J. Membrane Biol. 48, 215–236 (1979)

On Two-Dimensional Passive Random Walk in Lipid Bilayers and Fluid Pathways in Biomembranes

H.-J. Galla, W. Hartmann, U. Theilen, and E. Sackmann

Abteilung Exp. Physik III, Universität Ulm, Oberer Eselsberg, D-7900 Ulm, West Germany

Received 24 October 1978; revised 23 January 1979

Summary. The lateral mobility of pyrene, pyrene decanoic acid, and 1-palmitoyl-2-pyrene decanoyl-phosphatidyl choline (pyrene lecithin) in lipid bilayers is determined by the excimer formation technique. This method is applied to vesicles of lecithins differing in chain length and in the degree of saturation of the hydrocarbon chains. These values are compared with results in cephalins of different chain length and in dipalmitoyl phosphatidic acid at variable pH. The influence of cholesterol is investigated. The results are analyzed in terms of the Montroll model of two-dimensional random walk. The jump frequency of the probe molecule within the lipid lattice is obtained. The advantage of this measure of transport in lipid layers is that it does not involve lipid lattice parameters.

The main results of the present work are: (i) The lateral mobility of a given solute molecule in lamellae of saturated lecithins is independent of hydrocarbon chain length and rather a universal function of temperature. (ii) In unsaturated dioleyl lecithin the amphiphatic molecules have lateral mobilities of the same size as in saturated lipids. The jump frequency of pyrene, however, is by a factor of two larger in the unsaturated lecithin. (iii) The jump frequencies in phosphatidyl ethanolamines are about equal to those in lecithins. (iv) In phosphatidic acid layers the hopping frequencies depend on the charges of the head groups of both the lipids and the probes. (v) Cholesterol strongly reduces the jump frequency in fluid layers. (vi) The lateral mobility in biological membranes is comparable to that in artificial lipid bilayers.

The experimental results are discussed in terms of the free volume model of diffusion in fluids. Good agreement with the predictions made from this model is found. A striking result is the observation of a tilt in dioleyl-lecithin bilayer membranes from the hopping frequencies of pyrene and pyrene lecithin. A tilt angle of $\varphi = 17°$ is estimated.

The passive lateral mobility of membrane-bound particles (proteins, drugs, and metabolites) is essential for a large number of biological functions. Most popular present examples are membrane assembly (Taylor *et al.*, 1971) or initial processes leading to cap formation in immune response (Edelmann, 1976). The membrane-bound diffusion may well compete with the transport in the cell interior which is expected to be not so effective due to the high viscosity of the cytoplasmic matrix (Keith

0022-2631/79/0048-0215/$04.40

& Snipes, 1973). Now the diffusions of membrane-bound particles is driven by the lateral motion of the lipids, which in turn is activated by the very fast movement of defects along the hydrocarbon chains (Träuble, 1971; Galla & Sackmann, 1974). Consequently, the lateral mobility is closely related to the (transversal) transport of small molecules across membranes.

The first measurements of lipid lateral diffusion coefficients were performed by the spin-label technique (Deveaux & McConnell, 1972; Sackmann & Träuble, 1972). Later on, two types of fluorescence technique were developed. The first type is based on an analysis of diffusion controlled bimolecular reactions, i.e., of the kinetics of excimer formation (Galla & Sackmann, 1974) and of triplet-triplet-annihilation (Nagvi, Behr & Chapman, 1974). Here the lipid mobility is measured in terms of jump frequencies rather than of diffusion coefficients. Both methods are especially suited to measure local diffusion coefficients in membranes exhibiting mosaic-like structures. The second type of technique introduced more recently is based on the measurement of the influx (or outflux) of labeled molecules into a predetermined volume either using fluorescence correlation techniques (Fahey *et al.*, 1976) or fluorescence recovery after photobleaching (Axelrod *et al.*, 1976; Wu, Jacobson & Papahadjopoulos, 1977).

The purpose of the present paper is to present a comparative experimental study of the lateral mobility of lipophilic excimer probes incorporated into pure and mixed lipid layers. The work was done in order to learn more about correlations between lipid structure and lateral diffusion. Our results are compared with the measurements performed by the other techniques. Moreover, results obtained for biological membranes are presented.

I. Materials and Methods

Lipids, Fluorescent Probes and Vesicle Preparation

Lipids purchased from Fluka were checked by thin-layer chromatography and used without further purification. Pyrene decanoic acid and 1-palmitoyl-2-pyrene decanoyl-lecithin was synthesized by ourselves as described (Galla & Hartmann, 1979). Erythrocyte membranes were prepared and labeled by J. Luisetti of our department. For these experiments, lipid analysis was performed. The pyrene decanoic acid concentrations were determined by taking absorption spectra of solutions of the probe which were extracted from the cell-suspensions by a given volume of chloroform. Doped vesicles were prepared from chloroform solutions of both the lipid and the excimer forming probe molecules.

After evaporation of the solvent, a dispersion in 2×10^{-3} M CsCl solution was prepared by sonification with a Branson Sonifier for about 5 min above the lipid phase transition.

Phosphatidic acid was dispersed in buffer solutions of the given pH (Rauen, 1974) (KCl/HCl: pH 2.0; acetate buffer: pH 5.0; Tris/buffer/HCl, pH 7.0; and $Na_2B_4O_7/H_3BO_3$ in 0.05 NaCl solution: pH 9.0). All buffers had an ionic strength between $I=0.1$ and $I=0.14$. All buffers and salt solutions were freed from oxygen by extensive nitrogen bubbling. The experiments were performed under a nitrogen atmosphere.

Summary of Excimer Fluorescence Technique

Excimer formation between a ground-state molecule and an excited molecule is a suitable physical parameter to investigate lipid phase transitions (Galla & Sackmann, 1974), lateral diffusion (Galla & Sackmann, 1974), as well as phase separation phenomena (Galla & Sackmann, 1975). The ratio of the excimer yield, ϕ', to the monomer quantum yield, ϕ, is related to the association rate $k_a \cdot c$ according to (Birks, Dyson & Munroe, 1963; Förster & Seidel, 1965; Förster, 1969)

$$\frac{\phi'}{\phi} = \frac{k'_f}{k_f} \cdot \tau'_0 \cdot k_a \cdot c \tag{1}$$

where c is the probe concentration per unit area and k_a is the second-order rate constant for dimer formation. This equation holds if the dissociation of the complex is negligible. k_f and k'_f are the transition probabilities for the radiative decay of the excited monomer and the excimer, respectively. These values can be obtained from life time measurements. The fluorescence quantum yield ratio can be expressed in terms of the fluorescence intensity ratio as measured at the maxima of the monomer (I) and the excimer (I') band.

$$\frac{I'}{I} = \kappa \cdot \frac{\phi'}{\phi}. \tag{2}$$

κ is a proportionality constant which is characteristic for each probe. Moreover, κ depends on the wave-length dependence of the spectrometer sensitivity (Galla & Sackmann, 1974).

