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MOLECULAR MOTION AND ORDER IN ORIENTED LIPID MULTIBILAYER MEMBRANES EVALUATED BY SIMULATIONS OF SPIN LABEL ESR SPECTRA

EFFECTS OF TEMPERATURE, CHOLESTEROL AND MAGNETIC FIELD

YUHEI SHIMOYAMA *, L.E. GÖRAN ERIKSSON and ANDERS EHRENBERG

Department of Biophysics, Stockholm University, Arrhenius Laboratory, S-106 91 Stockholm (Sweden)

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Summary

A simulation method to interpret electron spin resonance (ESR) of spin labelled amphiphilic molecules in oriented phosphatidylcholine multibilayers in terms of a restricted motional model is presented. Order and motion of the cholestane spin label (3-spiro-doxyl-5α-cholestane) incorporated into egg yolk phosphatidylcholine, dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine, pure and in mixture with cholesterol, were studied at various temperatures. With egg yolk phosphatidylcholine identical sets of motional parameters were obtained from simulations of ESR spectra obtained at three microwave frequencies (X-, K- and Q-band). With dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine analyses of the spectra show that phase transitions occur in samples containing up to 30 mol % cholesterol. The activation energy for the motion of the spin label is about three times larger above than below the phase transition, indicating a more collective motion in the liquid crystalline state than in the gel state. In the liquid crystalline state the activation energy is larger in the pure phosphatidylcholines than with cholesterol added. Additions of cholesterol to egg phosphatidylcholine induces a higher molecular order but does not appreciably affect correlation times. This is in contrast to dipalmitoylphosphatidylcholine where both order and correlation times are affected by the presence of cholesterol. The activation energies follow the same order as the transition temperatures: dipalmitoylphosphatidylcholine > dimyristoylphosphatidylcholine > egg yolk phosphatidylcholine, suggesting a similar order of the cooperativity of the motion of the lipid molecules. Magnetic field-induced effects on egg phosphatidylcholine multibilayers

* Present address: Materials Science Laboratory, Faculty of Engineering, Hokkaido University, Sapporo 060, Japan.

were found at Q-band measurements above 40°C. The cholestane spin label mimics order and motion of cholesterol molecules incorporated into the lipid bilayers. This reflects order and motion of the portions of the lipid molecules on the same depth of the bilayer as the rigid steroid portions of the intercalated molecules.

Introduction

The present knowledge about the physical properties of biological membranes originates to a great extent from studies of model membranes in the form of multibilayers, vesicles, etc., prepared from isolated lipids. In this context the spin labelling technique has proved to be informative [1]. Spin-labelled amphiphilic molecules intercalated into lipid bilayers exhibit restricted anisotropic motion. In this case there is no straightforward method to easily extract from the ESR spectrum information on the nature of the motional restrictions and the molecular rotation. For this purpose it is necessary to resort to some method to simulate the recorded spectra.

With this aim we have previously [2,3] developed a model for the rapid restricted motion of a spin-labelled molecule. This motion is composed of a rotation around the long molecular axis and a simultaneous tumbling of this axis within the confines of a cone. We applied the model to the evaluation of ESR spectra from spin labels incorporated into single bilayer lipid vesicles. The model is now extended and applied to the oriented multibilayer system. We account for the distribution of the cone axes with respect to the director of the oriented multibilayer. The simulation of the ESR spectra furnishes information on correlation times and order parameters. Attempts to evaluate motional correlation times from spin label ESR spectra of membrane systems without use of a simulation procedure are liable to highly erroneous results [3]. We here show that application of the cone model gives a deepened insight into the physical nature of the membranes on the molecular level.

When using molecular probes, such as amphiphilic molecules with attached nitroxide spin label there is always an inherent risk that the probe perturbs the system under investigation. By designing probes that mimic as closely as possible natural membrane components this potential risk may be minimized. In the present study we have used the so-called cholestane spin label (3-spiro-

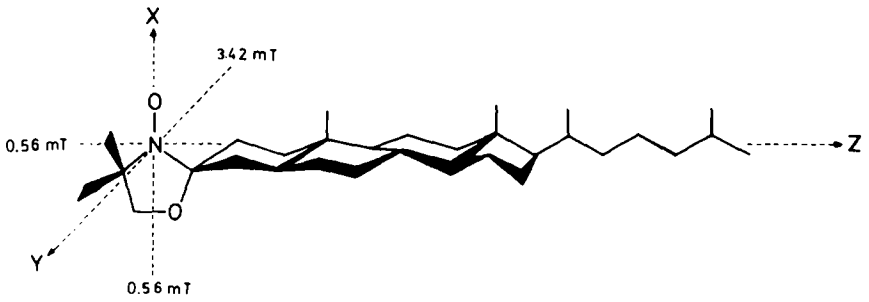

Fig. 1. Formula for the cholestane spin label (3-spiro-doxyl-5α-cholestane). The principal axes for the hyperfine- and g-tensors are assumed to coincide with the molecular reference frame.

doxyl-5α-cholestane; Fig. 1) which has been reported [4] to closely resemble cholesterol in its surface chemistry properties.

The cholestane spin label molecule may thus not only report on the state of motion and order of the lipid bilayer but specifically simulates the behaviour of cholesterol and similar steroid molecules. The fact that the major axis of the hyperfine tensor is perpendicular to the long molecular axis of the molecule (see Fig. 1) is a particular advantage which gives the possibility to resolve both the rotation around the long molecular axis and perpendicular to this axis.

Since the motional parameters obtained by simulation of ESR spectra are to a certain extent model dependent, a specific aim of the present investigation was to test whether our model furnishes physically realistic results. This was done by comparing the motional parameters obtained with the oriented multibilayer in different defined directions in the magnetic field and at three microwave frequencies. A further test was made by studying the well known thermal transition between gel phase and liquid crystalline phase exhibited by dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine.

Incorporation of cholesterol in the lipid bilayer is known to affect membrane properties dramatically. This influence has been studied by various physical methods [5] but the details of the molecular interactions are still largely unknown. We have now followed changes in order and motion of the intercalated cholestane spin label molecule at various contents of cholesterol in the lipid multibilayer.

In addition to the evaluation of the spectra by the simulation procedure we have followed the temperature dependence of the hyperfine splitting measured with magnetic field parallel with and perpendicular to the multibilayer surface. This furnishes additional evidence for the transition of the sample between gel and liquid crystalline phases, and is of particular value when the quality of the spectra is not good enough to make the simulation procedure meaningful.

A preliminary account of part of this investigation has been presented [6].

Materials and Methods

Egg yolk phosphatidylcholine was prepared according to the method of Singleton et al. [7] or bought from Lipid Products, England. In either case the purity was checked by thin-layer chromatography. Dipalmitoylphosphatidylcholine (DL-α-) and dimyristoylphosphatidylcholine (L-α-) were purchased from Sigma Chem. Co., U.S.A. with a purity of 99 and 98 wt.%, respectively. They were used without further purification. Cholesterol was purified by three times, recrystallization from methanol solution. The cholestane spin label (3-spiro-doxyl-5α-cholestane) was synthesized by the method of Keana et al. [8].

The oriented multibilayers were produced as follows: Dilute solutions were prepared of egg phosphatidylcholine in chloroform and of dipalmitoylphosphatidylcholine in ethanol. These solutions were mixed in appropriate proportions with stock solutions of cholesterol and spin label in chloroform. The molar ratio of spin label to phosphatidylcholine plus cholesterol was always kept at about 1%. The organic solvent was evaporated and the mixture redissolved at a

higher concentration in chloroform, or chloroform plus 30 vol.% ethanol. This solution was transferred to the supporting glass plate and the solvent was evaporated under a stream of wet nitrogen gas. The samples were annealed for 30 min under controlled relative humidity at 60°C, i.e. above the transition temperature of the phosphatidylcholine. In case of dipalmitoyl- and dimyristoylphosphatidylcholine the multibilayer structure was further improved by cycling the temperature a few times through the transition point.

In order to satisfy lipid hydration, the samples were kept equilibrated at a constant relative humidity. This was achieved by having a suitable salt solution at the bottom of the ESR tube in which the sample was mounted. A saturated aqueous solution of potassium sulfate was employed. This has been reported [9] to give a fairly constant relative humidity of 98% within the temperature range 2—100°C. The sample tube had to be arranged so that when the sample was centered in the ESR cavity the salt solution was well outside the microwave field of the cavity.

