The Dynamic Structure of Fatty Acyl Chains in a Phospholipid Bilayer Measured by Deuterium Magnetic Resonance†

Anna Seelig and Joachim Seelig*

ABSTRACT: Deuterium magnetic resonance of selectively deuterated lipids opens a new avenue for probing the structure of lipid membranes. The method provides quantitative information on the ordering and the motional anisotropy of the various parts of the lipid molecules without resorting to perturbing probes. This is demonstrated for nonsonicated bilayers of L-α-dipalmitoyllecithin. The lipid molecules are deuterated at nine different carbon atoms of the fatty acyl chains. The deuterium quadrupole splittings of the corresponding bilayer phases are measured yielding the following results. (1) The two fatty acyl chains are not completely equivalent physically, since they give rise to slightly different quadrupole splittings in some parts of the chain. (2) The segmental order parameters are constant for the first nine chain segments ($S_{mol} \approx 0.45$ at 41°). The order parameter decreases in the central part of the bilayer. A rise in temperature reduces the order parameter. (3) The chain ordering is explained on the basis of the rotational isomeric model for hydrocarbon chains. In the region of the constant order parameter gauche conformations can occur only in complementary pairs (*i.e.*, kink, jog), leaving the hydrocarbon chains essentially parallel to each other. This leads to a well-ordered bilayer with disordered hydrocarbon chains. The decrease of the order parameter in the central region is due to increasing contributions of gauche planes. The total number of gauche isomeric states is found to be three to six per chain. (4) The quantitative evaluation of the deuterium data yields a thickness of the hydrocarbon region of the bilayer of about 34–35 Å and a linear thermal expansion coefficient of -2.5×10^{-3} °K^{-1}, in good agreement with X-ray diffraction experiments. The linear thermal expansion coefficient can be determined for each chain segment separately. (5) The deuterium data differ from spin-label electron spin resonance experiments. This may be attributed to a perturbation of the bilayer by the spin-label group.

The motions of hydrocarbon chains in phospholipid bilayers have been investigated in detail using spin-label electron spin resonance (esr) and nuclear magnetic resonance (nmr) (Hubbell and McConnell, 1971; Jost *et al.*, 1971; McFarland and McConnell, 1971; Oldfield *et al.*, 1971; Horwitz *et al.*, 1972, 1973; Metcalfe *et al.*, 1971, 1973; Chan *et al.*, 1972, 1973; Godici and Landsberger, 1974). All studies agree qualitatively insofar as they characterize the hydrocarbon chains as less ordered and more mobile in the central part of the bilayer than close to the lipid–water interface. However, the quantitative comparison of the experimental data has been controversial for various reasons. (1) Spin-labels allow a direct measurement of the anisotropy of motion, characterized by the order parameter S_3 (Seelig, 1970). It is not known, however, to which extent the hydrocarbon chains in a bilayer are distorted by the bulky spin-label group (*cf.* Cadenhead and Müller-Landau, 1973). (2) Nmr studies have primarily been concerned with T_1 and T_2 relaxation times, which depend on the anisotropy and the rate of motion and also on the mechanism of relaxation. The evaluation of T_1 and T_2 experiments in terms of relevant molecular parameters is difficult and open to criticism. (3) In order to obtain high-resolution spectra almost all nmr studies have been performed with sonicated bilayers (vesicles). There is evidence that the organization of the hydrocarbon chains in such highly curved bilayers is different from that in planar bilayer arrangements. Unsonicated phospholipid dispersions are probably better models of membrane structure than phospholipid vesicles (Feigenson and Chan, 1974).