The rate of the excimer formation, $k_a \cdot c$, is proportional to the collision rate ν_{col} (Förster, 1969; Galla & Sackmann, 1974)

$$\nu_{col} = k_a \cdot c. \tag{3}$$

Combining Eqs. (1), (2) and (3) leads to

$$\nu_{col} = \frac{I'}{I \cdot \kappa} \cdot \frac{k_f}{k_{f'}} \cdot \frac{1}{\tau_{0'}}. \tag{4}$$

Note that the collision rate is only a function of measurable quantities.

Experimental Procedure

The fluorescence spectra were taken with a Schoeffel Instrument (RSS 1000) fluorescence spectrometer equipped with two monochromator/photomultiplier systems arranged perpendicular to the irradiation light path. This allows us to measure the excimer

(I') and the monomer (I) intensity simultaneously. The ratio I'/I is calculated by an analogue computer included in the fluorescence spectrometer and is plotted as function of temperature. Irradiation was performed at $\lambda = 322$ nm in the case of pyrene, $\lambda = 329$ nm for pyrene decanoic acid and pyrene lecithin. The excimer intensity was recorded at $\lambda = 478$ nm, whereas the monomer intensity was taken at $\lambda = 395$ nm. The excimer life time, τ'_0, was determined by a Lambda Physics N_2-laser flash spectrometer for different fluorescence probe concentrations.

II. Evaluation of Data in Terms of the Two-Dimensional Random Walk Model (Montroll-Model)

Normally, bimolecular reactions are analyzed in terms of the Smoluchowski theory. This procedure is not applicable to the case of two-dimensional systems (Naqvi *et al.*, 1974). For that purpose, a strongly simplified random-walk model was adopted in earlier work in order to evaluate spin-label- or excimer-diffusion measurements (Sackmann, 1976). A true statistical model of diffusion in two dimensions has been worked out earlier (Montroll, 1969). This model has been applied to photophysical processes, especially to energy transfer problems (Pearlstein, 1968).

The model starts from a two-dimensional periodic lattice of unit cells which contain N points. $(N-1)$ points are sites of exciton creation, whereas one point is considered to be a trap. Montroll has derived an expression for the average number of steps, $\langle n_s \rangle$, necessary for an exciton to reach a trapping center for the first time. It turned out that the average step number, $\langle n_s \rangle$, depends very sensitively on the dimension of the system. For a square lattice a logarithmic law holds:

$$\langle n_s \rangle \propto \frac{1}{\pi} \cdot N \cdot \ln N. \tag{5}$$

The model may be directly applied to the problem of excimer formation in membranes if suitable redefinitions of the parameters introduced by Montroll are applied:

1) The excited monomers (P^*) are considered as traps for ground state molecules (P). The latter in turn plays the role of the excitons in the Montroll model. Each trapping process leads to the formation of an excited dimer $(PP)^*$ which is assumed to contribute to the excimer emission.

2) The role of the lattice is taken over by the lipid molecules. In the fluid state the lattice is rather irregular. Now, a certain fraction of lattice

points is occupied by ground-state label molecules (P). In a random solution each lattice point can be occupied with the same probability. This corresponds to the Montroll-assumption that a photon may be absorbed with equal probability by any chlorophyll molecule.

3) The probability that two excited molecules, P^*, collide is nearly zero under the given experimental conditions.

Now, assume that every N-th position of the lipid lattice is occupied by a label molecule. Consider a sub-lattice of $2N$ points which will contain two label molecules on the average. Consider one label molecule (situated at the origin of the sub-lattice) to be excited. In the same sub-lattice a nonexcited probe is situated with the probability of 1. If a membrane contains a mole fraction, x_{La}, of label molecules, the sub-lattice ("unit cell") must contain $2N = 2/x_{La}$ lattice points. The average number of steps may then be expressed in terms of the molar fraction, x_{La}:

$$\langle n_s \rangle \propto \frac{1}{\pi} 2N \ln 2N = \frac{2}{\pi x_{La}} \cdot \ln \frac{2}{x_{La}}. \tag{6}$$

The average step number $\langle n_s \rangle$ may be related to the collision frequency ν_{col} (defined in Eq. (3)) by introducing the jump frequency ν_j of the label molecules. ν_j is the number of diffusional steps per second

$$\nu_{col} = \langle n_s \rangle^{-1} \cdot \nu_j. \tag{7}$$

Combining Eq. (4) and (7) leads to

$$\nu_j = \langle n_s \rangle \frac{I'}{I\kappa} \frac{1}{\tau_0'} \frac{k_f}{k_f'}. \tag{8}$$

In contrast to our earlier method of evaluation (Galla & Sackmann, 1974), the experimental parameter characterizing the lateral transport in membranes is now the jump frequency ν_j. The lateral diffusion coefficient is then obtained from the jump frequency according to

$$D_{diff} = \tfrac{1}{4} \nu_j \cdot \lambda^2 \tag{9}$$

where λ is the average jump length. It is given by the average distance of the lipid lattice and is of the order of $\lambda = 8$ Å. The main advantage of the present method of evaluation is that the jump frequency does not depend on any unknown parameter characterizing the lipid lattice (e.g., lattice constant).

III. Experimental Results and Discussion

Summary of Outstanding Results

The jump frequencies, ν_j, measured in the present work are summarized in Tables 1–6. For comparison, some data of our earlier measurements are included in the tables. In Tables 1–3, values of the jump frequencies of three representative molecules (namely, pyrene, pyrene decanoic acid, and pyrene lecithin) are collected for membranes of lecithins and phosphatidyl ethanolamines at temperatures above the lipid phase transition. In particular, lamellae of saturated lipids of different chain length are compared for both types of lipids. For the lecithins, the data of the saturated lipids are related to those of unsaturated DOPC[1] and egg lecithin. Results obtained for phosphatidic acid layers at different pH of the water phase are compiled in Table 4. The influence of cholesterol on the jump frequency is demonstrated in Table 5. In Table 6 the jump frequencies of the three lables are compared in case of DPPC.