The water contents of the samples were determined by gravimetric analyses of larger but otherwise identical samples placed in glass vessels that could be humidity equilibrated as described and sealed with covers. The dry weights were obtained after heating the samples in a dry atmosphere at 100°C until constant weights were recorded.

ESR measurements were made at three microwave frequencies with the following equipment: JEOL ME-1X spectrometer (X-band, 9.5 GHz; 0.32 T); Varian E-9 spectrometer with a microwave bridge constructed in this laboratory (K-band, 24.1 GHz; 0.81 T); and Varian E-9 spectrometer (Q-band, 35 GHz; 1.18 T). For measurements at X- and Q-band the temperature was regulated by a nitrogen gas flow and a heater/sensor system. The temperature was calibrated to ±0.2°C by means of a Pt resistance that could be placed at the sample position.

The sample supporting plates were cut from thin cover glasses commonly used for light microscopy. Sizes were 3 mm × 30 mm for X- and K-band and 1 mm × 20 mm for Q-band measurements. The plates were mounted on plexiglass rods and inserted into ESR sample tubes (internal diameter 3.1 mm for X- and K-band, 1.3 mm for Q-band). At top a seal could be made between the tube and the rod. In some experiments the small size Q-band sample was measured also at X- and K-band. The sample could be oriented with the magnetic field perpendicular ($\perp$) and parallel ($/\!/$) to the sample plane, in the X- and K-band equipments by means of a goniometer holding the sample and in the Q-band spectrometer by rotating the magnet. These orientations were selected by carefully observing the extrema for the angular variation of the hyperfine separation.

Simulation of ESR spectra. In refs. 2 and 3 we developed the theory for a model in which the molecular motion of a spin label in a lipid bilayer, depending on the temperature regime, is described as follows:

(1) At low temperature there is a rapid restricted rotation or twisting about the long molecular axis (the z-axis in Fig. 1) with the angular amplitude $\pm\phi_0$ and correlation time τ_a.

(2) At high temperature, when the axial rotation becomes unrestricted, $\phi_0 = 90°C$, there is added a restricted rumbling (wobbling) motion of the long

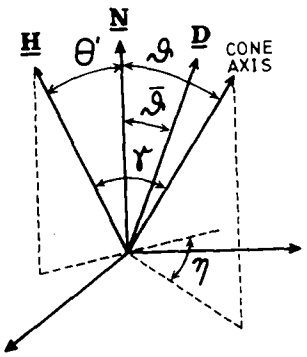

Fig. 2. The axis system defining the orientation of the cone axis relative to the magnetic field $\underline{H}$. The bilayer normal axis and the director are denoted $\underline{N}$ and $\underline{D}$, respectively.

molecular axis within a cone of a semi-cone angle β_0. The correlation time is τ_t for the tumbling motion and τ_{at} for the axial rotation coupled to the tumbling.

This model was successfully applied to spin labels in vesicular membrane systems. In the present case of oriented samples the line positions and the line widths of the ESR spectra are calculated essentially as before, but it was necessary to introduce certain changes.

In case of the vesicle membranes, the orientation of the cone axes relative to the local bilayer normal is averaged out in a random distribution. However, in the oriented samples, the multibilayers possess a macroscopic order which is represented by the director ($\underline{D}$). A relevant coordinate system for the oriented multibilayers is shown in Fig. 2. The normal axis ($\underline{N}$) to the multibilayer surface (sample plate) may either coincide with the director or the director may tilt by an angle $\bar{\vartheta}$. The angle between the cone axis and the $\underline{N}$ axis is denoted by ϑ. With the magnetic field $\underline{H}$ oriented with respect to the sample plate and the cone axis by angles θ' and γ, respectively, we can find a simple trigonometrical relation

$$\cos\gamma = \cos\vartheta\cos\theta' + \sin\vartheta\sin\theta'\cos\eta \tag{1}$$

Here η is the angle between the two planes containing $\underline{H}$ and $\underline{N}$, and the cone axis and $\underline{N}$, respectively.

It is necessary to account for the distribution of the cone axes with respect to the $\underline{N}$ axis. For this purpose we introduced a Gaussian distribution function [1]

$$P(\vartheta) = \sin\vartheta\exp\left\{-\frac{(\vartheta-\bar{\vartheta})^2}{2\vartheta_0^2}\right\} \tag{2}$$

Here ϑ_0 is the spread angle defining the width of the distribution. The weighting function $\sin\vartheta$ accounts for the solid angle over a whole sphere.

It is reported that the residual linewidth Γ_r is mainly attributed to the unresolved proton hyperfine splittings and is orientation dependent. We account for this effect in the linesidth calculation by the following expression [10,11]

$$\Gamma_r = X_0 + X_2 \cos^2\theta' \,. \tag{3}$$

The signs and absolute values of X_0 and X_2 depend on the spin label used and the polarity of the local surrounding of the spin label.

For the simulation of a random distribution system previously studied [2,3], we performed spectral integrations over the angular variables γ and η. However, the angle γ is now replaced by ϑ according to Eqn. 1. Consequently, the integrations are now made in terms of ϑ and η by variation within the limits $0° \leqslant \vartheta \leqslant 90°$ and $0° \leqslant \eta \leqslant 180°$ with increments 2.5° and 10°, respectively. Each spectrum is weighted by Eqn. 2 with given parameters $\bar{\vartheta}$ and ϑ_0.

With these extensions of the model, the spectral simulations are performed on an IBM 360/75 computer equipped with a Calcomp X-Y plotter. The simulated spectra on a plotting sheet are scaled according to the maximum peak-to-peak height. This enables a direct comparison by placing simulated and experimental spectra on top of each other.

The g- and T-tensor components are defined in a molecular reference frame as shown in Fig. 1, which is identical with the coordinate system applied in ref. 3 for description of the model for analysis of ESR spectra of spin labels with restricted motion in lipid bilayers. (Note however that we have now reversed the x-axis to obtain a right handed system.) In our previous communication [3] we reported analysis of ESR spectra from cholestane spin label in sonicated vesicles of egg yolk phosphatidylcholine. A set of g and T values was defined from measurements (g_{yy} and T_{yy}) and best fit criteria:

$g_{xx} = 2.0083 \qquad g_{yy} = 2.0021 \qquad g_{zz} = 2.0064$

$T_{xx} = 0.56$ mT $\qquad T_{yy} = 3.42$ mT $\qquad T_{zz} = 0.56$ mT

In the present work the same set of values was found to be valid. Now g_{zz} and T_{zz} were measured directly ($\perp$ direction, $\beta_0 = 0$) and the other values were also checked at low temperatures. There was no need to use different g and T values for different samples or conditions investigated, indicating that within the detection limit of the procedure the nitroxide group of the cholestane spin label in all cases was in surroundings of equal polarity.

For a simulation of a spectral pair taken with the field parallel ($/\!/$) and perpendicular ($\perp$) to the plate, starting estimates ϕ_0' and β_0' of the motional parameters ϕ_0 and β_0 are first obtained. These parameters are the main determinants of the line splittings $2T_{/\!/}'$ and $2T_\perp'$ observed in the two directions between the zero crossings of high ($M_I = -1$) and low ($M_I = +1$) field lines. When these zero crossings are not well defined in the parallel direction the amplitude extrema are used instead. Two cases are considered:

(I) When $T_{/\!/}' > \frac{1}{2}(T_{xx} + T_{yy})$ and $T_\perp' \sim T_{zz}$, $\beta_0' = 0$ and the order parameter $S_{zz} = 1$. The value ϕ_0' is evaluated using the formula (ref. 3, Eqn. 13)

$$\sigma' = \frac{\sin 2\phi_0'}{2\phi_0'} = \frac{2T_{/\!/}' - (T_{xx} + T_{yy})}{T_{yy} - T_{xx}} \tag{4}$$

This case implies that the steroid molecule does not tumble appreciably ($\beta_0 = 0$) and only undergoes restricted rotational motion about the z-axis.