Recently we have demonstrated that deuterium magnetic resonance (dmr) combined with selective deuteration can provide the same straightforward information as the spin-label method, but with the additional advantage of not perturbing the system (Seelig and Niederberger, 1974a). This method can be applied to liquid crystalline phases in general and does not require sonication to produce resolved resonances. We have used deuterium magnetic resonance to study a simple liquid crystalline bilayer (Seelig and Niederberger, 1974b) and have reported preliminary results with L-α-dipalmitoyllecithin (Seelig and Seelig, 1974). Here we present a more detailed study of the motion of the fatty acyl chains in dipalmitoyllecithin (DPL)[1] bilayers. We have synthesized a series of DPL molecules, selectively deuterated at nine different positions in the hydrocarbon chains, and have measured the deuterium magnetic resonance signal of the corresponding bilayer phases. From the residual quadrupole splitting the order parameter S_{mol} of the labeled chain segment is evaluated. The results are used to calculate the average dimensions of the hydrocarbon chains and to deduce an approximate picture of the chain packing. The dmr data are also compared with spin-label esr and ^{1}H and ^{13}C nmr measurements of similar bilayer systems.

Materials and Methods

Synthesis of Selectively Deuterated L-α-*Dipalmitoyllec-*

† From the Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Klingelbergstrasse 70, Switzerland. *Received July 2, 1974.* This work was supported by the Swiss National Science Foundation Grant 3.8620.72.

[1] Abbreviation used is: DPL, L-α-dipalmitoyllecithin.

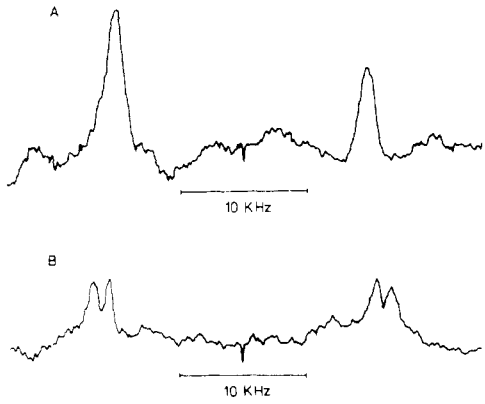


FIGURE 1: Deuterium magnetic resonance spectra of bilayers of L-α-dipalmitoyllecithin selectively deuterated in both fatty acyl chains. (A) C-10 deuterated DPL; 57°; 20,000 free induction decays. (B) C-3 deuterated DPL; 65°; 40,000 free induction decays.

ithin. Selectively deuterated palmitic acids were prepared by Kolbe electrolysis (Nguyên-Dinh-Nguyên, 1968) or by deuterium exchange followed by chain elongation (Seelig and Seelig, 1974; P. Bader and J. Seelig, to be published). The dideuteriopalmitic acids were characterized by their melting points and their infrared, proton nmr and dmr spectra. DPL selectively deuterated in both palmitic acyl chains was synthesized from glycerophosphorylcholine and dideuteriopalmitic acid anhydride according to Cubero-Robles and van den Berg (1969). The purified DPLs were characterized by thin-layer chromatography, by proton nmr and dmr, and, in part, by optical rotation measurements and elemental analysis.

Preparation of L-α-*Dipalmitoyllecithin Bilayers.* DPL bilayers were prepared by thoroughly mixing DPL (48.5 wt %) and water (51.5 wt %) and carefully heating the mixture in a sealed ampoule. The liquid crystalline bilayer phase is formed at temperatures greater than 41° (Chapman *et al.*, 1967). Approximately 500 mg of liquid crystalline phase was used for measuring powder type spectra. For the dmr experiments the ampoule was placed inside a 10-mm nmr tube.

Deuterium Magnetic Resonance Measurements. Dmr measurements were performed at 13.78 MHz with a Bruker HX-90-FT spectrometer equipped with a variable-temperature unit. The temperature unit was calibrated with a standard thermometer. Our previous DPL measurements were made with an uncalibrated instrument and the actual temperatures have now been found to be a few degrees higher than those indicated in the paper (Seelig and Seelig, 1974). The pulse width was 18 μsec (90° pulse). Proton decoupling experiments were performed with a Bruker B-FS-100 synthesizer and modulator unit. The noise modulation width was ±1.5 kHz at 3 W power. Normally 10,000–40,000 free induction decays were accumulated on a Bruker BNC-73 computer. The C-2 to C-5 deuterated DPL bilayers required 40,000–140,000 scans.

