conclusions reached about the relationship between the conformation and spatial orientation of the polar fragments of surface-active monomers and their polymorphism in water made it possible to understand the regular features of their emulsion polymerisation.[35,36,48,52]

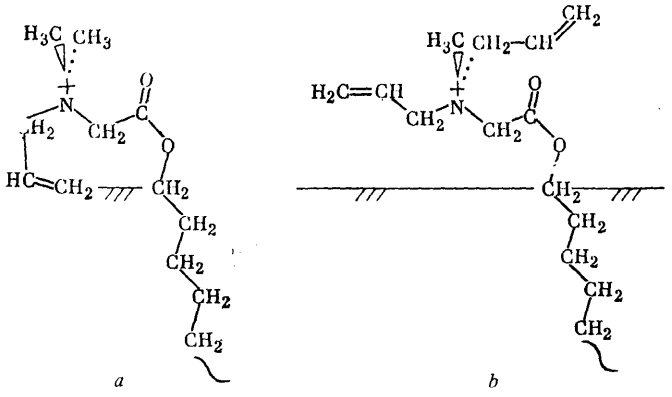


Figure 2. The conformation of alkylammonium SAS in asymmetric micelles: *a*) $n = 1.3$; *b*) $n = 2$.[36]

Table 2. Values of the broadening induced by Mn^{2+} cations for the ^1H NMR signals of dispersions of N-cetyl-N,N-dimethylmethacryloyloxyethylammonium bromide in water.[36]

Proton group	$\Delta\Delta\nu_{1/2}$, Hz*	
	asymmetric micelles	lamellae
CH_3 (terminal)	0.01	0.02
$(CH_2)_n$	4.28	0.60
CCH_3	1.04	1.50
$\overset{+}{N}CH_3$	1.50	2.45
α-CH_2	8.06	broadened
=CH(trans)	5.18	broadened
=CH(cis)	5.18	broadened

*$\Delta\Delta\nu_{1/2} = \Delta\nu_{1/2}([Mn^{2+}] \neq 0) - \Delta\nu_{1/2}([Mn^{2+}] = 0)$.

An analysis of the conformations of the alkyl fragments of sodium dibutyl phosphate in solution and in a micelle using Ni^{2+} and VO^{2+} ions[50] made it possible to select the most probable conformations of the POCCCC chain. Table 3 shows that the probability of a number of conformers increases on going from the SAS solution to the micelles. The use of Mn^{2+} ions as paramagnetic probes showed that the alkyl fragments of the SAS do not enter the aqueous phase. The first segment of the chain has an almost *trans* conformation, although the conformation of the subsequent segment is the same as in the liquid hydrocarbon.[51]

Thus polymorphic transformations in colloidal systems produce a change in the conformation of not only the hydrophilic but also the hydrophobic fragments of the SAS molecule.

Table 3. Probabilities of the alkyl chain conformers of sodium dibutyl phosphate.[50]

Conformer	P	
	monomer	micelle
TTT	0.132	0.200
$TTG^+ + TTG^-$	0.067	0.086
$TG^+T + TG^-T$	0.057	0.086
$G^+TT + G^-TT$	0.057	0.062
$TG^+G^+ + TG^-G^-$	0.029	0.037

Note: *P* is the probability of the conformer, *T* is the *trans*-rotamer, and *G* the *gauche* rotamer.

III. THE HYDROPHILIC PROBING OF LIPOSOMES

The method of hydrophilic paramagnetic probing opens up extensive possibilities for the study of the structure and molecular dynamics of lipid membranes, which are closed lamellae. It makes possible the differential observation of the signals from molecules situated on the external and internal surfaces of a bilayer vesicle.[2–6,8,9] From the ratio of the integral intensities of these signals it is possible to estimate the size of the membrane particles, and also to detect asymmetry of the molecular composition, that is differences in the distribution of lipids, proteins, and other components between the external and internal surfaces of a membrane. The method makes it possible to estimate the conformation and spatial orientation of the polar fragments of lipid molecules facing the aqueous phase. It makes it possible to study the processes of trans- and inter-membrane transfer of ions, lipid molecules, proteins, and all possible biologically active compounds.

Various aspects of the application of the hydrophilic paramagnetic probing of vesicles and biological membranes have been reviewed.[9,53–58]

1. The Differentiation of the Surfaces of Membranes by NMR Spectroscopy and the Estimation of the Dimensions of Liposomes

The testing of the bilayer character of liposomes of amphoteric lipids in water can be carried out using paramagnetic lanthanide ions.[3–5,8,9,59–73] and for anionic lipids using divalent manganese ions[2,3,6,9,74–82] or inorganic anions[46,80–83] which do not bring about flocculation of the colloidal system.

When shifting ions are introduced into the external volume of a dispersion of phosphatidylcholine (PC) in water, subjected to ultrasonic treatment, the ^1H NMR spectrum (Fig.3c) shows two signals from the protons of the polar group. The chemical shift of one of these signals coincides with that of the signal from the "pure" dispersion (Fig.3*a*), and corresponds to polar groups facing into the vesicle. The other is due to external polar groups coordinated to the lanthanide ions.[3,9,59,60] The directions of the shift of the signals are of opposite sign, and the broadening effect of the ion is determined by the absolute magnitude of the anisotropy factor of the magnetic susceptibility.[9,60]

A similar differentiation of the internal and external surfaces of a vesicle can be carried out from the ^{13}C [8] (Fig.4) and ^{31}P [4] (Fig.5) spectra. The observed ratios of the integral intensities of the external and internal signals from the polar groups (I_{ext}/I_{int}) are 2.09 for PC and 2.03 for sphingomyelin (SM) in water. [9]

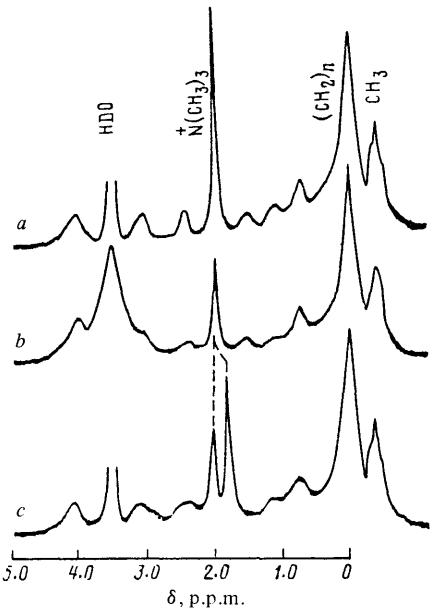


Figure 3. ^1H NMR spectra of a 5% dispersion of egg PC subjected to ultrasonic treatment: *a*) in D_2O; *b*) after the addition of 10^{-3} M $MnSO_4$ or *c*) 10^{-2} M $Eu(NO_3)_3$ to the external volume of the specimen.[3]

Nd^{3+} and Eu^{3+} ions have been used[61-63] to test the bilayer character of liposomes of synthetic saturated PC (C_{10}–C_{16}) and phospholipids containing polymerised itaconic acid fragments. Increase in the length of the alkyl chains in the PC molecules leads to the formation of more stable vesicles.[61] The addition of dipalmitoyl-lecithin, bis(2,4-octadienoyl)-PC, or cholesterol to unstable vesicles of itaconate-containing phospholipids gives two kinds of stable bilayer liposomes with diameters of 40 nm and 140 nm.[62,63]

By placing in the vesicles and in the external medium different paramagnetic probes (for example Pr^{3+} and Eu^{3+}) which shift the signals from the polar groups to weak and strong fields respectively, it is possible to make the method highly sensitive and at the same time to follow processes taking place on the external and internal surfaces of the bilayer.[64,65]

The influence of paramagnetic probes on the thermal phase transformations in aqueous dispersions of lipids has been studied.[59,67-70] For example, for dipalmitoyl-lecithin the introduction of Pr^{3+} ions raises the phase transition point by 3–5 K.[59,67]