(II) When $T'_{/\!/} \leqslant \frac{1}{2}(T_{xx} + T_{yy})$ and $T'_{\perp} > T_{zz}$, the axial rotation is fully developed ($\phi'_0 = 90°$) and the tumbling motion is liberated. The preliminary values of S_{zz}, and hence β_0, are calculated by the following expression (ref. 3, Eqn. 14) for the order parameter

$$S'_{zz} = \frac{T'_{/\!/} - T'_{\perp}}{\frac{1}{2}(T_{xx} + T_{yy}) - T_{zz}} = \tfrac{1}{2}\cos\beta'_0(1 + \cos\beta'_0) \qquad (5)$$

The simulations are made for both directions with the identical set of parameters, having $\bar{\vartheta}$ and $\vartheta_0 = 0$. The correlation times used in the first pair of simulations are set to be short, $\tau < 10^{-10}$ s. Starting values of the residual linewidth parameters X_0 and X_2 are determined by the widths of the central peak ($M_I = 0$) by means of Eqn. 3. The line positions are adjusted by changing ϕ_0, β_0 and ϑ_0. Then the correlation times and the residual linewidth are varied to fit lineshape and the proportions of the derivative amplitude. Again, ϕ_0, β_0 and ϑ_0 are adjusted, etc.

The calculation of the lineshape function is based on a combination of the Lorentzian and Gaussian type functions. In the simulations, we adjust the composition ratio according to the shapes of the outermost lines (i.e. $M_I = \pm 1$ components). It is noted that one and the same ratio Gaussian/Lorentzian = 1/4 gave the best result throughout this study.

For the analysis of spectra of cholestane spin label in lipid vesicles it was found [3] that some of the parameters in a sense were interdependent of each other, i.e. for small variations they have similar, but not identical overall effects on the spectra. The interdependence is considerably less pronounced for the oriented samples where the dominant effects of different parameters are separated in the two main directions. Thus it is most important and significant that ϕ_0 and τ_a (or τ_{at}) dominate in the parallel direction and β_0 and τ_t in the perpendicular direction.

It is difficult to give precise values of the accuracy of the values compiled in Tables I—III. However, the effects of variation of parameters in the simulation procedure reveal that a change in τ_t or τ_{at} (τ_a) by more than 15% or a change of β_0 (ϕ_0) by more than 2—5° (depending on the value of β_0 (ϕ_0)) led to less satisfactory fit. The Arrhenius graphs (examplified in Fig. 4) and the results at the three microwave frequencies (Table III) corroborate that these limits of error are not underestimates.

Results

Water content of lipid multibilayers

The amount of water adsorbed per mg of dry sample at 98% relative humidity was found to be for dipalmitoylphosphatidylcholine alone 0.17 and 0.22 mg at 25 and 50°C, respectively. For a mixture of dipalmitoylphosphatidylcholine and cholesterol (46 mol%) the corresponding amounts were 0.20 and 0.18 mg. These figures show that adsorption of water does not change drastically when going from gel to the liquid crystalline state or when cholesterol is added. Assuming 52 Å^2 per molecule of dipalmitoylphosphatidylcholine [12] and 37 Å^2 for cholesterol [5] a mean value for the thickness of the water layer of

8.6 Å is calculated. For pure dipalmitoylphosphatidylcholine we find approx. eight molecules of water per phosphatidylcholine molecule which is in agreement with the adsorption isotherms at 22°C reported by Jendrasiak and Mendible [13].

Evaluation of X-band ESR spectra of cholestane spin label in phosphatidylcholine multibilayers

Dipalmitoylphosphatidylcholine. ESR spectra of the cholestane spin label in oriented multibilayers of dipalmitoylphosphatidylcholine containing 0, 30 and 50 mol% cholesterol have been recorded in the temperature range −5 to +71°C. Spectra suitable for evaluation by simulation were most easily obtained with samples containing cholesterol. Hence we first discuss in detail the spectra from a sample containing 30 mol% cholesterol, some of which are shown in Fig. 3 by solid line.

At −5°C the spectra exhibit highly asymmetric lineshapes particularly in the // direction. In the ⊥ direction the spectrum shows a set of three narrow and closely spaced lines. At the high and low field wings there are indications of an extra component, possibly due to sample imperfections. These features were most pronounced at low humidity, low cholesterol content and low tempera-

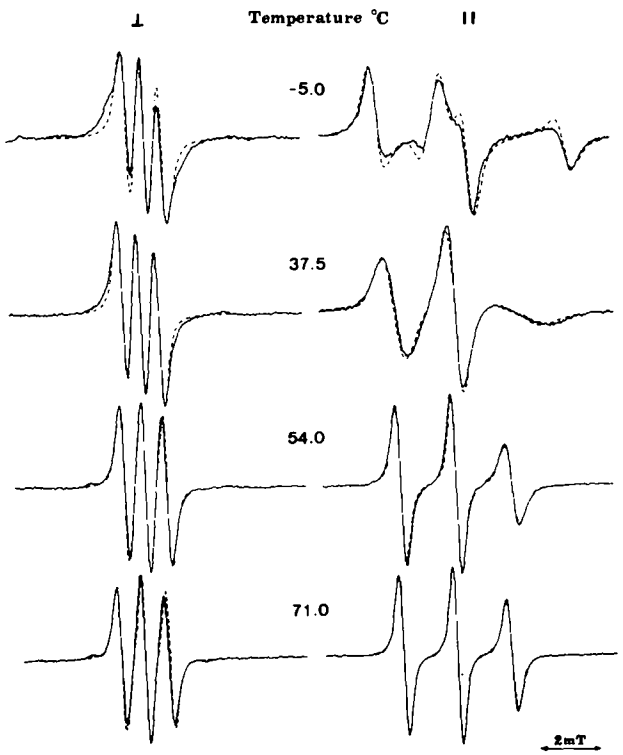

Fig. 3. Experimental (solid line) and simulated (broken line) ESR X-band spectra of the cholestane spin label (approx. 1 mol%) in oriented multibilayers of dipalmitoylphosphatidylcholine containing cholesterol (30 mol%) at various temperatures. The sample was equilibrated under the controlled relative humidity of 98% and was oriented with film plane parallel (//) are pendicular (⊥) to the magnetic field direction. The parameters used for the simulated spectra are summarized in Table I.

ture. They usually disappeared at higher temperature and were not considered for the spectral simulations.

In the ⊥ direction the measured values of hyperfine separation and g value are 0.56 mT and 2.0064, respectively, coincident with the values valid for the long axis of the probe molecules (vide supra and cf. ref. 1), which consequently must be well aligned along the bilayer normal $\underline{N}$. There is no indication of a tilted arrangement of the lipids in these experiments where cholesterol was present.

The spectrum in the // direction can only arise from a superposition of contributions from the x- and y-components of the hyperfine and g-tensors. This indicates that the orientations of corresponding axes of the spin label molecules (cf. Fig. 1) are randomly distributed within the bilayer plane.

Parameters used (Table I) for the simulated spectra (Fig. 3, broken lines) confirm that at −5°C the spin labels in the terms of the present model are completely aligned with no tumbling motion, $\beta_0 = 0$, and only perform a restricted rapid axial rotation with $\phi_0 = 30°$. When temperature increases from −5 to approx. 40°C the value of ϕ_0 gradually increases from 30° to 90°. In this temperature range, where the lipids are supposed to be in the gel phase, there is no evidence for a tumbling motion of the cholestane probe molecules, $\beta_0 = 0$ and $S_{zz} = 1$. In the higher temperature region from approx. 40 to 71°C the spectra are more symmetric about the baseline. The rapid axial rotation is fully developed ($\phi_0 = 90°$) and with increasing temperature the tumbling motion is gradually liberated, its amplitude β_0 increases and the order parameter S_{zz} decreases. ^{2}H NMR has also indicated comparatively high molecular order parameters in lamellar dispersions of dipalmitoyl as well as egg phosphatidylcholine, contain-

TABLE I

PARAMETERS USED FOR THE SIMULATION OF X-BAND SPECTRA FROM THE CHOLESTANE SPIN LABEL IN MULTIBILAYERS OF DIPALMITOYL PHOSPHATIDYLCHOLINE AT VARIOUS TEMPERATURES

Line shape function used for simulations is a mixture of Lorentzian and Gaussian shapes in a ratio of $\frac{4}{1}$: 1. See Eqn. 3 about X_0 and X_2. The effective velocity is related to the various correlations times by $\tau_{eff}^{-1} = \Sigma\tau^{-1}$.