The following general conclusions may be drawn from the data given in Tables 1–6:

1) The lateral mobility of a given solute molecule in lamellae of saturated lecithins does not depend on the hydrocarbon chain length. According to Table 1 the jump frequency, ν_j, is rather a universal function of temperature above the chain melting transition

$$\nu_j = \nu_0 \cdot e^{-\frac{A}{T}}. \tag{10}$$

An apparent activation energy $\Delta E = A \cdot k_B$ (where k_B is the Boltzmann constant) of $\Delta E = 33.5$ kJ/mole is obtained. This provides strong evidence that membrane fluidity is determined primarily by the dynamics of chain defects. This point is discussed in more detail in part IV.

2) An interesting case is DOPC. Amphiphatic molecules (e.g., PDA and PL) anchored in the membrane head-group region have hopping frequencies of about the same size as in the saturated lecithin. This can be most clearly seen in Fig. 1. In contrast, the hopping frequency of

[1] *Abbreviations used:* DOPC = dioleyl phosphatidyl choline; DLPC = dilauryl phosphatidyl choline; DMPC = dimyristoyl phosphatidyl choline; DPPC = dipalmitoyl phosphatidyl choline; DSPC = distearoyl phosphatidyl choline; DLPE = dilauryl phosphatidyl ethanolamine; DMPE = dimyristoyl phosphatidyl ethanolamine; DPPE = dipalmitoyl phosphatidyl ethanolamine; DPPA = dipalmitoyl phosphatidic acid; PDA = pyrene decanoic acid; PL = 1-palmitoyl-2-pyrene decanoyl-phosphatidyl choline.

Table 1. Jump frequencies of pyrene in saturated and unsaturated lecithin bilayer membranes for different temperatures above the chain melting transition temperature[a]

Temp. [°C]	15	25	30	35	40	45	50	55	60	65	70
Egg-lecithin	0.4	0.7	0.9	1.3	1.7	2.2	2.7	3.3	4.0	–	–
DOPC	1.3	2.2	2.7	2.9	3.5	4.0	–	–	–	–	–
DLPC	0.4	0.8	1.0	1.4	1.7	2.1	2.7	3.5	4.3	4.9	5.5
DMPC	–	0.6	0.8	1.0	1.4	1.7	2.1	2.8	3.6	4.5	5.3
DPPC	–	–	–	–	–	1.9	2.5	3.3	3.9	4.6	5.3
DSPC	–	–	–	–	–	–	–	–	3.5	4.2	5.0

[a] The jump frequencies ν_j are given in units of $[10^8\,\mathrm{sec}^{-1}]$. The lateral diffusion coefficient, D_{diff}, can be obtained directly by multiplying ν_j with $1/4\,\lambda^2 = 1.6 \times 10^{-15}\,\mathrm{cm}^2$ following Eq. (9). This corresponds to a jump width of $\lambda = 8\,\text{Å}$ for saturated lipids which follows from the area per lipid molecule of $64\,\text{Å}^2$ (Albrecht *et al.*, 1978). For the unsaturated lipids λ is about $10\,\text{Å}^2$. Note the exceptionally large values of the jump frequencies for the synthetic unsaturated lipid (DOPC).

Table 2. Jump frequencies ν_j (in units of $10^8\,\mathrm{sec}^{-1}$) of pyrene decanoic acid (PDA) in different lecithins and phosphatidyl ethanolamines[a]

Temp. [°C]	20	30	40	45	50	55	60	65	70
Egg-lecithin	0.5	0.7	1.1	1.2	1.4	1.7	2.1	–	–
DOPC	0.6	0.9	1.2	1.5	1.7	1.9	2.1	–	–
DPPC	–	–	–	1.4	1.6	2.0	2.4	2.8	3.2
DOPC/DPPC 1:1/mole/mole	–	0.8	1.1	1.3	1.5	1.7	1.9	–	–
DLPE	–	1.4	2.2	2.7	3.2	3.7	4.1	4.5	4.9
DMPE	–	–	–	–	–	3.7	4.2	4.4	4.6
DPPE	–	–	–	–	–	–	–	4.2	4.5

[a] Values for a one-to-one mixture of DOPC and DPPC are included.

Table 3. Jump frequencies of the pyrene lecithin label (PL) in egg lecithin, synthetic lecithins, and phosphatidyl ethanolamines $[10^8\,\mathrm{sec}^{-1}]$

Temp. [°C]	20	30	40	45	50	55	60	65	70
Egg-lecithin	0.6	0.8	1.2	1.4	1.6	2.0	2.7	–	–
DPPC	–	–	–	0.8	1.0	1.2	1.5	1.8	2.1
DLPE	–	1.7	2.6	3.1	3.3	3.5	3.7	3.9	4.0
DMPE	–	–	–	–	–	3.1	3.4	3.7	3.8
DPPE	–	–	–	–	–	–	–	3.7	3.8

Table 4. pH-dependence of the jump frequencies of pyrene, pyrene decanoic acid, and pyrene lecithin incorporated into phosphatidic acid vesicles at $T = 70\,°C$ [a]

pH	Pyrene	Pyrene decanoic acid	Pyrene lecithin
2	4.0	3.6	3.2
5	4.8	3.2	3.6
7	4.8	3.2	3.9
9	5.8	2.9	4.3

[a] ν_j is given in units of $10^8\,sec^{-1}$. In analogy to Table 1, the diffusion coefficients are obtained by multiplying ν_j with $1.3 \times 10^{-15}\,cm^2$ corresponding to an area per lipid molecule of $60\,Å^2$.

Table 5. Jump frequencies of pyrene in cholesterol-containing DPPC membranes [a]

Temp. [°C]	35	45	50	60	Activation energies (kJ/mole)
$x = 0.00$	–	1.9	2.7	3.7	35
$x = 0.01$	–	1.9	2.6	3.6	37
$x = 0.02$	–	1.8	2.4	3.5	38
$x = 0.05$	–	1.5	2.1	2.9	42
$x = 0.1$	0.7	1.3	1.8	2.5	45
$x = 0.3$	0.4	0.7	1.0	1.8	52
$x = 0.5$	0.3	0.5	0.7	1.1	54

[a] At a cholesterol content higher than 30 mole %, the chain melting phase transition of the mixture is abolished (*cf.* insert in Fig. 2). Again the ν_j is given in units of $10^8\,sec^{-1}$. x is the mole fraction of cholesterol. The apparent activation energies (*cf.* Eq. (10)) are given in the last column.