The systematic error of the determination of the ratio I_{ext}/I_{int} at ^{31}P nuclei,[73] produced as a result of the decrease in the nuclear Overhauser effect (NOE) on the signal from the nuclei in contact with the paramagnetic probe, with decoupling from the protons does not exceed 10% for PC.[9] To obtain a more accurate result, it has been suggested that the dispersion be titrated with a relaxation probe (Mn^{2+}, Ni^{2+}, Gd^{3+}) by recording the ^1H [2,3,74,75] or ^{31}P [74-77] NMR spectra. It should be noted that the measurement of the ratio of the intensities in Ref.74 was incorrect; instead of the peak intensities, the integral intensities should have been measured.[76]

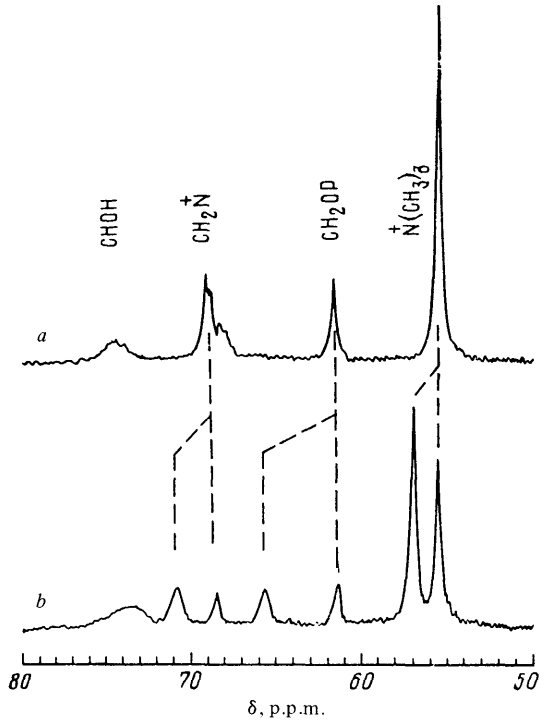


Figure 4. Range of the choline signals of the ^{13}C {H} NMR spectrum of a 20% SM dispersion subjected to ultrasonic treatment: *a*) in D_2O; *b*) after the addition of 5 x 10^{-2} M $Pr(NO_3)_3$ to the external volume of the specimen.[8]

Fig.6 shows that the dependence of the intensity of the ^{31}P signal on the concentration of Mn^{2+} in the external volume comes to a plateau corresponding to the intensity of the signal from the internal phosphate groups.[76,77] For phosphatidylinositol (PI), I_{ext}/I_{int} is 2.50.

Since according to X-ray diffraction data the thickness of the bilayer is 3.6 nm,[78] the external diameters are 24 nm for vesicles of PC and SM, and 19 nm ($\pm$1 nm) for vesicles of PI.[9] The values obtained agree with the results obtained by other methods.[79]

The titration of a system containing bilayer vesicles of PC gives two ^1H NMR signals from $\overset{+}{N}(CH_3)_3$ groups (the spectra were recorded at 250 MHz).[80] The signal from the internal $\overset{+}{N}(CH_3)_3$ groups is displaced by 0.01–0.02 p.p.m. towards higher fields. The magnitude of the shift increases with the time of ultrasonic treatment of the dispersion. Measurement of the relaxation times T_1 of the ^{13}C nuclei of the external and internal $\overset{+}{N}(^{13}CH_3)_3$ groups of enriched PC[71] showed that this shift is due to a difference in the packing and mobility of the molecules on the external and internal surfaces of the bilayer. This was previously noted in a study[81] using Mn^{2+} ions,

and not Yb^{3+} ions, as in Ref.71. Two-component ^{13}CO [8] and ^{31}P [77,82] signals were also obtained in the spectra of phospholipid vesicles. The observed influence of the density of packing of the molecules on the magnitude of the chemical shift[80,81] made it possible to discriminate the NMR signals (500 MHz) from monomers, micelles, and vesicles of PC, phosphatidylaminoethanol (PA), and SM using Pr^{3+} cations.[72] In these systems the vesicles were obtained without ultrasonic treatment by adding short-chain phospholipids.

A method has been proposed[34] for estimating the average dimensions and number of bilayers n in multilayer liposomes. The integral intensity of the signal from the polar groups, remaining in the titration with Mn^{2+} ions, makes it possible to determine the ratio I_{ext}/I_{int}. This ratio depends on n and R/d, where R is the external radius of the liposome, and d the thickness of the bilayer. It was shown that the ratio of the lengths of the alkyl chains in lipid-like SAS determines the structure of the liposome. Thus for liposomes of N-cetyl-N,N-diallyldodecylacetoammonium bromide with an average diameter of 1000 nm, $n = 13 \pm 1$, whereas liposomes of N-dodecyl-N,N-diallylcetylacetoammonium bromide with an average diameter of 25 nm are bilayer.

2. Factors Influencing the Lifetime of the Hydrophilic Probe in the Bound State

The lifetime of the paramagnetic probe in the state combined with the membrane determines the magnitude of the induced shift of the signals in the NMR spectra. The point of binding of the paramagnetic ions is the phosphate group of the phospholipid molecule.[4,5,8] The maximum number of Mn^{2+} ion binding positions on the surface of a PC membrane is 0.005 per PC molecule,[85] and the corresponding number for the lanthanides (Pr^{3+}, Nd^{3+}) is 0.13.[59] The presence of a single shifted signal in the NMR spectra when the lanthanides are introduced into a phospholipid dispersion indicates a high rate of lateral exchange of the ions between the binding position. The lifetime of the ions in the bound state is independent of their concentration in solution, but decreases with increase in the PC concentration from 0.17 s (at 0.06 M PC) to 0.02 s (at 0.18 M PC).[9] The lifetime of the probe in the bound state is a maximum at pD = 3.5 and decreases on heating.[9]

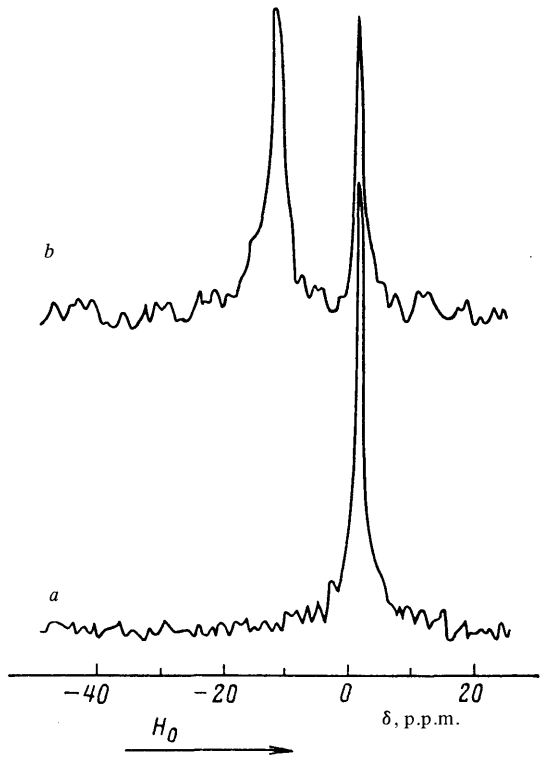


Figure 5. ^{31}P $\{^1H\}$ NMR spectra of a 10% dispersion of egg PC subjected to ultrasonic treatment: *a*) in D_2O; *b*) after the addition of 10^{-2} M $Pr(NO_3)_3$ to the external volume of the specimen.[4]