Cholesterol concentration (mol%)	Temperature (°C)	Correlation times (ns)				Motional angles (degree)		Spread angle (degree)	Residual linewidth parameters (mT)		Order parameter
		τ_a	τ_t	τ_{at}	τ_{eff}	ϕ_0	β_0	ϑ_0	X_0	X_2	S_{zz}
0	45.5	—	1.0	0.4	0.29	90	35	5.0	0.45	−0.02	0.75
0	54.0	—	0.8	0.3	0.22	90	40	5.0	0.42	0.00	0.68
0	62.5	—	0.6	0.2	0.15	90	43	5.0	0.40	0.00	0.63
0	71.0	—	0.5	0.2	0.14	90	46	5.0	0.40	0.00	0.59
30	−5.0	5.0	—	—	5.0	30	0	5.0	0.45	−0.15	1.00
30	11.5	4.0	—	—	4.0	35	0	5.0	0.45	−0.15	1.00
30	28.5	3.0	—	—	3.0	40	0	5.0	0.45	−0.15	1.00
30	37.5	2.0	—	—	2.0	70	0	5.0	0.45	−0.12	1.00
30	45.5	—	3.0	1.0	0.75	90	20	5.0	0.35	−0.05	0.91
30	54.0	—	2.0	0.4	0.33	90	26	6.0	0.30	−0.02	0.85
30	62.5	—	1.5	0.3	0.25	90	30	6.5	0.29	−0.02	0.81
30	71.0	—	1.0	0.2	0.17	90	33	7.5	0.28	−0.02	0.77

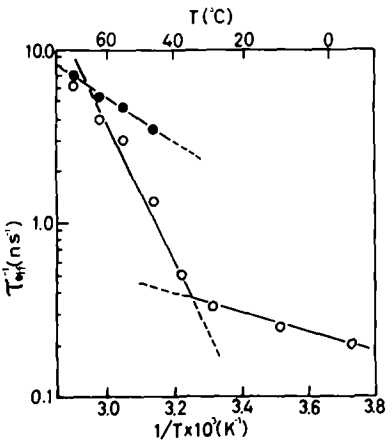

Fig. 4. Arrhenius graph of the reciprocal correlation time τ_{eff}^{-1} from the simulation (Table I) vs. reciprocal temperature for the cholestane spin label in oriented multibilayers of dipalmitoylphosphatidylcholine containing 30 mol% (○———○) and 0 mol% (●———●) cholesterol.

ing cholesterol. Specifically deuterated cholesterol [14,15] and intercalated fatty acids [15] were employed.

The correlation time continuously decreases with increasing temperature, as shown in Table I. In Fig. 4 the effective velocity $\tau_{eff}^{-1} = \Sigma\tau^{-1}$ (cf. Table I and ref. 3) is plotted in an Arrhenius graph. The points can be fitted with two lines of different slopes and an intersection at approx. 35°C. The break indicates a phase transition at this temperature within the multibilayer of dipalmitoyl phosphatidylcholine and cholesterol in molar proportions 7 : 3. It should be noted that τ_a and τ_{at} are the main determinants of the slopes and only minor changes are observed if τ_t is neglected. Below and above the transition temperature the activation energies are calculated to be 19 and 65 kJ/mol, respectively (see Table IV).

For pure dipalmitoylphosphatidylcholine, without any cholesterol but only with cholestane spin label added, it was possible to simulate the experimental spectra only in the temperature region 45—75°C, i.e. above the phase transition of dipalmitoylphosphatidylcholine (Table I). The spectra below this temperature range exhibited complicated line shapes with an anomalous line broadening. Introduction of a tilt angle ϑ according to Eqn. 2 in the simulation did not improve the results.

The Arrhenius graph for the motion of cholestane spin label in pure dipalmitoylphosphatidylcholine (Fig. 4) indicates a linear change in the temperature range 45—75°C with an activation energy of 25 kJ/mol (Table IV).

The temperature variations of the apparent hyperfine separations $T'_\perp$ and $T'_\parallel$ for the various cholesterol concentrations (0, 30 and 50 mol%) are given in Figs. 5A and 5B. The $T'_\perp$ values are measured as half the distance between the zero crossing points of $M_I = \pm 1$ components for the spectra obtained with the magnetic field perpendicular to the bilayers. The $T'_\parallel$ are taken as half the distance between the maximum and minimum extrema of the peaks for the same components in the // direction. The latter parameter is a proper measure of the hyperfine splitting $T_\parallel$ only in the lower temperature range, where there are no

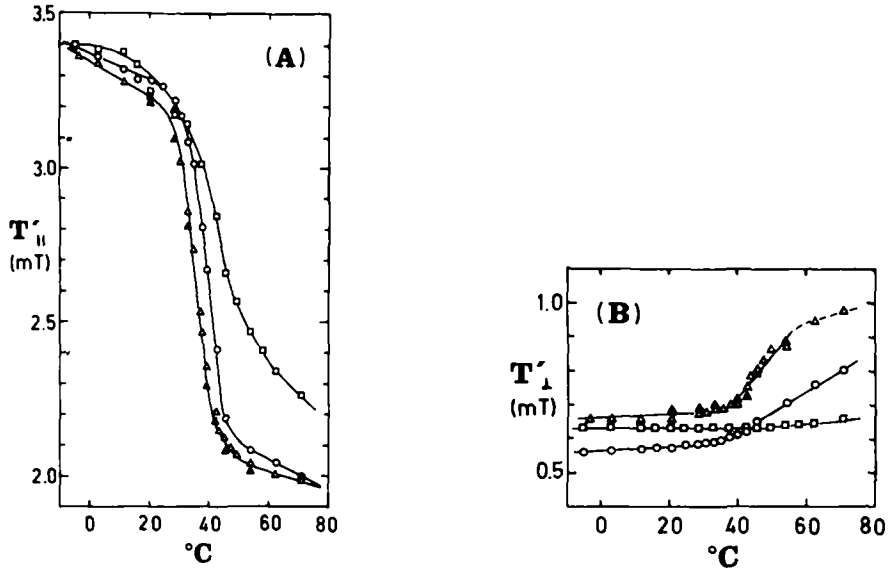

Fig. 5. (A, B) Temperature variation of apparent hyperfine separations $T'_{/\!/}$ (A) and $T'_{\perp}$ (B) measured from the ESR spectra of cholestane spin label in oriented multibilayers of dipalmitoylphosphatidylcholine with varying contents of cholesterol: △,▲, 0 mol%; ○, 30 mol%; □, 50 mol%. For 0 mol% cholesterol present points for descending (△) as well as ascending (▲) temperatures are indicated. $T'_{/\!/}$ is half of the distance between the derivative peaks at high and low field extrema of spectra taken in the parallel direction. $T'_{\perp}$ is half of the distance between the high and low field zero crossings of spectra taken in the perpendicular direction.

well defined zero crossings (see e.g. the spectrum at −5°C in Fig. 3) but was used for all spectra because it could be measured in a continuous manner throughout the whole temperature range*.

For pure dipalmitoylphosphatidylcholine and also with 30 mol% cholesterol added the value of $T'_{/\!/}$ (Fig. 5A) decreases slowly and approximately linearly with increasing temperature in the low temperature region of −5 to 20°C as well as in the high temperature region of 50—70°C. The two levels of the splitting are, however, quite different and there is a steep change in the intermediate temperature region. For a cooperative transition, as that from the gel to the liquid crystalline state, the temperature for which the measured value is half way between the two levels should approximate the transition point [16,17]. The transition temperatures thus estimated are 36 and 39°C for 0 and 30 mol% cholesterol, respectively. In the case of 50 mol% cholesterol both the levels of $T'_{/\!/}$ and the change between them are ill defined and no transition point could be determined.

The splitting $T'_{\perp}$ shows a different temperature dependence (Fig. 5B). Below the transition point its value is independent of temperature and above the transition it increases in an approximately linear fashion. The crossing point between the two lines gives a value of the transition temperature. For 0 and 30

* In this way the values of $T'_{/\!/}$ at high temperatures will be too large and approximately equal to $T_{/\!/}$ + ½ΔH, where ΔH is the mean linewidth. The estimated transition temperatures might be slightly too high.

mol% cholesterol we obtain 36 and 35°C, respectively, whereas for 50 mol% cholesterol the change in slope is too small to give any accurate value.

Below the transition point there is no definite effect on $T'_{\perp}$ upon addition of cholesterol. Above the transition point increasing proportion of cholesterol gradually depresses the temperature dependence.