Table 6. Comparison of jump frequencies of pyrene, pyrene decanoic acid, and pyrene lecithin in DPPC lamellae at 50 °C [a]

	Pyrene	Pyrene decanoic acid	Pyrene lecithin
Mol wt (M)	202	372	876
$\sqrt{M}$	14.2	19.3	29.6
$\nu_j \cdot [10^8\,sec^{-1}]$	2.5	1.6	1.0
$\nu_j \cdot \sqrt{M}$	35.5	31.0	29.6

[a] ν_j is given in units of $10^8\,sec^{-1}$. M is the mol wt.

pyrene in DOPC is by a factor of two larger than the values of ν_j in the saturated lecithins at the same temperature. This indicates that small hydrophobic molecules are confined mainly to the central part of the membrane. As will be shown below, the higher pyrene mobility can be explained in terms of a higher free volume in the membrane core caused by a partially tilted structure.

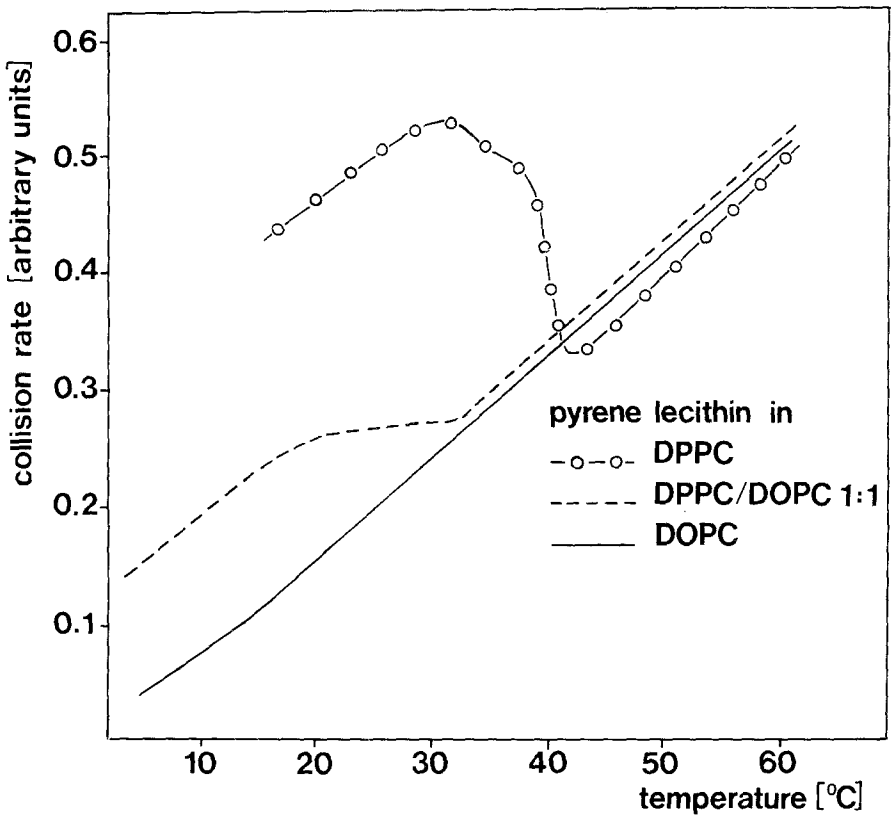

Fig. 1. Temperature dependence of the collision rate of pyrene lecithin in membranes of DPPC, DOPC, and a one-to-one mixture of both. Equal values of ν_{col} are found at a given temperature above the lipid phase transition temperature

3) For all probes studied, the jump frequencies in egg lecithin agree well with the values of ν_j in the saturated lipids at a given temperature. Thus we can state that incorporation of one unsaturated chain in a phospholipid does not change the fluidity considerably.

4) At a given temperature, the hopping frequencies in phosphatidyl ethanolamines, phosphatidic acids, and lecithins are nearly equal. Thus, for PDA the values of ν_j in DPPE, DPPA, and DPPC at $T=70\,°C$ differ at most by 20%. This shows again that the lateral transport coefficient in lipid layers is rather a universal function of temperature than of lipid head-group structure.

5) In phosphatidic acid layers the hopping frequencies depend in a characteristic way on the charges of the polar head groups of both the lipid and the probes. For the negatively charged probe molecule (PDA), ν_j decreases with increasing pH, i.e., with increasing fixed charges at the

Table 7. Comparison between jump frequencies, ν_j, (10^8 sec^{-1}) of pyrene, pyrene decanoic acid, and pyrene lecithin incorporated into DPPC membranes and DPPA membranes at pH 5 and 9.0[a]

	DPPC	DPPA, pH 5	DPPA, pH 9
Pyrene lecithin	2.1	3.6	4.3
Pyrene decanoic acid	3.2	3.2	2.9
Pyrene	5.3	4.8	5.8

[a] The temperature is 70 °C.

membrane surface. In contrast, ν_j increases for pyrene and pyrene lecithin with increasing pH (e.g., Table 7).

6) Addition of cholesterol to lecithin bilayers has a strong effect on the hopping frequency. This is directly demonstrated in Fig. 2. An increase of the cholesterol content from 0 to 10 mole % leads to a decrease in the hopping frequency by about 30 %. A smaller decrease of about 20 % is observed if the cholesterol content is increased between 10 and 50 mole %. The apparent activation energy is considerably increased (by about 40 %) upon cholesterol addition (*cf.* Table 5). In a free volume model of diffusion given below, this will be interpreted as a consequence of the well-known condensing effect of cholesterol.

The jump frequency of pyrene decanoic acid in erythrocyte plasma membranes at 50 °C is $\nu_j = 2.9 \times 10^8$ sec^{-1} (Luisetti, 1979). Surprisingly, the lateral mobility in erythrocyte membranes is of the same size as in fluid artificial membranes. This value of the jump frequency of PDA in erythrocytes is in rather good agreement with the value of ν_j observed for fatty-acid spin label in liver microsomes (Stier & Sackmann, 1973) ($\nu_j = 1 \times 10^8$ sec^{-1} at 40 °C). The mobilities observed by our method seem to be in contrast to the very low lateral diffusion coefficient measured in erythrocytes by photobleaching experiments (Peters *et al.*, 1974). One reason for this apparent discrepancy may be that the present technique measures local mobilities while long range transport is determined by the photobleaching experiments. Preliminary photobleaching experiments (Kapitza & Sackmann, 1979) showed, however, that the long-range lipid diffusion coefficient, D, in erythrocytes is, in fact, of about the same size as the value of D given above.