Results

Figure 1 shows dmr spectra of DPL bilayers deuterated at different carbon atoms in the fatty acyl chain. Each DPL molecule carries two CD_2 groups, which *a priori* cannot be expected to have the same quadrupole splitting. Nevertheless most samples give rise to simple spectra consisting of just two absorption peaks (Figure 1A), the separation of which is the residual quadrupole coupling $\Delta\nu$. The lines are quite broad suggesting that they are actually composed of two signals with slightly different quadrupole couplings. Two doublets are indeed resolved for some of the deuterated DPL bilayers (Figure 1B and Table I), although the differences in the quadrupole splittings are not large. The situation is even more complicated for C-2 deuterated DPL (*cf.* Seelig and Seelig, 1974). This indicates that the two fatty acyl chains are not completely equivalent. Due to conformational twisting in the vicinity of the polar region the CD_2 groups of the two fatty acyl chains may be positioned at slightly different distances from the lipid–water interface, experiencing different fluctuating movements. In order to clarify this problem we are at present synthesizing deuterated DPL molecules labeled in one fatty acyl chain only.

TABLE I: Quadrupole Splittings and Order Parameters for Bilayers of L-α-Dipalmitoyllecithin.

Labeled Carbon Atom	Quadrupole Splitting $\Delta\nu$ [kHz][a]			Order Parameter S_{mol}[b]		
	41°	50°	57°	41°	50°	57°
C-2	30.1	27.2	26.2	0.47	0.43	0.41
	23.2	19.2	17.0			
	14.2	12.0	12.1			
C-3	27.3	26.2	23.9	0.43	0.39	0.36
		23.2	22.2			
C-4	30.3	27.2	26.0	0.47	0.43	0.41
C-5	29.5	26.2	24.2	0.46	0.41	0.38
C-9	29.7	24.2	21.6	0.45	0.38	0.35
	27.7					
C-10	27.7	22.9	20.3	0.43	0.36	0.32
	27.0					
C-12	22.3	18.3	15.4	0.35	0.28	0.24
		17.0				
C-14	19.8	14.9	12.1	0.30	0.22	0.19
	17.8	13.3				
C-15	14.9	11.1	9.7	0.21	0.16	0.14
	12.3	9.7	8.4			

[a] Experimental error ±0.15 kHz. [b] For C-3 and C-9–C-15 average value of both splittings.

From the residual quadrupole splitting $\Delta\nu$ the order parameter S_{CD} of the C–D bond can be calculated according to

$$\Delta\nu = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

The deuteron quadrupole splitting constant (e^2qQ/h) has been found to be 170 KHz for paraffin chains (Burnett and Muller, 1971). The average orientation of the CD bond is essentially perpendicular to the bilayer normal; hence it is reasonable to assume that S_{CD} is negative. S_{CD} can be approximately related to the segmental order parameter S_{mol} (Seelig and Niederberger, 1974a).

$$S_{mol} = -2S_{CD} \quad (2)$$

The segment direction is given by the normal to the plane spanned by the two CD bonds of a methylene unit. With this definition the orientations of the individual chain segments will coincide with the long molecular axis if the chain is frozen in the extended all-trans conformation. The experimental quadrupole splittings and the corresponding order parameters for different label positions are summarized in

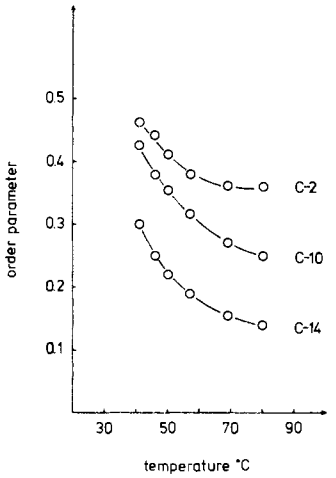


FIGURE 2: Temperature dependence of the order parameter S_{mol} of DPL bilayers labeled at three different carbon atoms of the fatty acyl chains.