Dimyristoyl phosphatidylcholine. The analysis of the ESR spectra from the cholestane spin label incorporated into oriented multibilayers of dimyristoyl phosphatidylcholine gave results analogous to those with dipalmitoyl phosphatidylcholine. The Arrhenius graph based on the correlation times derived from the simulations shows for a sample with 30 mol% cholesterol a transition point at 26°C and the activation energies 17 and 47 kJ/mol below and above the transition point, respectively. The temperature variation of the splittings $T'_{/\!/}$ and $T'_{\perp}$ indicate a transition point at 25°C both with 0 and 30 mol% cholesterol (Table IV).

Egg yolk phosphatidylcholine. The temperature variation of the X-band ESR spectra from the cholestane spin label incorporated into multibilayers of pure egg yolk phosphatidylcholine without cholesterol and with 30 mol% cholesterol added was studied from 25 to 60°C. In this temperature range egg yolk phosphatidylcholine bilayers are known to be in the liquid crystalline state. A recent Raman spectroscopic study [18] has revealed a transition at −11 ± 2°C. Examples of the spectra are shown in Fig. 6. The basic features are similar to those of the spectra obtained with dipalmitoylphosphatidylcholine and dimyristoyl phosphatidylcholine above their transition temperatures. The simulated spectra (Fig. 6, broken line) agree well with the experimental ones. The param-

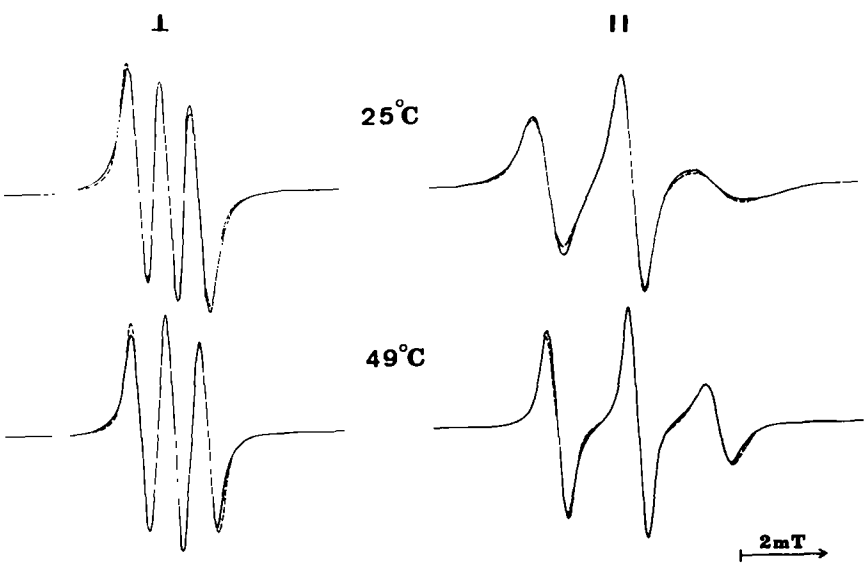

Fig. 6. Temperature variation of experimental (solid line) and simulated (broken line) ESR X-band spectra of the cholestane spin label in oriented multibilayers of egg yolk phosphatidylcholine with 30 mol% cholesterol. The sample was equilibrated under a controlled relative humidity of 98% and was oriented parallel (//) and perpendicular (⊥) to the magnetic field direction. Parameters used for the simulations are as shown in Table II..

TABLE II

PARAMETERS USED FOR THE SIMULATIONS OF X-BAND SPECTRA FROM THE CHOLESTANE SPIN LABEL IN EGG YOLK PHOSPHATIDYLCHOLINE MULTIBILAYERS

Besides the parameters listed below the following common parameters were employed: $\phi_0 = 90$, $\vartheta_0 = 5.0$, $\bar{\vartheta} = 0.0$ (degrees). The composite ratio of Lorentzian and Gaussian line shape function was 4 : 1. See Eqn. 3 about X_0 and X_2.

Cholesterol concentration (mol%)	Temperature (°C)	Correlation times (ns)			Motional angle (degree)	Residual linewidth parameters (mT)		Order parameter
		τ_t	τ_{at}	τ_{eff}	β_0	X_0	X_2	S_{zz}
0	25.0	8.00	1.20	1.04	37	0.40	−0.05	0.72
0	30.0	6.00	0.80	0.70	40	0.36	−0.04	0.68
0	40.0	4.00	0.50	0.44	43	0.33	−0.03	0.63
0	47.0	3.00	0.40	0.35	45	0.31	−0.02	0.60
0	54.0	2.00	0.35	0.29	48	0.30	−0.02	0.56
30	25.0	8.00	2.00	1.60	19	0.45	−0.08	0.92
30	29.0	6.00	1.30	1.07	24	0.40	−0.06	0.87
30	39.0	4.00	0.90	0.73	27	0.35	−0.05	0.84
30	49.5	3.00	0.60	0.50	30	0.34	−0.03	0.81
30	58.0	2.00	0.40	0.33	35	0.31	−0.02	0.75

eters for best-fit are presented in Table II. The correlation times result in linear Arrhenius graphs which correspond to an activation energy of 36 kJ/mol, both in the absence and the presence of cholesterol.

Evaluation of K- and Q-band ESR spectra of cholestane spin label in egg yolk phosphatidylcholine multibilayers

Comparison between X-, K- and Q-band spectra. Comparison of results at the three microwave frequencies had to be done at ambient temperature (23°C) since the K-band spectrometer was not equipped with temperature control. For these experiments we used egg yolk phosphatidylcholine since at this temperature it is in the liquid crystalline state and the spin label molecule performs both modes of motion, $\phi_0 = 90°$ and $\beta_0 > 0°$. One and the same sample specimen was measured at all three frequencies. Hence the small sample supporting plates for Q-band had to be used and it was a particular advantage that egg yolk phosphatidylcholine easily formed well oriented multibilayers both in the absence and in the presence of cholesterol (30 mol%). The ESR spectra recorded at the three frequencies are presented in Fig. 7 (full lines).

Spectral simulations were carried out independently at all three frequencies. Only the starting estimates of ϕ_0 and β_0 were the same and those determined at X-band. The best fit spectra are shown in Fig. 7 (broken lines) and the corresponding parameters in Table III. It should be noticed that the parameters derived from the X-band spectrum at 23°C (Table III) deviate somewhat from the corresponding value in Table II (25°C). The differences may be taken to illustrate the variation of results for different samples. Table III is based on experiments on a Q-band specimen.

Temperature effects on Q-band spectra. At Q-band the multibilayers of egg yolk phosphatidylcholine without and with 30 mol% cholesterol were investigated over the temperature range 23—60°C. The spectra are examplified in Fig.

TABLE III

SIMULATION PARAMETERS USED FOR ORIENTED (Q-BAND SPECIMEN) EGG YOLK PHOSPHATIDYLCHOLINE MEASURED AT VARIOUS MICROWAVE FREQUENCIES AND TEMPERATURES

Superposition of Lorentizian and Gaussian line shapes in a ratio 4 : 1.

Cholesterol concentration (mol%)	Temperature (°C)	Resonance frequency (GHz)	Correlation times (ns)			Motional angles (degree)		Residual linewidth parameters (mT)		Order parameter	
			τ_t	τ_{at}	τ_{eff}	β_0 ($\perp$)	β_0 ($\parallel$)	X_0	X_2	S_{zz} ($\perp$)	S_{zz} ($\parallel$)
0	23.0	9.5	8.0	1.50	1.26	30	30	0.37	−0.04	0.81	0.81
0	23.0	24.1	8.0	1.50	1.26	30	30	0.35	−0.03	0.81	0.81
0	23.0	35.0	8.0	1.50	1.26	30	30	0.35	−0.03	0.81	0.81
0	40.0	35.0	4.0	0.65	0.56	38	46	0.30	−0.02	0.70	0.59
0	60.0	35.0	2.0	0.25	0.22	43	50	0.28	−0.02	0.63	0.53
30	23.0	9.5	8.0	1.50	1.26	23	23	0.40	−0.04	0.86	0.86
30	23.0	24.1	8.0	1.50	1.26	23	23	0.37	−0.05	0.86	0.86
30	23.0	35.0	8.0	1.50	1.26	23	23	0.37	−0.05	0.86	0.86
30	40.0	35.0	4.0	0.70	0.60	30	35	0.35	−0.03	0.81	0.75
30	60.0	35.0	2.0	0.30	0.26	36	43	0.30	−0.02	0.73	0.63