Application of the Free Volume Model of Diffusion in Fluids (Cohen and Turnbull, 1959) to Lipid Layers

The diffusion of a molecule in a fluid system may be divided up into a three-step process (Cohen & Turnbull, 1959):

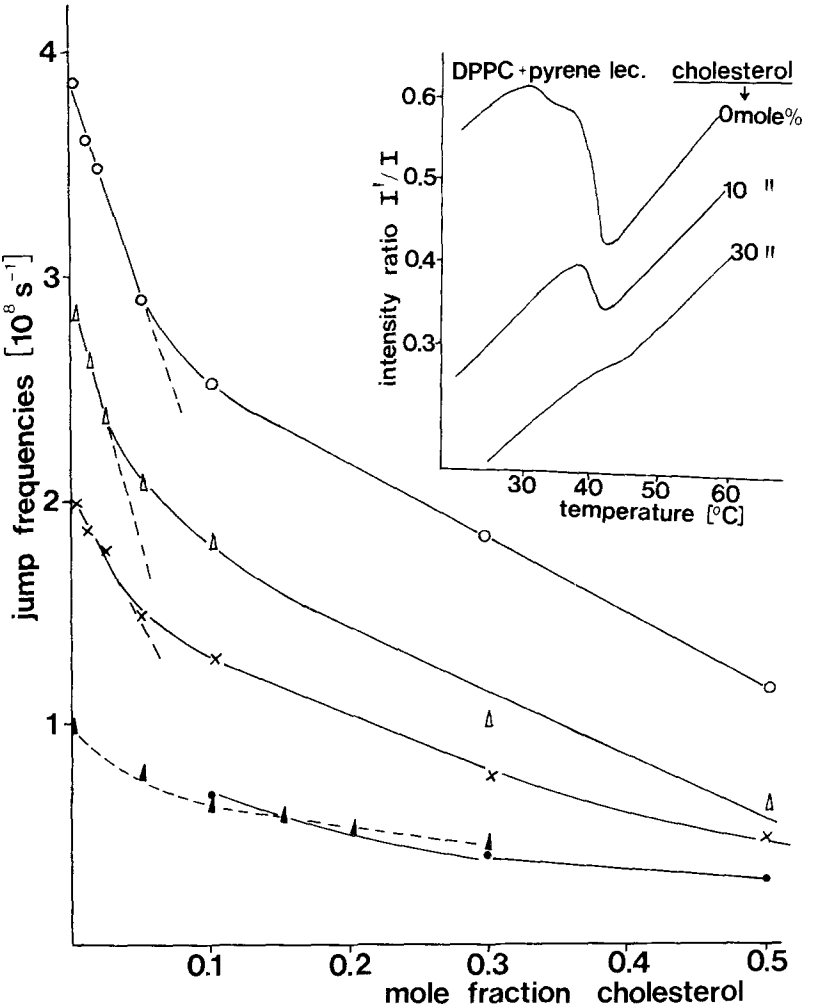

Fig. 2. Influence of cholesterol on the hopping frequencies of pyrene and pyrene lecithin in dipalmitoyl lecithin bilayer vesicles. Pyrene: (○—○—○) $T=60\,°C$; (△—△—△) $T=50\,°C$; (×—×—×) $T=45\,°C$; (●—●—●) $T=35\,°C$. Pyrene lecithin: (▲—▲—▲) $T=50\,°C$. Note that 10 mole% cholesterol reduces the jump frequency by about 30%. The insert shows the influence of cholesterol on the lipid-phase transition as determined by the use of pyrene lecithin (5 mole %) as optical probe. The height of the step in I'/I at the phase transition diminishes with increasing cholesterol content and is absent at more than 30% cholesterol

1) the creation of local free volume by density fluctuations which open up a hole within the cage where a solute molecule is situated.

2) the second step is the jump of the molecule into this hole, creating a void at the previous position.

3) to stabilize this lateral displacement, this void created has to be filled by another solvent molecule.

The free volume per molecule in a lipid bilayer may be defined as

$$v_f = \bar{v} - v_o = L(\bar{a} - a_o). \tag{11}$$

$\bar{v}$ and $\bar{a}$ denote the average volume and area, respectively, occupied by one lipid molecule. v_o and a_o are the corresponding Van der Waals dimensions. Cohen and Turnbull calculated the probability, $P(v)$, for redistribution of the average free volume, $\bar{v}$, in such a way that a local void of free volume, v, is created within a fluid system. If the volume of this void exceeds a critical value ($v \geqq v^*$), a molecule can perform a diffusional jump into this new position. The diffusion coefficient is thus a function of this local free volume v. In a lipid layer the free volume can be created by kink formation in the hydrocarbon chains as illustrated in Fig. 5. The average diffusion coefficient, $\bar{D}$, is then

$$\bar{D} = \int_{v^*}^{\infty} D(v)\, P(v)\, dv. \tag{12}$$

$P(v)$ is the probability of finding a value of the "local free volume" between v and $v + dv$. A statistical mechanical consideration leads to

$$P(v) = (\gamma / v_f) \exp[-\gamma \cdot v / v_f]. \tag{13}$$

Here γ is a numerical factor introduced to account for a possible overlap of the free volume ($1/2 < \gamma < 1$). Now, $D(v)$ is considered as a step-like function: $D(v) = 0$ for $v < v^*$ and $D(v) = D(v^*)$ for $v > v^*$. The total probability of finding a hole of a volume exceeding v^* is

$$P(v^*) = \int_{v^*}^{\infty} P(v)\, dv = \exp[-\gamma \cdot v^* / v_f]. \tag{14}$$

According to Cohen and Turnbull the diffusion coefficient $D(v^*)$ is easily estimated if the molecules may be considered as hard rods. $D(v^*)$ may then be related to the gas kinetic velocity, u, according to

$$D(v^*) = g \cdot d(v^*) \cdot u \tag{15}$$

where g is a geometric factor and $d(v^*)$ is the diameter of the cage. Combining Eqs. (12), (14) and (15) and assuming a step-like function for $D(v)$, as noted above, leads to

$$D = D(v^*)\, P(v^*) = g \cdot d^* \cdot u \cdot \exp[-\gamma \cdot v^* / v_f] \tag{16}$$

where d^* approximately equals the molecular diameter. This is the equation derived by Cohen and Turnbull for simple liquids.