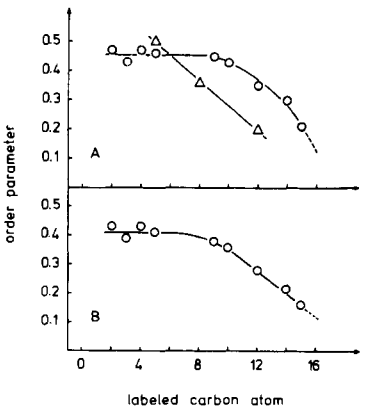


FIGURE 3: Order parameter S_{mol} of DPL bilayers as a function of the position of the labeled carbon atom. (A) 41°; (○) deuterium data (48.5 wt % DPL; 51.5 wt % H_2O); (△) spin-label data (20 wt % DPL; 80 wt % H_2O) (Hubbell and McConnell, 1971). (B) 50°C; (○) deuterium data (48.5 wt % DPL; 51.5 wt % H_2O).

Table I for 41, 50, and 57°. For some bilayer systems we have measured the temperature dependence of the quadrupole splitting for temperatures up to 80°. Representative results are shown in Figure 2. Below 41°, that is below the liquid crystalline → gel phase transition point, the resonance signals become too broad to be detectable with our instrument.

The line width at half-height of the dmr resonances in the liquid crystalline phase is about 1-2 kHz, but the quality of the spectra is as yet insufficient to allow a precise quantitative evaluation. Proton decoupling which produces a considerable sharpening of dmr resonances in simple liquid crystalline bilayers (Diehl and Niederberger, 1974) has no effect in DPL bilayers. This behavior can be explained by the different rates of motion in the two systems. Due to the higher viscosity of DPL bilayers the intrinsic line width seems to be determined mainly by quadrupole relaxation, while in soap-like systems the dominant contribution comes from unresolved proton-deuteron couplings.

Discussion

In oriented samples of C-5 deuterated DPL bilayers the quadrupole splitting was found to collapse if the bilayers were inclined at the magic angle with respect to the magnetic field (Seelig and Seelig, 1974). It was concluded that the axis of motional averaging was identical with the bilayer normal and that the average orientation of the hydrocarbon chains was perpendicular to the plane of the bilayer (at least from C-5 to C-16). The order parameters S_{mol} can then be interpreted in terms of a simple geometric picture. If θ denotes the momentary angle between the direction of a chain segment and the bilayer normal then S_{mol} is related to the *average* orientation according to

$$S_{mol} = (\tfrac{1}{2})(3\langle\cos^2\theta\rangle - 1) \qquad (3)$$

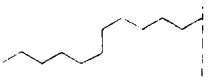
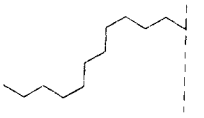
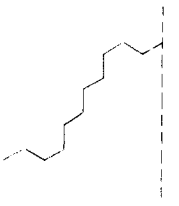
where the angular brackets indicate the time average. In Figure 3 the order parameter S_{mol} is plotted as a function of the position of the labeled carbon atom for 41 and 50°. These data are intimately connected with the conformational properties of the fatty acyl chains and reflect the chain flexibility in the bilayer. Also included in Figure 3 are spin-label esr results on DPL bilayers at 41° (Hubbell and McConnell, 1971).

Conformational Dynamics of Fatty Acyl Chains. The angular fluctuations arise essentially from rotational isomerisations around carbon-carbon bonds. The rotations are subject to the following constraints. (1) As a consequence of the bond rotation potential, carbon-carbon bonds can occur in three rotational states only, conventionally denoted as trans (t) and gauche$^{\pm}$ (g$^{\pm}$) (*cf.* Flory, 1969). For a free chain the average conformation is determined by the energy difference between trans and gauche isomers and also by cooperative interactions between nearest neighbors. (2) In a bilayer the conformational freedom is further restricted by intermolecular interactions (van der Waals attraction, steric repulsion) between adjacent hydrocarbon chains. Only those conformations are probable which do not distort the parallel packing of the chains too much and thus do not create too large a defect volume.