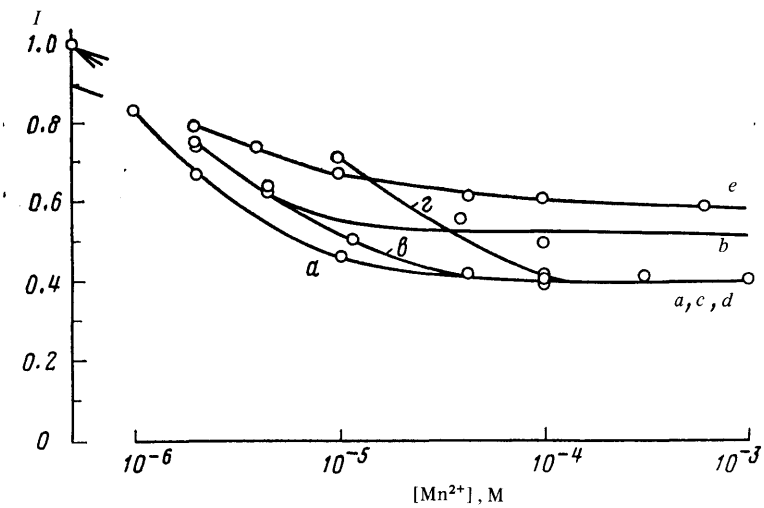


Figure 6. Dependence of the integral intensity (I) of the ^{31}P NMR signal on the Mn^{2+} concentration for dispersions of 0.08 M PI (*a*);[73] 0.05 M (PC + PG) mixture, 1:1, mole/mole, *b*) PC, *c*) PG; and the corresponding dependence for the $\overset{+}{N}(CH_3)_3$ ^1H NMR signal for dispersions of 0.05 M PC (d) and a (PC + PG) 1:1 mole/mole mixture (*e*)[71] in D_2O.

The inclusion of anionic lipids (PI and phosphatidytlserine PS) in the lipid bilayer leads to an increase in the paramagnetic shifts of the signals from the polar groups.[8,76,77] These lipids, by giving the surface of the bilayer a negative charge, influence the distribution of ions in the Stern layer of the vesicle. As a result, the lifetime of the probe in the bound state increases.[9] The same phenomenon is responsible for the influence of inorganic anions on the magnitude of induced shifts.[4,8,46,53,86,87] In this case, however, the redistribution of the ions takes place as a result of the selective Langmuir adsorption of anions on the surface of the lipid bilayer.[87] The ratio of the anion binding constants K correlates satisfactorily with the ratio of the electrophoretic mobilities of liposomes in the presence of these anions (Table 4).[86,87]

Table 4. Electrophoretic mobility of PC liposomes in solutions of different potassium salts and the constants of the Langmuir adsorption isotherms for different anions (300 K, pD = 6).[87]

Anion	Mobility, $\mu m\ s^{-1}\ V^{-1}\ cm$	Δ, p.p.m.	K, $mole^{-1}$
Cl^-	−0.15±0.01	0 15±0.01	1.67±0.45
NO_3^-	−0.46±0.03	1.01±0.02	4.03±0.26
SCN^-	−1.85±0.11	0.99±0.01	23.7±1.1
SO_4^{2-}	1.25±0.09	−0.43±0.01	14.9±0.2

3. Determination of the Conformation and Spatial Orientation of Polar Fragments

The dipole–dipole broadening of the signals in the NMR spectra of colloidal systems prevents the direct use of spin–spin coupling constants to estimate the conformation of polar groups. Thus in Refs.5, 8, and 49 the conformation and spatial orientation of phosphorylcholine zwitterions on the surface of vesicles were determined using the relative pseudo-contact shifts induced by Eu^{3+}, Pr^{3+}, and Yb^{3+} for the signals in the ^1H and ^{13}C NMR spectra. The experimental values of the shifts together with the values calculated from Eqn.(1) for all possible 54 P–O, O–C, and C–C bond rotamers (Fig.7) were plotted on maps of the relative induced shifts of the ^{13}C and ^1H nuclei (Fig.8).

The main contribution to the conformation of the zwitterion is made by the *trans*-forms 111, 211, 611 (70–83%),[5,49] and their probability increases with the degree of unsaturated character of the hydrocarbon chains and with increase in temperature. This is apparently related to the increase in the area corresponding to the SAS molecule[28] and agrees with D and ^{31}P NMR data.[88]

The conclusions obtained are confirmed for O–C bond rotamers by an analysis of the vicinal spin–spin coupling constants $^3J_{POOCC}$ [49] and $^3J_{POCH}$,[89] found without the introduction of a paramagnetic probe. Thus paramagnetic ions have no significant influence on the conformation of a polar group on the surface of a vesicle, so that the fears expressed on this account in Refs.15 and 90 are unfounded.

In micelles of lysolecithin and glycerophosphorylcholine diacetate, instead of the *gauche* forms 132 ≡ 123 and 323 ≡ 532, the different set 523 ≡ 332 is produced,[49] in agreement with the results of studies[91,92] in which a paramagnetic probe was not used.

The fact that the NMR signals from the phosphorylcholine nuclei and from the CH_2CH_2CO groups of the aliphatic acid chains have induced shifts with different directions indicates, in accordance with Eqn.(1), that for all selected conformations the phosphorylcholine zwitterion is arranged parallel to the membrane surface.[5,8,49] This conclusion was subsequently confirmed by other methods: measurement of the ^{31}P NOE in vesicles,[93,94] the ^{31}P NMR of oriented bilayers,[95,96] neutron diffraction (for PC),[97] X-ray diffraction (for PA),[98] measurement of the anisotropy of the ^{31}P shift and D NMR for both PA[99] and PC,[100,101] and quantum-chemical calculations.[102] Thus paramagnetic ions do not alter the orientation of a polar group at the phase interface.[5,9,49,103]

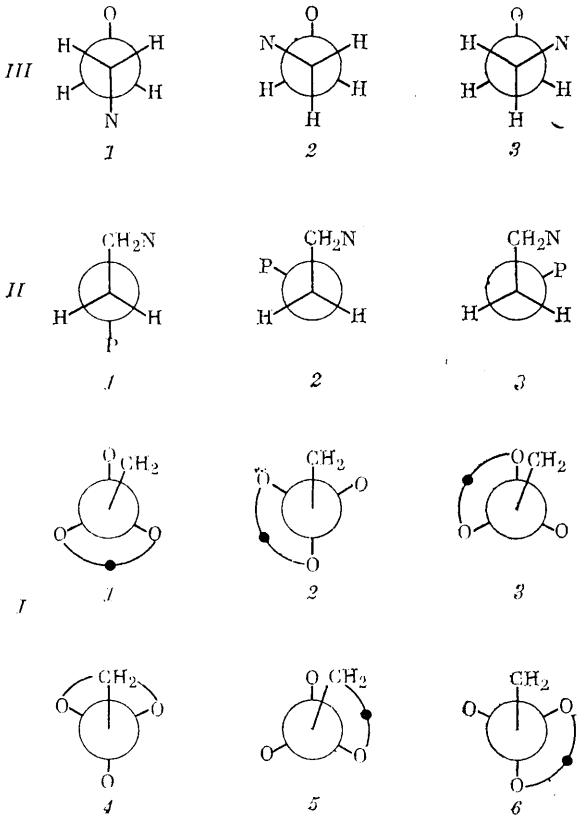


Figure 7. Rotamers of the phosphorylcholine group with respect to the bonds P–O (I), O–C (*II*), and C–C (*III*).[49].

From this it follows that it is not correct to transfer the conclusion about the normal arrangement of the zwitterion on vesicles, obtained for planar bilayers.[104,105] It was noted above that on going from non-closed lamellar structures to bilayer liposomes, the spatial orientation of the polar fragment changes from normal to the surface of the bilayer to planar.[36]

The main reason for the stabilisation of the unfolded *trans*-conformation and the planar orientation of the phosphorylcholine zwitterion on the surface of the vesicle is apparently the intermolecular interaction between the phosphate and $\overset{+}{N}(CH_3)_3$ groups of neighbouring molecules.[49,102] This conclusion is of fundamental importance for an understanding of the molecular organisation and functioning of biological membranes.

4. The Exchange of Lipid Molecules and the Compositional Asymmetry of Membranes

Liposomes, like micelles, are dynamically stable structures. The molecules of SAS in liposomes are extremely mobile, however. They take part in three types of transfer: lateral diffusion,[106] trans-membrane transfer,[107] and inter-membrane transfer.[76] The ratio of the rates of these processes depends on the structure of the lipid molecules and the colloidal system, the molecular composition of the bilayer, and external factors (temperature, ionic strength, the presence of transport proteins, etc.). This ratio determines the possible formation of liposomes with an asymmetric composition of the external and internal monolayers, and also of molecular clusters in the monolayer.