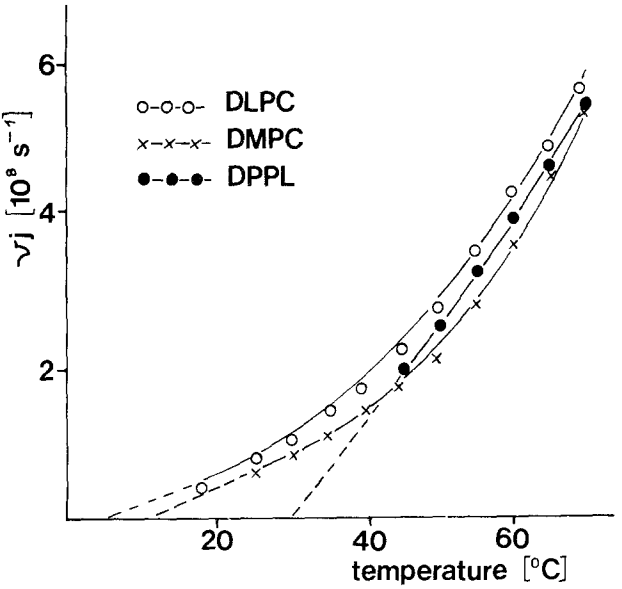

Fig. 3. Temperature dependence of the jump frequency, ν_j, of pyrene in DLPC, DMPC, and DPPC membranes. The curves extrapolated to $\nu_j=0$ lead to the pretransition temperatures of the corresponding lipids

Now let us consider the special case of a fluid lipid matrix. The lipid molecules may be regarded as hard rods of length, L. The values v^* and v_f, respectively, must then be replaced by critical corresponding values of the free area a^* and average free area a_f per molecule. The temperature dependence of the diffusion coefficients enters into the model via the law of linear thermal expansion according to which a_f is given by

$$a_f = \alpha_{\text{lat}} \cdot \bar{a}_m (T - T_o). \tag{17}$$

In this equation $\bar{a}_m$ is the value of the molecular area below the critical temperature T_o.[2] T_o is the so-called glass transition temperature below which the free volume becomes very small. The lateral thermal expansion coefficient α_{lat} can be determined from monolayer studies (Albrecht, Gruler & Sackmann, 1978). At constant pressure, Eqs. (16) and (17) lead to

$$D = g \cdot a^* \cdot u \cdot \exp\left[\frac{-\gamma \cdot v^*}{a_m \cdot \alpha (T - T_o)}\right]. \tag{18}$$

[2] In the case of lipid layers T_o may be defined by extrapolating the experimental jump frequencies, ν_j, *vs.* T curves towards $\nu_j=0$ as shown in Fig. 3. Interestingly, the critical temperature obtained in such a way agrees rather well with the lipid pretransition temperature.

Comparison of the Free Volume Model with the Experiments

a) Eq. (18) is in complete accordance with the experimental evidence found for lecithin layers according to which the jump frequency, ν_j, is a universal function of temperature given by $\nu_j = \nu_{jo} \exp\{-B/(T-T_o)\}$.

b) The ratio $\gamma v^*/v_m$ may be determined by fitting Eq. (18) to the experimental curves of Fig. 3 where the lateral expansion coefficient as obtained from film balance experiments can be used. For DPPC the lateral expansion coefficient is given by $\alpha_{\text{lat}} = 2.3 \times 10^{-3} K^{-1}$ (Albrecht *et al.*, 1978). The thickness of the membrane is not expected to change considerably with temperature. For a membrane thickness of $d = 20$ Å one then obtains a value of the volume expansion coefficient of $\alpha_{\text{vol}} = 1.15 \times 10^{-4} K^{-1}$. Taking into account the experimentally obtained apparent activation energy of $\Delta E_A = 35$ kJ/mole one obtains $v^* \cdot \gamma/v_m = 0.4$. This is in reasonable agreement with the value of simple liquids where $v^* \cdot \gamma/v_m = 0.6$ (Cohen & Turnbull, 1959).

c) The free volume model predicts that the diffusion coefficients of solute molecules which are comparable in size to the solvent are equal to the solvent self-diffusion coefficient, provided solvent and solute have same mass, m (Cohen & Turnbull, 1959). Table 6 compares the jump frequencies of the labels used in this work in DPPC bilayers at 50 °C. The diffusion coefficient decreases by a factor of 2.5 upon going from pyrene to pyrene lecithin. Now the gas kinetic velocity [in Eq. (16)] is estimated according to $u = \sqrt{2kT/m}$. If the exponential factor in Eq. (16) is independent of size, the product $D \cdot \sqrt{m}$ should be constant. Table 6 shows that this condition is fulfilled very well for PL and PDA. For pyrene, $\nu_j \cdot \sqrt{m}$ is by about 20 % larger. This difference can be attributed to the influence of the polar head group.

Tilted Structure of Dioleyl-Lecithin Lamellae

As shown by the interpretation of our experimental results, the free volume model can well be applied to anisotropic fluids. Now the application of this model leads to the conclusion that the hydrocarbon chains of DOPC are tilted with respect to the normal of the membrane surface. The tilt extends between the double bond and the polar surface as indicated in Fig. 4: This follows from the exceptionally large hopping frequency of pyrene in DOPC bilayers. It is by a factor of two larger