The statistical-mechanical analysis of intra- and intermolecular forces in lipid bilayers requires the application of cooperative models (Adam, 1973; Nagle, 1973; Marčelja, 1974; Bothorel *et al.*, 1974). The deuterium data may serve as a convenient starting point to test the theoretical predictions of the various models. This has already been done quite successfully (Marčelja, 1974) for the previously described soap-like bilayer. However, the order parameter S_{mol} can provide fairly accurate information on some aspects of the bilayer structure even without resorting to sophisticated cooperative theories. The argument is based on essentially geometric considerations of the chain structure, since the order parameter is related directly to the average chain geometry. In Table II various fatty acyl chain conformations are listed which are in accordance with the rotational isomeric model (*cf.* also Pechhold, 1968). If the direction of the first segment is defined as the direction of the bilayer normal, then Table II illustrates that only two segment orientations are observed in all the conformations listed. The segments are either aligned parallel to the bilayer normal (henceforth called A segments) or they are inclined at an angle of 60° (B segments). This is even better revealed by inspection of molecular models. Table II does not include conformations containing g$^{\pm}$ g$^{\mp}$ or g$^{\pm}$ g$^{\pm}$ sequences. The former are forbidden even in a free chain (*cf.* Flory, 1969), the latter lead to a bending of a straight chain perpendicular to the bilayer normal. This orientation is energetically unfavorable in most parts of the bilayer except perhaps in the region of the terminal methyl group.

The various conformations are in a rapid dynamic equilibrium. The jump frequency for transitions between the rotational states is much faster than the time scale of the dmr

TABLE II: Chain Conformations in a Bilayer.

Structural Defect[a]	Conformation	Chain Skeleton
All-trans	t t t t t t t t t t t	
2g1 kink	t t t t g^+ t g^- t t t t t t t t g^- t g^+ t t t t	
2g2 jog	t t t g^+ t t t g^- t t t t t t g^- t t t g^+ t t t	
3g2	t t t g^+ t g^+ t g^+ t t t t t t g^- t g^- t g^- t t t	
2g3 jog	t t g^+ t t t t t g^- t t t t g^- t t t t t g^+ t t	
3g3	t t g^+ t t t g^+ t g^+ t t t t g^- t t t g^- t g^- t t t t g^+ t g^+ t t t g^+ t t t t g^- t g^- t t t g^- t t	
4g3	t t g^+ t g^+ t g^- t g^- t t t t g^- t g^- t g^+ t g^+ t t	

[a] The first number gives the total of gauche conformations. The second number indicates the shortening of the chain in units of l = 1.25 Å compared to the all-trans conformation (Pechhold, 1968).

experiment, and only the time average of A and B orientations is detected. Let us denote the probabilities of a segment being in state A or B with p_A and p_B, respectively. Since other orientations have been neglected it follows

$$p_A + p_B = 1 \quad (4)$$

The time average of the various orientations is thus given by

$$S_{mol} = p_A(\tfrac{1}{2})(3\cos^2 0° - 1) + p_B(\tfrac{1}{2})(3\cos^2 60° - 1) \quad (5)$$

With eq 4 it then follows

$$p_B = (1 - S_{mol})/1.125 \quad (6)$$

It should be noted that p_B is *not* identical with the probability of carbon–carbon bonds being in the gauche isomeric state. As is obvious from Table II, trans isomers can also be inclined to the bilayer normal, as for example in 2g2 or 2g3 jogs.

In deriving eq 6 we have neglected the possibility of rigid body motions of the DPL molecules. This type of motion would decrease the order parameter of *all* segments by the same factor S_0. In simple liquid crystalline bilayers S_0 was estimated to be 0.7–0.8 (Seelig and Niederberger, 1974a; Niederberger and Seelig, 1974). The chain length of a DPL molecule is almost twice that of the previous soap-like molecules and the viscosity of DPL bilayers is also larger by a factor of 10 compared to our previous system. Therefore concerted fluctuations of large amplitude and high frequency are even less likely in DPL bilayers and S_0 can be expected to approach unity. The neglect of S_0 seems not to be too serious a deficiency in our model. Let us then apply this model to investigate some bilayer properties.