The hydrophilic paramagnetic probe method is the most effective both for the study of the three types of molecular exchange[5,65,76,77,108–110] and in the analysis of the molecular topography of colloidal systems.[64,65,73–77, 111–114]

The rate of the trans-membrane transfer of lipid molecules is low. The time constant of the process is greater than 2.2×10^4 s for egg PC[107] (determined by the spin probe method) and 4.3×10^4 s for [N–$^{13}CH_3$] dioleoyl PC[108] (determined from the penetration of Pr^{3+} ions through the bilayer) at 350 K. This rate can be increased, however, by modelling the bilayer by a system of inverse micelles[5,109] or by introducing small quantities of lysolecithin (LPC) and peroxides.[65,110]

In Refs.5 and 109, the rates and thermodynamic parameters of exchange were found using a mixture of inverse micelles of PC in benzene, with and without Mn^{2+} or Pr^{3+} ions in the solubilised aqueous vesicle.

The kinetics of the trans-membrane transfer of molecules of PC[110] and PI[65] in the presence of LPC or peroxides has been studied by introducing Pr^{3+} or Yb^{3+} ions on the outside of the vesicles. The use of a double probe technique[64] showed that in the transfer of PI molecules the decrease in the induced shift of the signal from the external $\overset{+}{N}(CH_3)_3$ groups is accompanied by an increase in the shift of the signal from the internal groups. The results obtained for both inverse micelles and vesicles indicate that the trans-membrane transfer of the molecules is cooperative in character.

Inter-membrane exchange by lipid molecules is initiated either by a transport lipid-binding protein or by LPC.[76,77] The analysis of the kinetics of the process was based on the increase in the induced paramagnetic shift of the signals from the polar groups when molecules of

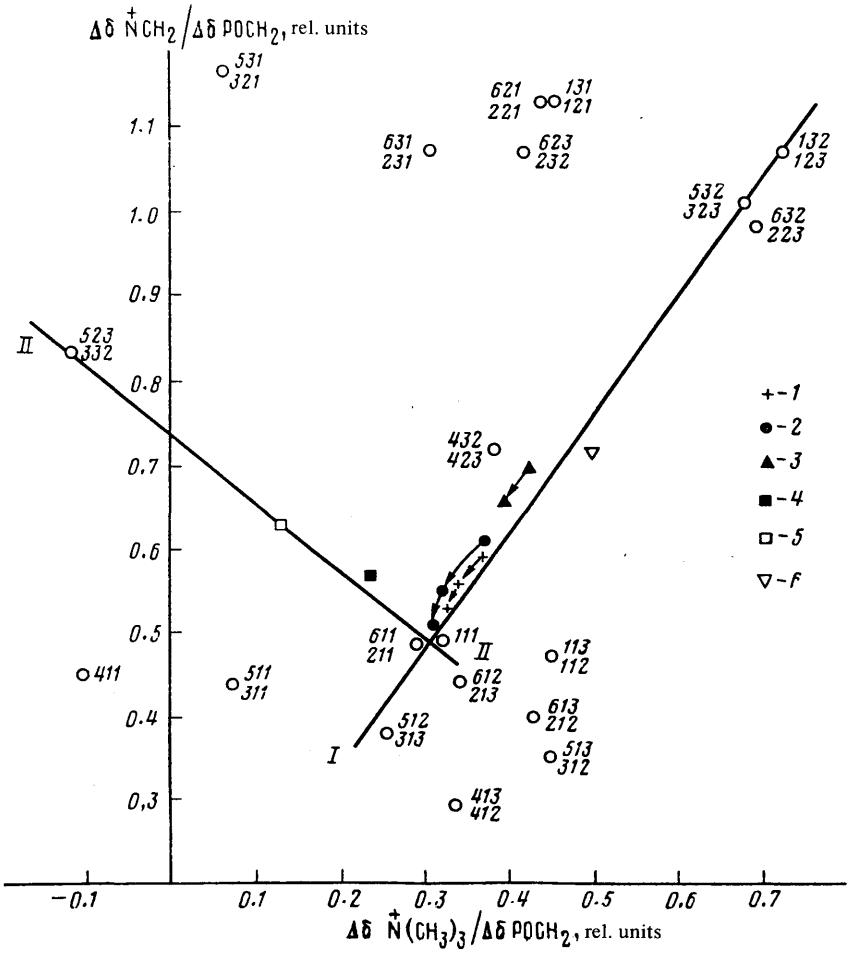


Figure 8. Map of the induced shifts for the ^1H nuclei of the zwitterion: *1*) egg PC, *2*) dioleoyl-PC, *3*) dipalmitoyl-PC, *4*) lysolecithin, *5*) glycerophosphorylcholine diacetate, *6*) stearylphosphocholine.[49]

an anionic lipid (PI) were built into the bilayer (Figs.9 and 10). The change in the magnitude of the induced shift and the ratios I_{ext}/I_{int} give information on the mechanism of inter-membrane exchange.

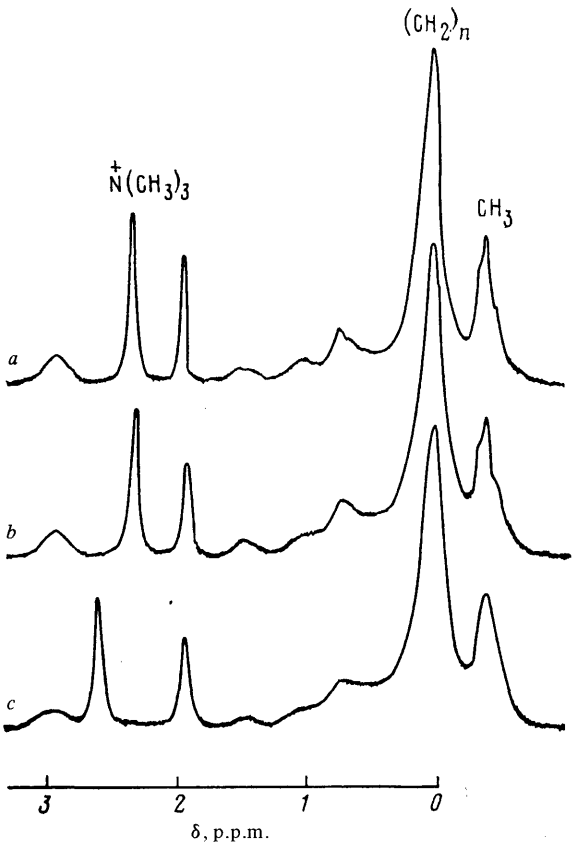


Figure 9. ^{1}H NMR spectra of phospholipid dispersions in D_2O after the addition of 10^{-2} M Pr^{3+} to the outside: *a*) PC vesicles, *b*) mixed vesicles of PC and PI before and *c*) after the exchange of PI by the action of a transport protein (323 K).[77]

In the exchange, vesicles with an asymmetric distribution of lipids are formed (Fig.11). In the spontaneous exchange of PC and PI molecules[65] or in the presence of a PI-transporting protein, PI is exclusively built into the external monolayer.[77] If the system contains micelles of LPC, PI is localised chiefly in the internal monolayer. Monomolecular exchange takes place in the first case, and cooperative exchange in the second. The $^{13}C[N-{}^{13}CH_3]$ NMR spectra of palmitoyl LPC, built into vesicles of dioleoyl-PC, in the presence of Dy^{3+} showed that LPC itself is localised in the external monolayer of the vesicle.[111]