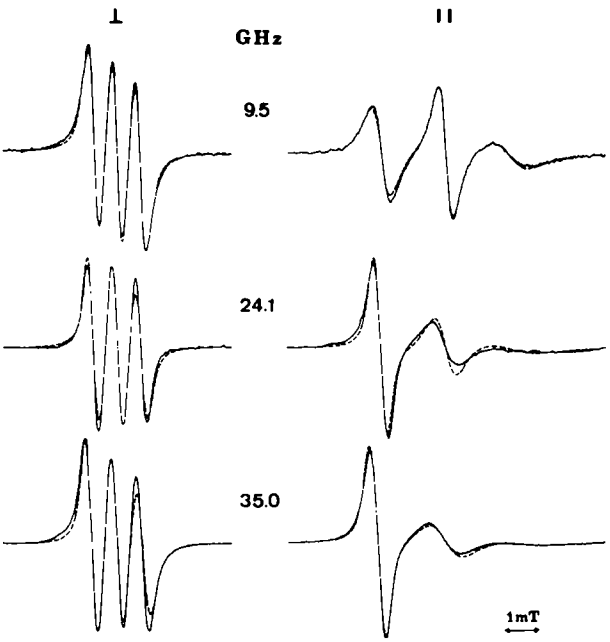

Fig. 7. Comparison of experimental (solid line) and simulated (broken line) ESR spectra of the cholestane spin label in multibilayers of egg yolk phosphatidylcholine containing 30 mol% cholesterol. The same specimen (plate for Q-band cavity size) was used in a course of measurements at three different frequencies (X-, K-, Q-band). Spectra were recorded with the magnetic field parallel (∥) and perpendicular (⊥) to the plate, at 23°C and under a controlled relative humidity of 98%. Parameters used in the simulated spectra are as shown in Table III.

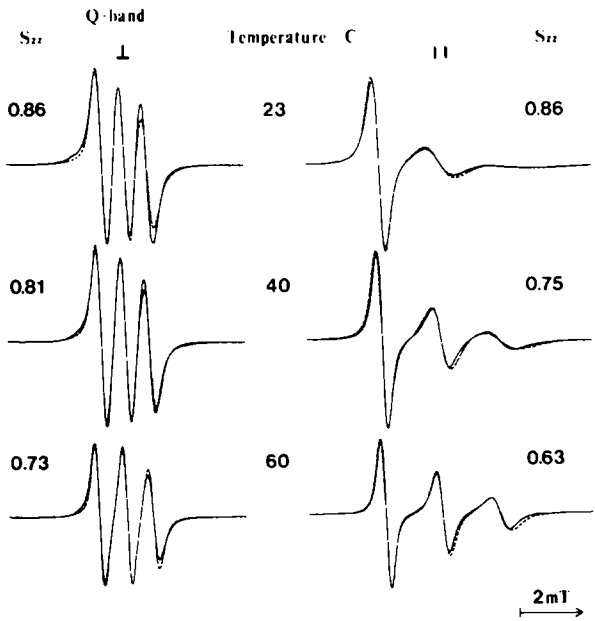

Fig. 8. Temperature (°C) variation of experimental (solid line) and simulated (broken line) ESR Q-band spectra of the cholestane spin label in oriented multibilayers of egg yolk phosphatidylcholine with 30 mol% cholesterol. The spectra were recorded with magnetic field parallel (∥) and perpendicular (⊥) to the sample plate under a controlled relative humidity of 98%. Parameters used in the simulation are found in Table III.

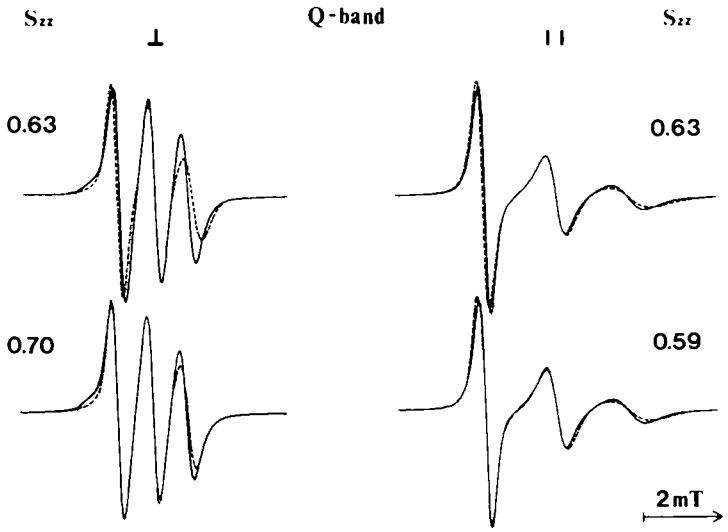

Fig. 9. Demonstration of magnetic field effect as revealed by the experimental (solid line) and simulated (broken line) ESR Q-band spectra of the cholestane spin label in oriented multibilayers of egg yolk phosphatidylcholine at a temperature of 40°C. The upper pair of simulated spectra were calculated with identical order parameter. In the lower row, however, the spectra were simulated with different order parameters in the magnetic field parallel (//) and perpendicular (⊥) to the sample plate.

8 for the case with cholesterol and in Fig. 9 without cholesterol (40°C). The simulation parameters for both types of sample are found in Table III.

In the Arrhenius graph the correlation times for the two samples practically coincide on one and the same line corresponding to an activation energy of 37 kJ/mol.

For each sample at 23°C the pair of Q-band spectra in the // and ⊥ directions could be simulated with a single set of parameters. As shown in the previous sections this was also true for pairs of X-band spectra of all samples at all temperatures. For the Q-band spectra of egg yolk phosphatidylcholine above 40°C this was, however, not possible. A single best-fit set of parameters for a pair of spectra invariably lead to simulated spectra with hyperfine separations which were too large compared with those of the experimental spectra. This is depicted in the upper part of Fig. 9. It was found that the only way to obtain satisfactory agreement between simulated and experimental spectra was to relax the requirement of a constant cone opening β_0 (order parameter S_{zz}) to accomodate the influence of a strong magnetic field while keeping all other parameters the same in the two directions. As shown in the lower part of Fig. 9 and Table III it was necessary to decrease β_0 (increase S_{zz}) in the ⊥ direction and increase β_0 (decrease S_{zz}) in the // direction.

Discussion

In order to treat the inherent dynamical features of the spectra time-dependent line shapes have to be accounted for. Several more or less sophisticated methods have been proposed and also applied to membrane studies. One way is to treat the fluctuations as small time-dependent perturbations with the mo-

tional-narrowing formalism (e.g. the Redfield theory), which has been applied in several papers [1,3,19—23]. For the motion of a nitroxide spin label the motional narrowing criterion usually sets $\tau \leqslant 3 \cdot 10^{-9}$ s as a limit for the applicability. For the motion of spin labels in lipid bilayers different models for the molecular reorientation have been considered. One approach is to use a diffusion equation [11,19—22] or the so-called jump model [23]. The most general and theoretically most satisfactory method would perhaps be to apply a stochastic treatment of the motion which is capable to accomodate a wide range of correlation times [1,24]. However, this method is so far very time demanding making compution of a large number of spectra prohibitively expensive. In our work we have, however, extended the cone model [1—3] and shown [3] that for a restricted molecular motion the motional narrowing equations may be applied for longer correlation times than would be true for isotropic systems.

Any treatment of the motion requires introduction of suitable orientational potentials for the rotations. The form of these potentials has to be assumed and their widths have to be adjusted to fit the ESR spectra. A distrubution function with a restoring potential has been applied in several cases [11,19,20,24]. In our case ϕ_0 and β_0 define suitable step function potentials. Our application of the cone model with the motional narrowing treatment should offer an alternative practical solution, the reliability of which we here have tested for well characterized lipid bilayer samples.

The comparative experiments at the three microwave frequencies X-, K- and Q-band, were made in order to test whether the model of the motion of the spin label molecule developed by Israelachvili et al. [2,3] is capable of giving consistent results under these various conditions. Fig. 7 shows that the greatest changes occur in the // direction, but there are also quite distinct changes in the $\perp$ direction, even if they are less clearly seen in the small scale reproductions.