Bilayer Thickness. The knowledge of p_A and p_B for each

chain segment can be utilized to determine the average thickness of the hydrocarbon region. For a segment in the A state the projected length on the bilayer normal is l = 1.25 Å, which corresponds to the *effective* length of a carbon–carbon bond in an all-trans chain. Alternatively the projected length in the B state is l cos 60°. The average length $\langle l_i \rangle$ of the ith segment is thus

$$\langle l_i \rangle = p_{iA} l + p_{iB} l \cos 60° = l(1 - 0.5 p_{iB}) \quad (7)$$

The total length $\langle L \rangle$ of a DPL hydrocarbon chain is then given by

$$\langle L \rangle = \sum_{i=1}^{15} \langle l_i \rangle = l\left(15 - 0.5 \sum_{i=1}^{15} p_{iB}\right) \quad (8)$$

Using eq 6 and the results of Figure 3B the average length is found to be $\langle L \rangle$ = 10.52 l = 13.15 Å at 50°. The same fatty acyl chain in the all-trans form would measure 15 l = 18.75 Å. The difference between these two conformations is 5.6 Å. The deuterium data together with our simple model therefore predict that the DPL bilayer in the liquid crystalline state should be approximately 11.2 Å shorter than the same bilayer with completely extended chains. This result can be compared with X-ray diffraction experiments. For DPL phases with 5% water content, electron density profiles have been calculated for temperatures below and above the phase transition (Cain *et al.*, 1972; Figure 1). The separation of the peaks of highest electron density, marking the positions of the polar groups, decreases from 48.3 Å at 35° to 36.7 Å at 49°. The difference of 11.6 Å is very close to our calculation. For DPL at 50% water content only the Bragg reflections are available (Chapman *et al.*, 1967; Figure 9). It is found that upon heating the sample through the phase transitions the lamellar spacing decreases from 64.0 to 56.2 Å. The thickness of the water layer and the polar region remain approximately constant and the difference of 7.8 Å can be attributed to a shortening of the lipid chains. In the gel phase with 50% water content the hydrocarbon chains are tilted with respect to the bilayer normal by an angle of 27° (Chapman *et al.*, 1967). This already reduces the effective bilayer thickness (that is, the separation of the glycerol groups across the hydrocarbon region) by 4.1 Å. The total reduction for the transition from a nontilted bilayer with extended chains to a liquid crystalline bilayer is thus again 11.9 Å. The thickness of the hydrocarbon region therefore decreases from 45.9 to about 34 Å. A thickness of 34–36 Å has also been determined for the hydrocarbon region of egg-yolk lecithin bilayers (Levine and Wilkins, 1971).

All X-ray experiments independently measure a shortening of the hydrocarbon region by 11–12 Å. The analysis of the deuterium order parameters leads to virtually the same result. We therefore conclude that our simple model is a useful first-order approximation to the chain geometry. In Figure 4 the shortening of the bilayer is illustrated by introducing into a straight hydrocarbon chain some of the conformations listed in Table II. Figure 4 must be regarded as a rather simplified picture since it represents only one out of many other possible conformations. The conformational distortions are presumably formed by a concerted rotation of adjacent chains. This minimizes the occurrence of energetically unfavorable defect volumes (*cf.* Blasenbrey and Pechhold, 1970). The three-dimensional fluctuations in a bilayer are, however, difficult to display with molecular models.

Temperature Dependence of the Hydrocarbon Chain Ordering. Figure 2 shows that a rise in temperature reduces the order parameter, that is the ordering of the hydrocarbon chains. By means of the above model the average fatty acyl chain length $\langle L \rangle$ is calculated to be 13.6, 13.15, and 12.9 Å at 41, 50, and 57°, respectively. The disordering of the chains is thus accompanied by a decrease in the effective chain length. This reduction of the bilayer thickness has already been observed in X-ray experiments and has been explained in terms of a rubber-like model for the hydrocarbon chain elasticity (Luzzati and Husson, 1962; Luzzati, 1968). The deuterium data support this model and provide direct experimental evidence for the entropy elasticity of the hydrocarbon chains.