The curvature of the bilayer also leads to an asymmetric distribution of lipids. In mixed PC–PG vesicles, the phosphatidylglycerol (PG) molecules are present chiefly on the external surface,[74,75] as shown by ^{31}P and ^1H NMR with Mn^{2+} This distribution is difficult to understand, since other anionic lipids of PS[64,82] and PI[61] are situated chiefly in the internal monolayer, as indicated by more accurate data with two probes, Pr^{3+} and Eu^{3+} (Fig.10). This distribution is attributed to the more compact packing of the molecules in the internal monolayer, as a result of which the bulky polar fragments of PC are more effectively arranged in the external monolayer. With increase in the concentration of PC or PI, however, the electrostatic forces of repulsion displace an increasing number of molecules of the anionic lipids into the external layer, and the asymmetry of the membrane decreases.[64,65]

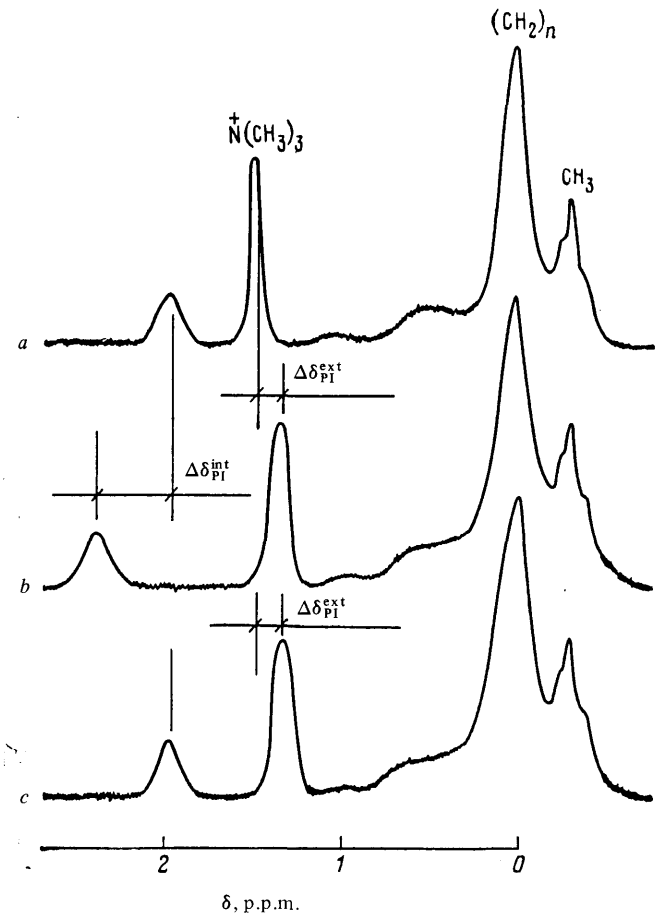


Figure 10. ^{1}H NMR spectra of phospholipid dispersions in D_2O: *a*) PC vesicles, *b*) mixed vesicles of PC and PI, subjected to ultrasonic treatment, *c*) vesicles of PC with spontaneously built-in PI after the combined incubation of vesicles of PC and PI for 5.8 x 10^4 s at 296 K, containing 10^{-2} M Pr^{3+} inside and 10^{-2} M Eu^{3+} outside.[65]

The asymmetry of the distribution of cholesterol in vesicles of PC was also first detected using a paramagnetic probe (Pr^{3+}).[112] Cholesterol is present in the external monolayer in vesicles of unsaturated PC, and is distributed throughout the vesicles of saturated PC.[113] The constant for the spontaneous inter-membrane exchange of cholesterol is 0.0117 min^{-1}.[113]

The rate of the lateral diffusion of cholesterol depends on the molecular structure of the PC.[53,114] Thus if the liposomes contain simultaneously molecules of the SS-, SU-, and UU-types (S and U represent respectively saturated and unsaturated alkyl chains), the cholesterol is more frequently in contact with molecules of the UU-type than with molecules of the SU- or SS-type. Thus if the membrane contains PC of different types, the cholesterol is present chiefly in the phase containing the more unsaturated molecular forms of PC.

5. Analysis of the Interaction of Liposomes with Low-Molecular-Weight and Biologically Active Compounds

The above example of the study of the behaviour of cholesterol molecules in the structure of liposomes indicates the promising possibilities provided by the use of the paramagnetic probe method to investigate the nature of the interaction of physiologically active compounds with a membrane. Most studies are based on the use of the effect on the trans-membrane transfer of lanthanide cations of ionophors,[66,115-117] local anaesthetics,[118,119] and other physiologically active compounds,[76,77,120] as in the case of LPC.[110]

The transport of Pr^{3+}, Eu^{3+}, Yb^{3+}, and Dy cations through a bilayer has been studied using the ionophor X-537A,[115] alamethicin,[116] the ionophor A23187 of the nigericin type,[117] glycophorin,[66] the local anaesthetics tetracaine, procaine, and lidocaine,[118] isoprenoids,[119] and sodium taurocholate.[120] Here, the lanthanide cations act as physiological analogues of calcium. The activation energy of the process is 85 kJ $mole^{-1}$.[119]

A different method has been proposed for determining the kinetics of the transport of Na^+ and Li^+ by the ionophor monensin using Gd^{3+} as relaxation probe. Since the order of magnitude of the rate of transfer was the same as that of the rate of relaxation of the ^{23}Na and ^7Li nuclei, the mobility of the ions made a contribution to the relaxation time. The values found, $v_{Na} = 4$ nm s^{-1} and $v_{Li} = 0.035$ nm s^{-1} correspond to the affinity of the ionophor for cations.[121]

^{13}C-enriched lipids have been used for the paramagnetic hydrophilic probing of the distribution of aliphatic acids in vesicles of PC with a pH gradient.[58] The relaxation probe Mn^{2+} established that the 1-anilinonaphthalene-8-sulphonate molecule is localised on the surface of the vesicles.[122]

IV. THE STRUCTURE OF BIOCOLLOIDAL SYSTEMS

The transport of ions through a membrane barrier plays a fundamental role in the vital activity of cells. It is equally important, however, to establish the mechanism of lipid–protein interaction and the behaviour of combined water in colloidal systems. The characteristics of the introduction of polypeptides (poly-L-glutamic acid, poly-L-leucine)[123] and a transport protein[76,77] into a liposome have been studied using Eu^{3+} and Pr^{3+} ions and ^1H and ^{31}P NMR spectroscopy. It was established that the mosaic model is applicable to bilayer vesicles. According to this model, the protein "icebergs" in the membrane float in a lipid sea. The mosaic model was also found to be applicable to blood serum lipoproteins,[124-127] the structure of which was determined by ^{13}C and ^{31}P NMR spectroscopy using Eu^{3+} ions and built-in [N–$^{13}CH_3$]-PC and -SM and 1,2-(1-oleoyl-^{13}C)-PC.[124,125] Experiments with the hexacyanoferrate(III) anion and Nd^{3+} [126] confirmed the published conclusions[124,125] that all the mobile $\dot{N}(CH_3)_3$ groups of the phospholipids in both low-density and high-density lipoproteins are situated on the external surface of the globule (Fig.12). The incomplete broadening of the ^{31}P signals in the spectra of lipoproteins in the presence of Mn^{2+} ions (80% in high-density lipoproteins and 50% in low-density lipoproteins)[127] may be due to an error in the organisation of the experiment resulting from the competition with the powerful Complexone ethylenediaminetetraacetic acid.