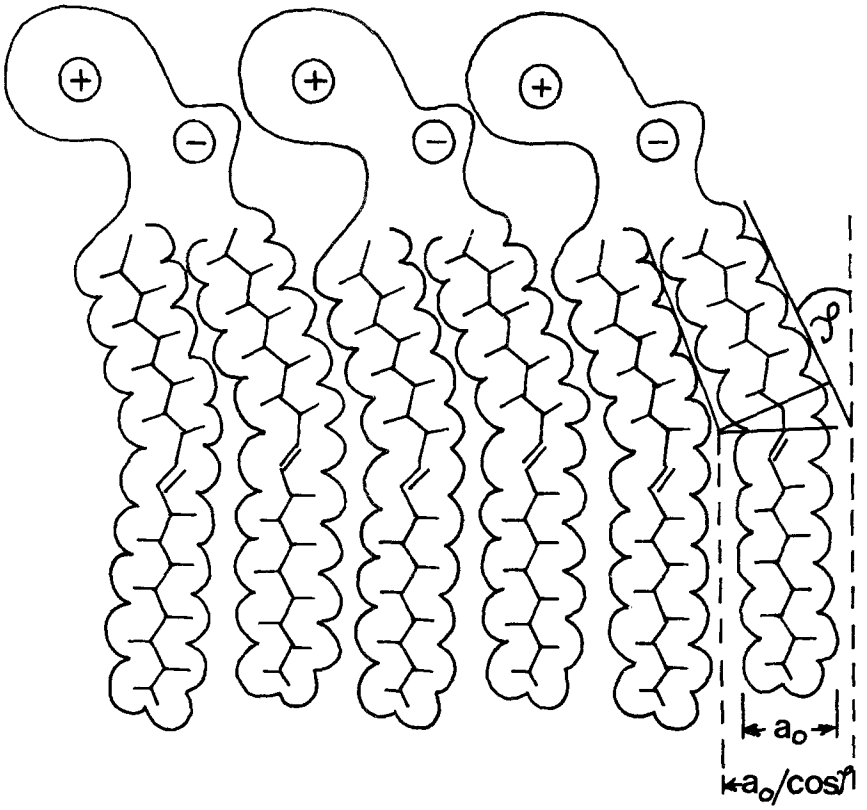

Fig. 4. Possible structure of dioleyl lecithin monolayer as suggested by differences in diffusion coefficients of hydrophobic (pyrene) and amphiphatic (PL, PDA) probe molecules. A bend in the chains imposes a tilt of the hydrocarbon chain region between the double bond and the headgroups. Note that the free area, a_f, of the lower chain region is thus by a factor of $1/\cos\varphi$ larger than that of the upper part

than the value of v_j in a saturated lipid layer (e.g., DPPC) at the same temperature. In striking contrast, the amphiphatic probes, PL and PDA, have nearly identical jump frequencies for the saturated and unsaturated lipids.

Recent fluorescence quenching experiments showed that pyrene is mainly confined to the central region of the bilayers (Luisetti *et al.*, 1979). It is the free area, a_f, per lipid hydrocarbon chain of this region which will determine the hopping frequency of pyrene. The lateral mobility of the elongated amphiphatic molecules, however, will be rather determined by the free area, a_f^0, in the outer region of the DOPC bilayer. According to Fig. 4 this part is more condensed than the central region characterized by the free area, a_f^c. Now the free area, a_f^c, of the central region is

related to the free area, a_f^o, in the outer region according to $a_f^c = a_f^o/\cos\varphi$. φ is the tilt angle defined in Fig. 4.

On the basis of the above considerations, the tilt angle φ may be estimated roughly from the ratio of the hopping frequencies of pyrene and PL (or PDA). Application of Eq. (16) leads to a value of $\varphi \approx 17°$ at 50 °C. This example shows that diffusion measurements may also provide valuable information on membrane structures.

Influence of Cholesterol on Lateral Mobility in Lecithin

The pronounced decrease in jump frequency, ν_j, upon addition of cholesterol can also be interpreted in terms of the free volume model. In the limit of low cholesterol concentrations ($x_c < 5$ mole %), a random mixture is observed and the measured jump frequency is a well-defined quantity. Now, it is well known that addition of cholesterol to fluid lecithin leads to a pronounced condensing effect (Marsh, 1974). This causes a reduction in free volume and subsequently a decrease in jump frequency. Below 5 mole %, ν_j decreases linearly with increasing cholesterol concentration, x_c (*cf.* Fig. 3). The reduction in ν_j may be estimated quantitatively by the free volume model.

Consider the case of DPPC. The reduction in ν_j at 50 °C is given by $\Delta\nu/\Delta x_c = -0.15 \times 10^8\ \mathrm{s}^{-1}$ per mole % cholesterol (*cf.* Fig. 2). According to Eq. (16), a change ($\Delta\nu_j$) in ν_j is related to a change in temperature (ΔT) by

$$\Delta\nu = \nu(T_o) \cdot \frac{B}{T_o} \cdot \frac{\Delta T}{T_o} = 0.08 \times 10^8 \cdot \Delta T\ [\mathrm{sec}^{-1}]. \tag{19}$$

The numerical value is valid for 50 °C where $\nu(T_o) = 2.5 \times 10^8\ \mathrm{sec}^{-1}$ and $B/T_o = 14$ for an activation energy of 35 kJ/mole. Application of the linear thermal expansion law leads to a relation between the temperature change and the change in free area for DPPC at 50 °C (it is $1\,K \triangleq 15\,\text{Å}^2$) (Albrecht *et al.*, 1978). Film balance studies of mixed cholesterol/DPPC monolayers showed that addition of cholesterol leads to a linear decrease in average area A, per molecule according to $\Delta A/\Delta x_c = -0.8\,\text{Å}^2$/mole % cholesterol. Identifying ΔA with a decrease in average free area (Δa_f) per molecule leads to the following value of the change in ν_j with cholesterol concentration: $\Delta\nu_j/\Delta x_c = 0.27 \times 10^8\ \mathrm{sec}^{-1} \cdot \text{mole } \%^{-1}$. This is in good agreement with the experimental value of $\Delta\nu_j/\Delta x_c = 0.15 \times 10^8\ \mathrm{sec}^{-1} \cdot \text{mole } \%^{-1}$. This example shows again the usefulness of the free-volume model for a discussion of lateral mobility in membranes.

The diffusion measurements cannot be interpreted at higher cholesterol concentrations. Monolayer studies led to the conclusion that lateral phase separation causes a heterogeneous lateral organization of the components. An interesting mixture is that with 30 mole % cholesterol and 70 mole % DPPC which is supposed to be randomly organized. The pyrene jump frequency in this mixture is $\nu_j = 10^8\ \mathrm{sec}^{-1}$ at 50 °C.

Cholesterol is expected to destroy the crystal structure of the pure lipid below the lipid phase transition. This is indicated by the fact that the phase transition is abolished above 30 mole % of cholesterol. Above this limit, the lipid layer allows an appreciable lateral mobility even at temperature where the pure lipid is completely immobilized. For the example of a 1 : 1 mixture of DPPC and cholesterol the jump frequency at 35 °C is $0.3 \times 10^8\ \mathrm{sec}^{-1}$. Note that this composition corresponds to the situation in erythrocytes.