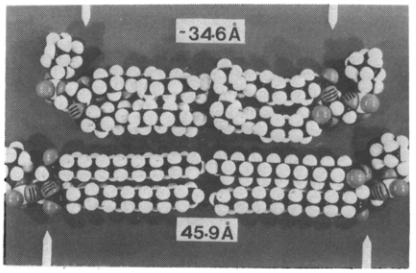


FIGURE 4: Corey–Pauling–Koltun models of bilayers. The all-trans conformation of the fatty acyl chains is compared with chains containing two 2g1 kinks and a terminal gauche plane.

The linear thermal expansion coefficient α of a bilayer is defined as

$$\alpha = \Delta d / d \Delta T \quad (9)$$

Here d is the bilayer thickness and ΔT denotes the change in temperature. For a bilayer composed of natural phospholipids α was determined by X-ray diffraction experiments as -3.0×10^{-3} °K^{-1} (Luzzati and Husson, 1962). This may be compared with the deuterium data. For a bilayer thickness of about 34 Å the expansion coefficient is given by

$$\alpha = 2\Delta \langle L \rangle / 34 \Delta T \quad (10)$$

yielding an average value of -2.5×10^{-3} °K^{-1} for the above numbers. The deuterium data are therefore in quantitative agreement with the X-ray measurements.

X-Ray diffraction detects the average expansion of the whole bilayer. Using deuterium-labeled lipids the linear expansion coefficient can now be determined for each chain segment separately. Let us define the segmental expansion coefficient as

$$\alpha_i = \Delta \langle l_i \rangle / l \Delta T \quad (11)$$

With eq 6 and 7 it then follows

$$\alpha_i = \Delta S_{mol} / 2.25 \Delta T \quad (12)$$

Using the results of Figure 2, α_i is found to range from 0 to -4.0×10^{-3} °K^{-1} depending on the temperature and the segment position investigated.

Disordered Hydrocarbon Chains in an Ordered Bilayer. The order parameter remains approximately constant for the first nine segments. This is at variance with previous spin-label results (*cf.* Figure 3) but agrees well with dmr studies of soap-like bilayers (Charvolin *et al.*, 1973; Seelig and Niederberger, 1974b). A region of constant order parameter is probably typical for bilayers containing saturated fatty acyl chains of equal chain length. Cis double bonds may exert a completely different influence. This is at present under investigation. A region of constant order parameter requires the same angular fluctuations for all segments involved. As was shown previously, this excludes the occur-

rence of isolated gauche conformations, but can only be explained by fluctuating kink- or jog-like structures (Seelig and Niederberger, 1974b). Quantitatively the observed order parameter $S_{mol} = 0.4$ (at 50°) can be accounted for by incorporating two 2g1 kinks or one 2g2 jog into the chain, but a dynamic equilibrium between the two conformations seems more realistic. The innermost region of the bilayer is characterized by a gradual decrease of the order parameter, which can be rationalized by an increasing probability of gauche states.

If the all-trans conformation of a hydrocarbon chain is referred to as the *ordered* state, and if gauche conformations are defined as *disordered,* then the fatty acyl chains in a bilayer may be called *disordered.* However, this disorder is not uniform but increases toward the methyl terminal of the chains. On the other hand, the deuterium data indicate that gauche conformations occur pairwise only (kinks, jogs) for most of the chain. This has the consequence that the hydrocarbon chains remain packed in a rather parallel fashion. Compared to an isotropic liquid or a coiled macromolecule the bilayer as a whole appears to be rather *well-ordered,* at least for the first nine segments.

The deuterium order parameters can only be interpreted if the total number of gauche states per hydrocarbon chain of DPL is larger than 2. On the other hand, assuming a minimal energy difference of 500 cal mol^{-1} between t and $g^{\pm}$ states (*cf.* Flory, 1969), it follows from Boltzmann's law that the number of gauche states must be less than 7 (at 50°). We therefore estimate 3–6 gauche isomers per chain. This is in agreement with thermodynamic estimates (Nagle, 1973; Marčelja, 1974). More accurate data on the probability of gauche states and its dependence on the segment position should become available if the deuterium data are analyzed in terms of the above mentioned cooperative models.