Mn^{2+} ions have been used to determine the location of the membrane proton of erythrocyte ghosts.[128] Comparison of the broadening of the signals in the ^1H NMR spectra of model membranes of PC

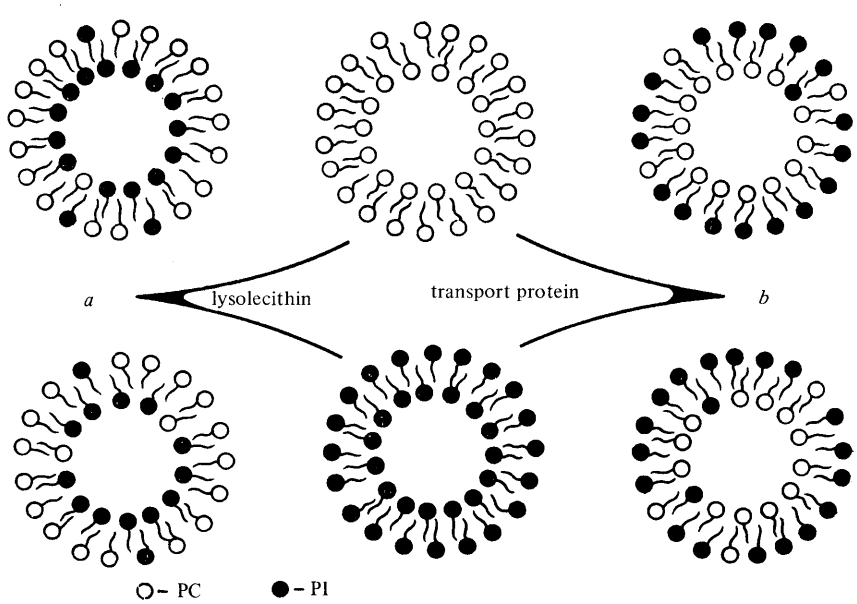


Figure 11. Diagram of inter-membrane exchange under the influence of: *a*) LPC, and *b*) a transport proteins.[53]

and the erythrocyte ghosts showed that the protein is situated on the surface of the biomembrane. This agrees with the results of an analysis of the membrane of sarcoplasm reticulum using an iminoxyl probe from its broadening effect on the ^{1}H and ^{13}C NMR signals.[129]

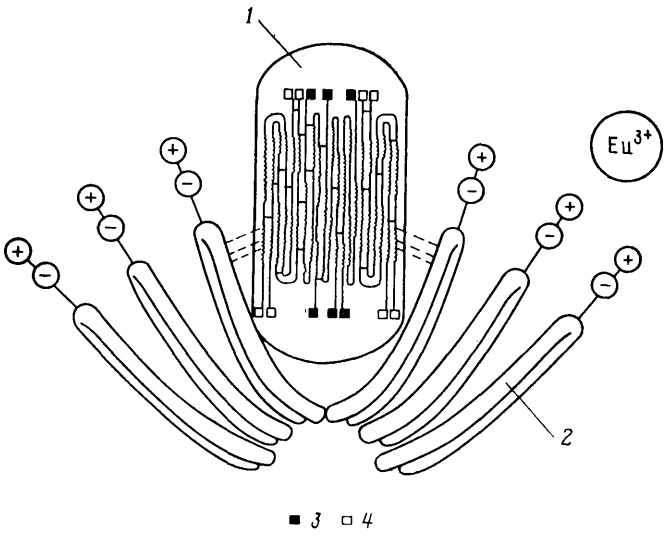


Figure 12. Model of a high density lipoprotein: *1*) protein, *2*) mixture of PC and SM, *3*) apoprotein I, *4*) apoprotein II.[125]

^{31}P NMR spectroscopy has been used[130] to measure the rate of the penetration of paramagnetic ions into heart cells. The penetration of Mn^{2+} ions made it possible to diagnose ischaemia.

Relaxation paramagnetic probes have been used many times[56-58,132,133] to study the behaviour of water in biological systems. In the first of these studies, Mn^{2+} ions were used to identify the signals from free and combined (intracellular, 4 p.p.m.) water in native rabbit sciatic nerve.[131] All other studies used similar methods. One of the most interesting is a study[132] in which NMR spectroscopy was used to investigate the intercellular transport of water in the roots of wheat plants. The introduction of Mn^{2+} ions into the space outside the cell makes it possible to eliminate from consideration the vacuolar transport of water and to control the symplast path. It is known that manganese is necessary for the liberation of oxygen in photosynthesis in the chloroplast membrane. The state of the manganese ions combined with the membrane was estimated using the values of the spin–lattice relaxation time T_1 of the protons of the water in a suspension of the thylakoid membranes of chloroplast.[133] It was shown that the manganese atoms have oxidation states II and III.

A large number of studies have been devoted to the investigation of the structure and topography of colloidal protein systems using paramagnetic hydrophilic propbes.[14,15] These studies, which are all of the same type, will not be characterised, but the results of one such study, where the possibilities provided by the method were most fully used, can be considered.[134] The authors cited used Mn^{2+}- and Cr^{3+}-ATP probes to determine the distances to different regions of carbamoyl-phosphate synthetase (CPS) by ^{1}H, ^{13}C, ^{15}N, ^{6}Li, ^{7}Li, and ^{31}P NMR spectroscopy (Table 5). These results gave the first topographical map of the environment of the substrates and the positions of the activator ions and allosteric modifiers in the CPSA dimer from *E.coli*.

Table 5. Distance from the ions Mn^{2+} and Cr^{3+}-ATP (r) to the nuclei of the ligand in the complex of CPS from *E.coli*.[134]

Mn^{2+}-ligand			Cr^{3+}-ATP-ligand		
ligand	nucleus	r, nm	ligand	nucleus	r, nm
Glu	^{13}C (5)	0.86±0.06	Glu	^{13}C (5)	0.70±0.05
$Co(NH_3)_4$-ATP	^{31}P ($\alpha\beta$); ^{31}P (γ)	0.66±0.04; 0.64±0.04	HCO_3^-; NH_4^+	^{13}C; ^{15}N	0.47±0.03; 0.74±0.05
Li^+	^{7}Li; ^{6}Li	0.75±0.04; 0.80±0.05	—	—	—

Thus the use of paramagnetic shift and relaxation hydrophilic probes combined with traditional methods of studying colloidal systems by NMR spectroscopy opens up extensive possibilities for the analysis of their structure and molecular dynamics. The results obtained by this method are of fundamental interest for establishing the mechanisms of micellar catalysis and emulsion polymerisation. The method has made it possible to study a wide range of properties of membrane systems, important both for the investigation of the molecular structure and mechanism of the functioning of cell membranes and for the development of membrane technology.

REFERENCES

1. O.P.Yablonskii, Yu.E.Shapiro, and V.P.Konovalova, Kolloid. Zhur., 1984, **46**, 610.
2. L.D.Bergel'son, L.I.Barsukov, N.I.Dubrovina, and V.F.Bystrov, Dokl.Akad.Nauk SSSR, 1970, **194**, 222.
3. V.F.Bystrov, N.I.Dubrovina, L.I.Barsukov, and L.D.Bergel'son, Chem.Phys.Lipids., 1971, **6**, 343.
4. V.F.Bystrov, Yu.E.Shapiro, A.V.Viktorov, et al., FEBS Lett., 1972, **25**, 337.
5. L.I.Barsukov, Yu.E.Shapiro, A.V.Viktorov, et al., Dokl.Akad. Nauk SSSR, 1973, **208**, 717.
6. L.I.Barsukov, Yu.E.Shapiro, A.V.Viktorov, et al., Dokl.Akad. Nauk SSSR, 1973, **211**, 847.
7. E.E.Zaev and L.V.Spirikhin, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser.Khim., 1973, **12**, 32.
8. Yu.E.Shapiro, A.V.Viktorov, V.I.Volkova, et al., Chem.Phys. Lipids, 1975, **14**, 227.
9. Yu.E.Shapiro, A.V.Viktorov, L.I.Barsukov, and L.D.Bergel'son, "Biofizika Membran" (The Biophysics of Membranes), Izd.Nauka, Moscow, 1981, p.22.
10. N.Bloembergen and W.C.Dickinson, Phys.Rev., 1950, **79**, 179.
11. I.Ya.Slonim and A.Kh.Bulai, Uspekhi Khim., 1973, **42**, 1976 [Russ.Chem.Rev., 1973(11)].
12. J.Reuben, "Progress in NMR Spectroscopy", Vol.9, Pergamon Press, New York, 1973, Pt 1, p.1.
13. NMR Shift Reagents, Edited by R.E.Sievers, Acad.Press, 1973.
14. K.G.Orrell, "Annual Reports on NMR Spectroscopy", Vol.9 L, Acad.Press, 1979, p.1.