These differences are all effects of the g-anisotropies, $\delta g = g_{xx} - g_{yy} = 0.0062$ and $\Delta g = g_{zz} - \frac{1}{2}(g_{xx} + g_{yy}) = 0.0012$, which appear in the linewidth expression in terms multiplied by the frequency (cf. ref. 3, Eqns. 19—23). It is clear from Fig. 7 that the simulated spectra quite well reproduce all these features. It is striking that the best fit parameters for each sample are practically the same at all three frequencies as shown in Table III. Only the parameters of the residual linewidth, X_0 and X_2, defined by Eqn. 3, are not identically the same at the three frequencies. The small variations of these parameters and the fact that the best fit simulations are slightly less satisfactory at K- and Q-band as compared with X-band may be attributed to the neglect of the non-secular terms, which are known to have a larger contribution at higher frequencies [25].

The present results strongly support the view that the model developed by Israelachvili et al. [3] reflects in a reasonable way the physical situation of the probe molecule in its environment. This is particularly well demonstrated by the overall fit between simulated and experimental spectra and the agreement of motional parameters at the three frequencies (Fig. 7 and Table III).

A comparison of ESR spectra from the cholestane spin label in oriented multibilayers of egg yolk phosphatidylcholine at three microwave frequencies was earlier made by Mailer et al. [23]. From inspection of their published spectra it may be concluded that their samples were less well oriented as compared to

ours. Nevertheless, the major changes with microwave frequency are still observed. Their comparison is less extensive and they only evaluated the correlation time for rotation about the long molecular axis (approximately our τ_{at}) from measurements of the peak-to-peak linewidths of the spectral lines and application of a jumping spin model.

The present simulations of Q-band spectra of the cholestane spin label in egg yolk phosphatidylcholine multibilayers recorded at temperatures above 40°C showed (Figs. 8 and 9, Table III) that β_0 is smaller in the $\perp$ than in the // direction. This means that under those conditions at a magnet field of 1.18 T there is a tendency of the molecules to align their long molecular axis with the magnetic field. Theoretical considerations suggest that this is due to magnetic anisotropy of the spin label and the cholesterol and lipid molecules in domains of suitable size. The effect becomes noticeable at high magnetic fields in a system where there is a proper balance between motional freedom and remaining cooperativity. Effects of a magnetic field on phospholipid membranes have been discussed earlier by Gaffney and McConnell [22] but not previously demonstrated as clearly as here.

The transition temperatures and the activation energies derived from the Arrhenius graphs of the correlation times and the temperature plots of the hyperfine separations for the three phosphatidylcholines investigated are compiled in Table IV. For pure dipalmitoylphosphatidylcholine a transition is found at 36°C. This is close enough to be identified with the transition from gel state to liquid crystalline state at approx. 41°C [26] and/or the pretransition at approx. 35°C [27,28]. From ^{31}P NMR it has been suggested that there is an onset of rapid axial rotation of the phospholipids at a temperature approx. 5°C below the main transition temperature [29]. Both our methods to analyze the EPR spectra from the cholestane spin label give results that clearly show that the transition persists after addition of 30 mol% of cholesterol. The transition is only gradually washed out to a more diffuse change as observed for a sample with 50 mol% cholesterol.

Also the transition at about 25°C observed for pure dimyristoylphosphatidylcholine is in close agreement with the literature value of 24°C [26,28] for the gel to liquid crystalline transition of that liquid. Again our results show that the transition persists in the presence of 30 mol% cholesterol.

It is commonly believed that mixing with cholesterol washes out such transitions making the liquid crystalline state more gel-like and vice versa already at concentrations below 30 mol%. Our results show that the position and sharpness of the transition as sensed by the cholestane spin label is hardly influenced by such concentrations of cholesterol.

In the case of both dipalmitoyl- and dimyristoylphosphatidylcholine containing 30 mol% of cholesterol there is an approx. 3-fold increase of the activation energy of the motion of the cholestane spin label when going from the gel of the liquid crystalline state. This suggests that below the transition temperature there is a fairly well defined volume for the steroid spin label to rotate within, whereas above the transition interaction with the surrounding molecules is greatly increased so that the motion is more collective in character. This is not difficult to understand since above the transition temperature the tumbling motion is liberated, a motion which must be collective in nature and

TABLE IV

COMPILATION OF ACTIVATION ENERGIES AND TRANSITION DATA OBSERVED FOR VARIOUS LIPID MULTIBILAYERS

Phospholipid	Cholesterol (mol%)	Measuring frequency (GHz)	Procedure of evaluation *	Temperature region (°C)	Activation energy ** (kJ/mol)	Transition temperature *** (°C)
Dipalmitoyl-phosphatidylcholine	0	9.5	s	45—70	25	none
	0	9.5	hfs	—10—70	—	36
	30	9.5	s	—5—35	19	35
	30	9.5	s	35—71	65	35
	30	9.5	hfs	—10—70	—	35, 39
	50	9.5	hfs	—10—70	—	diffuse
Dimyristoyl-phosphatidylcholine	0	9.5	hfs	—10—70	—	25
	30	9.5	s	7—26	17	26
	30	9.5	s	26—70	47	26
	30	9.5	hfs	—10—70	—	25
Egg yolk phosphatidylcholine	0	9.5	s	20—60	36	none
	30	9.5	s	20—60	36	none
	0	35.0	s	20—60	37	none
	30	35.0	s	20—60	37	none

* s = by means of spectral simulation; hfs = by means of plotting hyperfine separation as function of temperature (Fig. 5).

** From the Arrhenius graphs the accuracy of the activation energies is estimated to be better than ±5%.

*** The accuracy of the transition temperatures is estimated to be better than ±3°C.

also must transmit the interaction with the surroundings to the rotational motion.

For dipalmitoylphosphatidylcholine we also observe that in the liquid crystalline state one effect of addition of 30 mol% cholesterol is to increase the activation energy of the spin label motion from 25 to 65 kJ/mol. This increase in activation energy shows that cholesterol induces cooperativity of the motion in the bilayer. The change also shows that the effect of cholesterol on the motion of the spin label will be temperature dependent. At about 70°C the correlation times are approximately the same in presence and absence of cholesterol and the only apparent effect of cholesterol is the well known increase of order by decreasing β_0. At a lower temperature, 45°C, slightly above the transition point of dipalmitoylphosphatidylcholine the effect of the added 30 mol% of cholesterol is not only to increase order but also to increase the correlation times of the spin label motion by a factor of approx. 3. This is seen clearly in Fig. 4 and Table I.

Unfortunately, the activation energy could at present not be determined in case of pure dipalmitoylphosphatidylcholine in the gel state due to the complexity of the spectra, particularly in the ⊥ direction. The spectra were not completely reproducible for different specimens, indicating variation in the molecular alignment. It has been suggested that the bilayers have a domain structure [30]. Results from X-ray [27,28] as well as spin label [30,31] studies indicate that there may exist a substantial tilt for the lipids in pure dipalmitoylphosphatidylcholine below the transition temperature. None of our experimental spectra so far with dipalmitoylphosphatidylcholine in the gel state can be simulated satisfactorily by introduction of a tilt angle in the procedure. Evidence on this point from the simulations has to await experiments on improved specimens so that reliable figures for the motional and orientational parameters can be obtained.

In the case of egg yolk phosphatidylcholine the picture of the cholesterol effect is different. The correlation times and the activation energy are practically independent of the 30 mol% of cholesterol added and the only effect is on the order, viz. S_{zz} and β_0. Again, measurements were only made on samples in the liquid crystalline state. These findings are supported by studies where ^2H [32], ^{13}C [33] and ^{31}P [29] NMR have been employed. Also a recent spin probe study of heterogeneous lipids from ox brain has indicated a similar behaviour [34]. However, Mailer et al. [23] found the correlation time for the cholestane spin label to be 0.6 ns in pure egg yolk phosphatidylcholine at 22°C and to increase with addition of cholesterol being 3.4 ns at 30 mol% and 7—9 ns at 50 mol% or higher. We are unable to corroborate this and find for egg yolk phosphatidylcholine τ_{at} = 1.5 ns both at 0 and 30 mol% cholesterol. However, the cone opening β_0 is decreased by cholesterol. A correct account of this in calculating the spin jump distance ΔH used by Mailer et al. [23] will diminish the increase in correlation time with cholesterol content. It will also be of importance how well the lipid bilayers are oriented within the sample.

For dipalmitoylphosphatidylcholine we find, however, that cholesterol increases the correlation time and that this increase is temperature dependent and abolished at high temperatures. Whether there is a cholesterol dependence of this kind also for egg yolk phosphatidylcholine it should be best developed

at lower temperatures, but then the accuracy in determination of τ-values is decreased.