Order Profile and Bilayer Fluidity. Since the disorder increases in the central region, it might be thought that this part of the bilayer could be called more *fluid* than the regions close to the lipid–water interface. We feel that this conclusion, based on the order parameter alone, is not justified. Fluidity in the normal sense refers to the *rate* of motion but not to the ordering of the molecular system. No simple relations between the two quantities are known. The independence of the two parameters is illustrated by comparing the DPL bilayers with sodium decanoate–decanol bilayers (Seelig and Niederberger, 1974b; Niederberger and Seelig, 1974). In the soap-like liquid crystal the average order parameter in the constant region is $S_{mol} \approx 0.6$, while the microviscosity is found to be $\eta \approx 0.05$ P (Schindler and Seelig, 1973,1974; *cf.* also Roberts, 1973). In the DPL bilayer the viscosity is probably more than ten times larger as judged from epr diffusion experiments (Träuble and Sackmann, 1972; Devaux and McConnell, 1972), but the system is also less ordered ($S_{mol} \approx 0.4$). This demonstrates that the fluidity of the bilayer to a certain extent is independent of the order parameter and must be determined by a separate measurement. In the case of deuterium magnetic resonance this additional information could be provided by measuring T_1 or T_2 relaxation times (*cf.* Saito *et al.,* 1973).

Comparison of Dmr with Spin-Label Epr and ^1H and ^{13}C Nmr. Spin-label esr and dmr are directly comparable since both measure the angular fluctuations of the chain segments (Seelig, 1970). However, due to its lower resonance frequency, dmr may detect slower motions than spin-label epr. This would lead to a smaller deuterium order parameter because slower fluctuations of larger amplitude are averaged out. In the DPL system this time scale effect is not observed experimentally. The deuterium order parameters are generally found to be larger than the spin-label order parameters (*cf.* Figure 3A). The two types of measurements differ also qualitatively in that the spin-labels detect a continuous decrease of the order parameter. A similar difference between deuterium labels and spin-labels was also obtained for soap-like bilayers (Seelig and Niederberger, 1974b). Although the different water content of the two DPL systems may also have a small effect on the ordering, we attribute this discrepancy essentially to the perturbing influence of the spin-label group.[2] At the position of the label group the diameter of the chain is almost twice that of a normal hydrocarbon chain. The spin-label group distorts the parallel packing of the chains and thus creates free space for a more disordered molecular motion.

The perturbing effect of the spin-label group is also revealed by statistical-mechanical calculations (Belle *et al.,* 1974; Belle and Bothorel, 1974). Taking into account intra- and intermolecular interactions the melting entropies and the segmental order parameters have been calculated as a function of the chain–chain separation in the lipid bilayer. It is found that the spin-label order parameters can only be fit if the chain–chain separation is made twice as large as is sufficient for the melting entropies. Although the model is quite crude, it accurately reflects the geometrical differences between a spin-labeled and a nonlabeled hydrocarbon chain.

^1H and ^{13}C nmr techniques avoid the use of distorting probes and are well suited to sense the physical state of the bilayer lipids. Unfortunately the interpretation of T_1 and T_2 relaxation times for both the ^1H and ^{13}C nuclei in terms of molecular motions is hampered by the fact that the order parameters and the correlation times cannot be measured separately. A comparison between the deuterium measurements and previous ^1H and ^{13}C nmr measurements is therefore difficult. Qualitatively, however, our conclusions agree very well with the results of Horwitz *et al.* (1973). Quantitatively the deuterium data may be helpful in reexamining previous interpretations of ^1H and ^{13}C relaxation times and may thus contribute to resolving the controversy surrounding this subject.

Acknowledgments

The authors gratefully acknowledge Mr. P. Bader and Mr. P. Fischlewitz for their excellent technical assistance in preparing the deuterated compounds and Dr. W. Niederberger for many valuable comments. They thank Dr. S. Marčelja and Dr. P. Bothorel for sending preprints of their work.

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[2] Measurements with C-5, C-10, and C-12 deuterated DPLs at 80 wt % water and 41° yield practically the same results as observed at 50 wt % water content. The low signal-to-noise ratio makes these measurements difficult.

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