15. V.F.Zolin and L.G.Koreneva, "Redkozemel'nyi Zond v Khimii i Biologii" (Lanthanide Probes in Chemistry and Biology), Izd. Nauka, Moscow, 1980, 350 pp.
16. Yu.E.Shapiro, Uspekhi Khim., 1984, **53,** 1407 [Russ.Chem. Rev., 1984(8)].
17. H.M.McConnell and R.E.Robertson, J.Chem.Phys., 1958, **29,** 1361.
18. I.Solomon, Phys.Rev., 1955, **99,** 559.
19. A.W.Overhauser, Phys.Rev., 1953, **91,** 476.
20. K.A.Dill and P.J.Flory, Proc.Nat.Acad.Sci.USA, 1981, **78,** 676.
21. E.E.Zaev, L.M.Khalilov, and L.V.Spirikhin, Kinetika i Kataliz, 1974, **15,** 1088.
22. E.E.Zaev and L.M.Khalilov, Kolloid.Zhur., 1974, **36,** 147.
23. E.E.Zaev, Kolloid.Zhur., 1979, **41,** 1179; 1980, **42,** 752.
24. F.M.Menger, Acc.Chem.Reson., 1979, **12,** 111.
25. P.Fromherz, Chem.Phys.Lett., 1981, **77,** 460.
26. H.Ziemiecki and W.R.Cherry, J.Amer.Chem.Soc., 1981, **103,** 4479.
27. B.Cabane, J.Physique (Orsay), 1981, **42,** 847.
28. D.J.Mitchell and B.W.Nonham, J.Chem.Soc.Faraday Trans.II, 1981, **77,** 601.
29. F.M.Menger and D.W.Doll, J.Amer.Chem.Soc., 1984, **106,** 1109.
30. O.V.Veselova, B.P.Nikolaev, and A.M.Shlyakov, Kolloid. Zhur., 1985, **47,** 1027.
31. M.Aizawa, T.Komatsu, T.Nakagawa, Bull.Chem.Soc.Jap., 1982, **55,** 3434.
32. Yu.E.Shapiro, O.K.Shvetsov, and N.P.Dozorova, Kolloid. Zhur., 1976, **38,** 943.
33. N.P.Dozorova, Yu.E.Shapiro, T.G.Balyberdina, et al., Kolloid. Zhur., 1979, **41,** 545.
34. Yu.E.Shapiro, N.P.Dozorova, N.M.Mironova, and T.G.Balyberdina, Vysokomol.Soed., 1981, **23A,** 1374.
35. Yu.E.Shapiro, V.P.Zubov, V.V.Egorov, and N.P.Dozorova, Kolloid.Zhur., 1981, **43,** 187.
36. Yu.E.Shapiro, Yu.N.Orlov, V.V.Egorov, and V.P.Zubov, Vestnik Moskov.Gos.Univ., Ser.2, Khimiya, 1984, **25,** 487, 595.
37. O.K.Shvetsov, N.M.Mironova, Yu.E.Shapiro, et al., Faserforsch.Textiltechn., 1977, **28,** 217.
38. Yu.E.Shapiro, O.K.Shvetsov, N.P.Dozorova, and A.A.Ershov, Vysokomol.Soed., 1978, **20B,** 328.
39. E.E.Zaev, Kolloid.Zhur., 1980, **42,** 752.
40. E.E.Zaev and L.M.Khalilov, Kolloid.Zhur., 1980, **42,** 1175.
41. E.E.Zaev and L.M.Khalilov, Zhur.Strukt.Khim., 1980, **21,** 53.
42. E.E.Zaev and L.M.Khalilov, Kolloid.Zhur., 1984, **46,** 427.
43. E.E.Zaev, V.A.Ponomareva, L.M.Obzhelyanskaya, and A.L.Shtangeev, Kolloid.Zhur., 1981, **43,** 251.
44. E.E.Zaev, L.V.Spirikhin, and Yu.G.Grechishnikov, Zhur.Fiz.Khim., 1977, **51,** 524 [Russ.J.Phys.Chem., 1977(2)].
45. T.Matsuo, K.Yudate, and T.Yagamura, J.Colloid.and Interf. Sci., 1981, **83,** 354.
46. G.L.Jendrasiak, Chem.Phys.Lipids, 1970, **5,** 85; 1971, **6,** 215.
47. S.Miyagishi and M.Nishida, J.Colloid and Interf.Sci., 1980, **73,** 270.
48. E.V.Batrakova, Yu.N.Orlov, V.V.Egorov, et al., Kolloid. Zhur., 1985, **47,** 130.
49. L.I.Barsukov, Yu.E.Shapiro, A.V.Viktorov, et al., Bioorg. Khim., 1976, **2,** 1404.
50. C.Chachaty, B.Perly, J.Quaegebeur, et al., Sci.et rech.num. spec., 1982, 30.
51. Y.Chevalier and C.Chachaty, J.Phys.Chem., 1985, **89,** 875.
52. V.V.Egorov, V.P.Zubov, V.B.Golubev, et al., Vysokomol. Soed., 1981, **23B,** 803.
53. Yu.E.Shapiro, "Fizicheskaya Khimiya" (Physical Chemistry), Yaroslavl', 1976, p.42.
54. L.D.Bergel'son and L.I.Barsukov, Science, 1977, **197,** 224.
55. W.C.Hutton, P.L.Yeagle, R.B.Martin, Chem.Phys.Lipids, 1977, **19,** 255.
56. H.Wennerström and G.Lindblom, Quat.Rev.Biophys., 1977, **10,** 67.
57. R.E.Jacobs and E.Oldfield, "Prog.Nucl.Magn.Reson.Spectrosc"., Pergamon Press, New York, 1980, **14**(3), 113.
58. M.Ptak and A.Sanson, "Magn.Reson. in Colloid and Interf. Sci"., Reidel Publ.Comp., 1980, p.285.
59. Y.K.Levine, A.G.Lee, N.J.M.Birdsall, J.C.Metcalfe, and J.D.Robinson, Biochim.et biophys.acta, 1973, **291,** 592.
60. S.B.Andrews, J.W.Faller, J.M.Gilliam, and R.J.Barnett, Proc. Nat.Acad.Sci.USA, 1973, **70,** 1814.
61. H.Hauser and M.D.Barratt, Biochem.Biophys.Res.Communs., 1973, **53,** 399.
62. E.Tsuchida, N.Seki, and H.Ohno, Makromol.Chem., 1986, **187,** 1351.
63. M.Takane, K.Shigehara, and E.Tsuchida, Makromol.Chem., 1986, **187,** 853.
64. A.V.Viktorov, I.A.Vasilenko, L.I.Barsukov, et al., Dokl.Akad. Nauk SSSR, 1977, **234,** 207.
65. A.V.Viktorov, I.A.aVasilenko, L.I.Barsukov, et al., Dokl.Akad. Nauk SSSR, 1979, **245,** 1243; 1980, **246,** 479.
66. W.J.Gerritsen, E.J.J.Van Zoelen, A.J.Vecklen, A.J.Verkleij, B.De Kruijff, and L.L.M.Van Deenen, Biochim.et biophys.acta, 1979, **551,** 248.
67. K.Arnold, Studia Biophysica, 1974, **42,** 229.
68. K.Arnold, W.Gründer, R.Göldner, and A.Hofmann, Z.Phys.Chemie (Leipzig), 1975, **256,** 522.
69. R.Lawaczeck, M.Kainosho, J.-L.Girardet, and S.I.Chan, Nature, 1975, **256,** 584.
70. M.C.Uhing, Chem.Phys.Lipids, 1975, **14,** 303.
71. B.Sears, W.C.Hutton, T.E.Thompson, Biochemistry, 1976, **15,** 1635.
72. N.E.Gabriel and M.F.Roberts, Biochemistry, 1984, **23,** 4011; 1986, **25,** 2812; 1987, **26,** 2432.
73. B.De Kruyff, P.R.Cullis, and G.K.Radda, Biochim.et biophys. acta, 1975, **406,** 6.
74. D.M.Michaelson, A.F.Horwitz, and M.P.Klein, Biochemistry, 1973, **12,** 2637; 1974, **13,** 2605.
75. A.F.Horwitz, M.P.Klein, D.M.Michaelson, and S.J.Kholer, Ann.New York Acad.Sci., 1973, **222,** 468.
76. L.I.Barsukov, Yu.E.Shapiro, A.V.Viktorov, et al., Biochem. Biophys.Res.Communs., 1974, **60,** 196.
77. L.I.Barsukov, Yu.E.Shapiro, A.V.Viktorov, et al., Chem.Phys. Lipids, 1975, **14,** 211.
78. Y.K.Levine and M.H.F.Wilkins, Nature, New Biol., 1971, **230,** 69.
79. H.Hauser, L.Irons, and G.Hoppe-Seyler, Physiol.Chem., 1972, **333,** 1579.
80. R.J.Kostelnik and S.M.Castellano, J.Magn.Reson., 1972, **8,** 219; 1973, **9,** 291.
81. M.P.Sheetz and S.I.Chan, Biochemistry, 1972, **11,** 4576.
82. J.A.Berden, R.W.Barker, and G.K.Radda, Biochim.et biophys.acta, 1975, **375,** 186.
83. Yu.E.Shapiro, A.V.Viktorov, L.I.Barsukov, and L.D.Bergel'son, Biofizika, 1978, **23,** 727.