The activation energy of 36 kJ/mol for the motion of the cholestane spin label in the multibilayers of egg yolk phosphatidylcholine should be compared with the activation energy 32 kJ/mol previously [3] (the value stated in this reference was in error) determined for the spin label in egg yolk phosphatidylcholine dispersions. The slight difference might be ascribed to sample differences, e.g. different degrees of hydration.

The activation energies for the spin label motion in the three phospholipids investigated, with and without cholesterol, see Table IV, may be compared for the liquid crystalline state. In dipalmitoylphosphatidylcholine it is largest and in egg yolk phosphatidylcholine it is smallest. We suggest that this reflects that the cooperativity of the motion of the lipid molecules decreases in the order dipalmitoyl phosphatidylcholine > dimyristoyl phosphatidylcholine > egg yolk phosphatidylcholine. This conclusion is further supported by the values of transition temperature from gel to liquid crystalline state which follow the same order.

Our results could be compared with those of two papers [34,35] where the stochastic treatment of the molecular motion has been applied to hydrated lipid bilayers. Cannon et al. [35] measured at 6 and 45°C on the cholestane spin label incorporated into multibilayers of lipids extracted from mitochondria. For the rotation around the long molecular axis (approximately our τ_{at}) and perpendicular to this axis (approximately our τ_t) they obtained $\tau_{/\!/} = 1.5$ and 0.4 ns, and $\tau_\perp = 36$ and 10 ns for 6 and 45°C, respectively. No phase transition has been indicated for mitochondrial lipids in this temperature range. It is further known that mitochondria contain very low amounts of cholesterol. The two points give 25 kJ/mol as an approximate value of the activation energy, which is close to the values we have obtained for dipalmitoylphosphatidylcholine and egg yolk phosphatidylcholine in the liquid crystalline phase without added cholesterol, 25 and 36 kJ/mol, respectively.

Neal et al. [34] have studied the cholestane spin label in multibilayers of ox brain lipids and simulated the ESR spectra using the stochastic formalism. We have simulated one of their spectral pairs (original spectra kindly supplied by I.C.P. Smith) using the present method. For the spectra of the sample containing 10% cholesterol we required the following input parameters; those of Neal et al. [34] are included in brackets for comparison: $\tau_{at} = 2.0$ ns ($\tau_{/\!/} = 1.2$ ns), $\tau_t = 20$ ns ($\tau_\perp = 300$ ns), $S = 0.88$ (0.85). The agreement between τ_{at} and S is excellent, whereas there is a significant discrepancy between the values of τ_t and $\tau_\perp$. It should be pointed out that the definitions of τ_{at} and $\tau_{/\!/}$, and τ_t and $\tau_\perp$ are not identical, whereas that of S is identical in both cases. In addition, Neal et al. [34] chose a rigid limit value for $\tau_{/\!/}$ derived from the approximate jumping spin method of Mailer et al. [23], and the simulations should be quite insensitive to values of $\tau_{/\!/}$ in this range. We feel therefore that the results of the present simulation and that of Neal et al. [34] are in reasonable agreement. This is particularly gratifying in view of the relative simplicity of the present model.

The spin label molecule (Fig. 1) is very similar to cholesterol. The hydrophilic hydroxyl group of the latter has been replaced by the doxyl group which

is hydrophilic as well. The doxyl group is bulky, but it is rigidly attached to the steroid framework, adding to the length of rigid part of the molecule without much change of its width. There are recent results [15] suggesting that there is no complex formed between lipid and cholesterol in the type of sample investigated here. Hence the cholestane spin label may be taken to mimic the mode of intercalation and mode of motion of the cholesterol molecule in lipid bilayers.

Since the spin label group is integrated with the rigid steroid portion of the molecule it may be supposed to faithfully reflect the order of the corresponding portions of the lipid molecules. These portions are probably next to the polar head groups of the lipids. The motion of the spin label reflects how well this molecule and consequently the cholesterol molecule fits into the mosaic of the bilayer.

Acknowledgements

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References

1 Berliner, L.J. (1976) Spin Labeling Theory and Applications, Academic Press, New York
2 Israelachvili, J., Sjösten, J., Eriksson, L.E.G., Ehrström, M., Gräslund, A. and Ehrenberg, A. (1974) Biochim. Biophys. Acta 339, 164—172
3 Israelachvili, J., Sjösten, J., Eriksson, L.E.G., Ehrström, M., Gräslund, A. and Ehrenberg, A. (1975) Biochim. Biophys. Acta 382, 125—141
4 Cadenhead, D.A., Demchak, R.J. and Müller-Landau, F. (1972) Ann. N.Y. Acad. Sci. 195, 218—222
5 Demel, R.A. and de Kruyff, B. (1976) Biochim. Biophys. Acta 457, 109—132
6 Ehrenberg, A., Shimoyama, Y. and Eriksson, L.E.G. (1977) in Structure of Biological Membranes (Abrahamsson, S. and Pascher, I., eds.), pp. 119—134, Plenum Press, New York
7 Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53—56
8 Keana, J.F.W., Keana, S.B. and Beethan, D. (1967) J. Am. Chem. Soc. 89, 3055—3056
9 O'Brien, F.E.M. (1948) J. Sci. Inst. 25, 73—76
10 Goldman, S.A., Bruno, G.V., Polnaszek, C.F. and Freed, J.H. (1972) J. Chem. Phys. 56, 716—735
11 Schindler, H. and Seelig, J. (1974) J. Chem. Phys. 61, 2946—2949
12 Marsh, D. (1974) Biochim. Biophys. Acta 363, 373—386
13 Jendrasiak, G.L. and Mendible, J.C. (1976) Biochim. Biophys. Acta 424, 133—158
14 Gally, H.U., Seelig, A. and Seelig, J. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1447—1450
15 Stockton, G.W. and Smith, I.C.P. (1976) Chem. Phys. Lipids 17, 251—263
16 Hill, T.L. (1963) Thermodynamics of Small Systems, Part I, W.A. Benjamin, New York
17 Lee, A.G. (1975) Prog. Biophys. Mol. Biol. 29, 3—56
18 Mendelsohn, R., Sunder, S. and Bernstein, H.J. (1976) Biochim. Biophys. Acta 443, 613—617
19 Schindler, H. and Seelig, J. (1973) J. Chem. Phys. 59, 1841—1850
20 Hemminga, M.A. (1974) Chem. Phys. 6, 87—99
21 Hemminga, M.A. (1975) Chem. Phys. Lipids 14, 141—173
22 Gaffney, B.J. and McConnel, H.M. (1974) J. Magn. Res. 16, 1—28
23 Mailer, C., Taylor, C.P.S., Schreier-Muccillo, S. and Smith, I.C.P. (1974) Arch. Biochem. Biophys. Acta 298, 133—144
24 Polnaszek, C.F., Bruno, G.V. and Freed, J.H. (1973) J. Chem. Phys. 58, 3185—3199
25 Kivelson, D. (1972) in Electron Paramagnetic Relaxation in Liquids (Muus, L.T. and Atkins, F.W., eds.), pp. 213—273, Plenum Press, New York

26 Spiker, Jr., R.C. and Levin, I.W. (1976) Biochim. Biophys. Acta 433, 457—468
27 Rand, R.P., Chapman, D. and Larsson, K. (1975) Biophys. J. 15, 1117—1124
28 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575—4580
29 Cullis, P.R. (1976) FEBS Lett. 70, 223—228
30 Birrell, G.B. and Griffith, O.H. (1976) Arch. Biochem. Biophys. 172, 455—462
31 Hemminga, M.A. (1977) J. Magn. Res. 25, 25—45
32 Stockton, G.W., Polnaszek, C.F., Leitch, L.C., Tulloch, A.P. and Smith, I.C.P. (1974) Biochem. Biophys. Res. Commun. 60, 844—850
33 Godici, P.E. and Landsberger, F.R. (1975) Biochemistry 14, 3927—3932
34 Neal, M.J., Butler, K.W., Polnaszek, C.F. and Smith, I.C.P. (1976) Mol. Pharmacol. 12, 144—155
35 Cannon, B., Polnaszek, C.F., Butler, K.W., Eriksson, L.E.G. and Smith, I.C.P. (1975) Arch. Biochem. Biophys. 167, 508—518