84. Yu.E.Shapiro, V.V.Egorov, and V.P.Zubov, Vestnik Moskov. Gos.Univ., Ser.2, Khimiya, 1987, **28,** 183.
85. P.W.Nolden and T.Ackermann, Biophys.Chem., 1975, **3,** 183.
86. H.Hauser, M.C.Phillips, B.A.Levine, R.J.P.Williams, Eur.J. Biochem., 1975, **58,** 133.
87. L.I.Barsukov, V.I.Volkova, Yu.E.Shapiro, et al., Bioorg. Khim., 1977, **3,** 1355.
88. H.-U.Gally, A.Niederberger, and J.Seelig, Biochemistry, 1975, **14,** 3647.
89. H.Akutsu and Y.Kyogoku, Chem.Phys.Lipids, 1977, **18,** 285.
90. M.F.Brown and J.Seelig, Nature, 1977, **269,** 721.
91. N.J.M.Birdsall, J.Feeney, and A.G.Lee, et al., J.Chem.Soc. Perkin Trans.II, 1972, 1441.
92. K.K.Yabusaki and M.A.Wells, Biochemistry, 1975, **14,** 162.
93. P.L.Yeagle, W.C.Hutton, C.-H.Huang, and B.Martin, Proc. Nat.Acad.Sci.USA, 1975, **72,** 3477.
94. P.L.Yeagle, "Biomolecular Structure and Function", Acad. Press, New York–London, 1978, p.65.
95. R.G.Griffin, L.Powers, J.Herzfeld, R.A.Haberkorn, and P.S.Pershan, Biophys.J., 1977, **17,** 84.
96. R.G.Griffin, L.Powers, and P.S.Pershan, Biochemistry, 1978, **17,** 2718.
97. G.Bueldt, H.-U.Gally, A.Seelig, J.Seelig, and G.Zaccai, Nature, 1978, **271,** 182.
98. P.B.Hitchcock, R.Mason, K.M.Thomas, and G.G.Shipley, Proc.Nat.Acad.Sci.USA, 1974, **71,** 3036.
99. J.Seelig and H.-U.Gally, Biochemistry, 1976, **15,** 5199.
100. J.Seelig, H.-U.Gally, and R.Wohlgemuth, Biochim. et biophys.acta, 1977, **467,** 109.
101. J.Seelig, Biochim.et biophys.acta, 1978, **515,** 105.
102. B.Pullman and H.Berthod, FEBS Lett., 1974, **44,** 266.
103. Yu.A.Shchipunov and G.Yu.Drachev, J.Colloid and Interf.Sci., 1986, **110,** 424.
104. M.C.Phillips, E.G.Finer, and H.Hauser, Biochim.et biophys. acta, 1972, **290,** 397.
105. H.Hauser, M.C.Phillips, B.A.Levine, R.J.P.Williams, Nature, 1976, **261,** 390.
106. R.D.Kornberg and H.M.McConnell, Proc.Nat.Acad.Sci.USA, 1971, **68,** 2564.
107. R.D.Kornberg and H.M.McConnell, Biochemistry, 1971, **10,** 1111.
108. B.De Kruijff and K.W.A.Wirtz, Biochim.et biophys.acta, 1977, **467,** 318.
109. L.R.C.Barclay, B.J.Balcom, and B.J.Forrest, J.Amer.Chem. Soc., 1986, **108,** 761.
110. L.I.Barsukov, Yu.E.Shapiro, A.M.Parfen'eva, et al., Biofizika, 1974, **19,** 456.
111. B.de Kruijff, H.P.van den Besselaar, and L.L.M.Van Deenen, Biochim.et biophys.acta, 1977, **465,** 443.
112. C.H.Huang, J.P.Sipe, S.T.Chow, and R.B.Martin, Proc.Nat. Acad.Sci.USA, 1974, **71,** 359.
113. L.K.Bar, Y.Barenholz, and T.E.Thompson, Biochemistry, 1986, **25,** 6701.
114. L.I.Barsukov, Yu.E.Shapiro, and A.V.Viktorov, Biofizika, 1977, **22,** 207.
115. M.S.Fernandez, H.Celis, and M.Montal, Biochim.et biophys. acta, 1973, **232,** 600.
116. A.L.Y.Lau and S.I.Chan, Proc.Nat.Acad.Sci.USA, 1975, **72,** 2170.
117. G.R.A.Hunt, FEBS Lett., 1975, **58,** 194.
118. M.S.Fernandez and J.Cerbon, Biochim.et biophys.acta, 1973, **298,** 8.
119. R.J.Cushley, B.J.Forrest, A.Gillis, and J.Tribe, Can.J.Chem., 1979, **57,** 458.
120. F.J.Castellino and B.N.Violand, Arch.Biochem.Biophys., 1979, **193,** 543.
121. H.Degani and G.A.Elgavish, FEBS Lett., 1978, **90,** 357.
122. G.L.Jendrasiak and T.N.Estep, Chem.Phys.Lipids, 1977, **18,** 181.
123. H.Ohno, T.Seki, and E.Tsuchida, Makromol.Chem., 1984, **185,** 329.
124. G.Assmann, E.A.Sokoloski, and H.B.Brewer, Proc.Nat.Acad. Sci.USA, 1974, **71,** 549.
125. G.Assmann and H.B.Brewer, Proc.Nat.Acad.Sci.USA, 1974, **71,** 989, 1534.
126. E.G.Finer, R.Henry, R.B.Leslie, and R.N.Robertson, Biochim. et biophys.acta, 1975, **380,** 320.
127. T.O.Henderson, A.W.Kruski, L.G.Davis, T.Glonek, and A.M.Scanu, Biochemistry, 1975, **14,** 1915.
128. M.P.Sheetz and S.I.Chan, Biochemistry, 1972, **11,** 548.
129. J.D.Robinson, N.J.M.Birdsall, A.G.Lee, and J.C.Metcalfe, 1972, **11,** 2903.
130. D.P.Hollis, B.H.Bulkley, R.L.Nummally, et al., Clin.Res., 1978, **26,** 240A.
131. P.Dea, S.I.Chan, and F.J.Dea, Science, 1972, **175,** 206.
132. A.V.Anisimov, A.S.Evarestov, I.D.Samuilova, and N.A.Gusev, Dokl.Akad.Nauk SSSR, 1983, **271,** 1246.
133. T.Wydrzynski, N.Zumbulyadis, P.G.Schmidt, et al., Biochim.et biophys.acta, 1975, **408,** 349.
134. F.M.Raushel, P.M.Anderson, and J.J.Villafranca, Biochemistry, 1983, **22,** 1872.

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