Dynamics of Chain Motion and Lateral Displacement of Free Volume

The formation of free volume is a consequence of the thermally induced rotational isomers of the hydrocarbon chains, the simplest being the *gtg*-kink. Kink formation leads to a lateral displacement of the chain, creating a local free volume (*cf.* Fig. 5). The solute molecule may jump into the pocket thus created. The diffusional jump is completed if the new void is closed by a suitable movement of a defect in an adjacent chain. Therefore the jump frequency of random motion can be related to the hopping frequency, ν_k, of the defects along the hydrocarbon chains (Galla & Sackmann, 1974):

$$\nu_j = \frac{2 d_k^2}{L^2} \nu_k . \tag{20}$$

L is the length of the diffusing molecule (e.g., pyrene), d_k is the lateral displacement of the hydrocarbon chain upon *gtg*-kink formation. According to Fig. 5, $d_k = 1.3$ Å. The hopping frequency ν_k, may be estimated from the activation energy ΔE_k of the kink diffusion along the chain: $\nu_k \approx f \cdot \exp\{-\Delta E_k / kT\}$, where f is the CH_2-rocking frequency. A value of $\nu_k \approx 2 \times 10^9\ \mathrm{sec}^{-1}$ was estimated (Galla & Sackmann, 1974). For pyrene ($L \approx 7$ Å), the jump frequency becomes $\nu_j = 1.5 \times 10^8\ \mathrm{sec}^{-1}$. This value is in good agreement with the experimental result ($\nu_j = 2.5 \times 10^8\ \mathrm{sec}^{-1}$ at 50 °C). The kink model also accounts for the finding that ν_j does not depend on the length of the lipid molecules. Obviously, it is

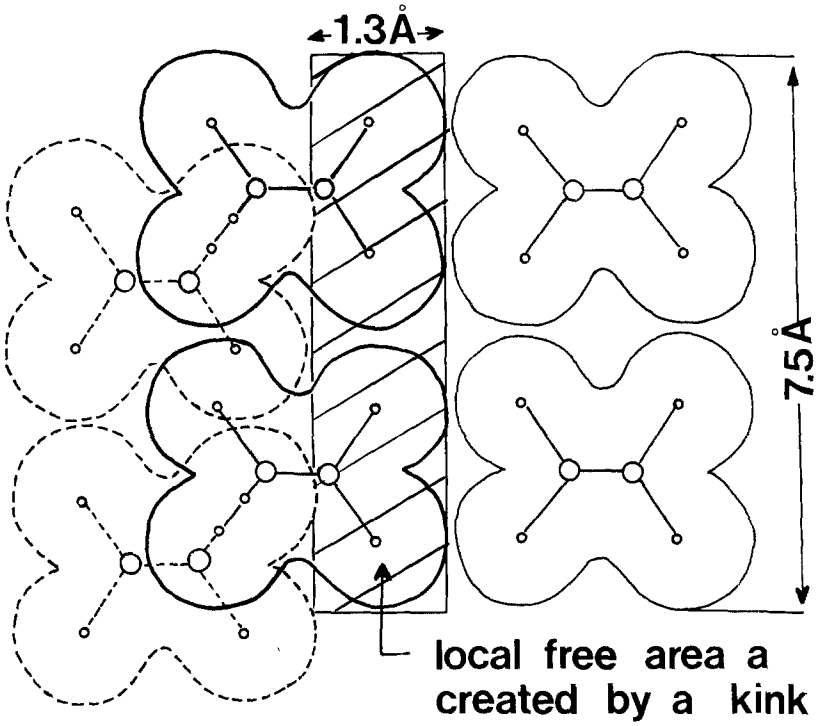

Fig. 5. Illustration of creation of free volume in the hydrocarbon region of lipid layers. The membrane is viewed perpendicular to the membrane surface. The contours shown correspond to the Van der Waals dimensions of an all-trans CH_2-chain in a direction perpendicular to the chain axis. The left side shows the lateral displacement of part of the chains by a kink formation. The new position of part of the chains after the displacement is shown by the dotted contours. The dimension of the free area is indicated by the hatched area. Its dimension is 1.3×7.5 Å^2 for two adjacent chains

the dynamics of the chain defects which determines the lateral diffusion in membranes. This also shows that the lateral and transversal transport in membranes are closely related.

V. The Passive Random Walk in Membranes

Molecular spread via lateral diffusion in membranes is extremely important for the following reasons: firstly, random motion in two dimensions may provide a more effective directional transport than three dimensional diffusion (Adam & Delbrück, 1968; Montroll, 1969); secondly, the viscosity of cell plasma is usually very high. From spin-label rotational correlation times (Keith & Snipes, 1973) a viscosity of $\eta \approx 40$ cp is found in *E. coli* protoplasma at 25 °C. According to Einstein's theory of Brownian motion, the high lipid mobility implies a fast random

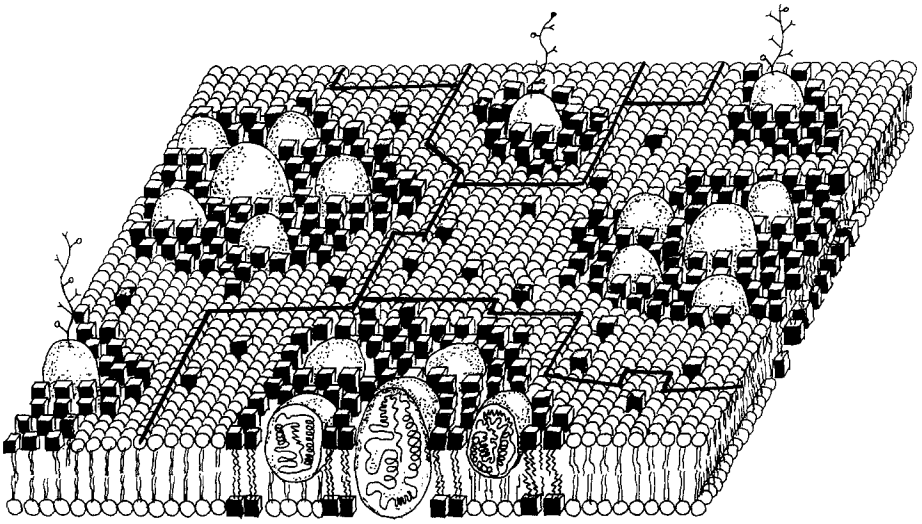

Fig. 6. Possible pathway of a molecule diffusing randomly in a biological membrane. Fluid channels between rigidified lipid-protein domains allow a high local mobility due to the low microviscosity in the channel. Collectively the membrane may be nonfluid

motion of proteins dissolved in the membrane. For cylindrically shaped membrane-bound proteins, the coefficients of lateral diffusion of the lipids (D_L) and the proteins (D_P) may be related to the molecular weights of these molecules (M_L and M_P) (*cf.*, e.g., Sackmann *et al.*, 1973, and Scandella, Devaux & McConnell, 1972) according to

$$D_P = \sqrt{\frac{M_L}{M_P}} \cdot D_L. \tag{21}$$

Now, remember that the diffusion coefficient of lipids in biological membranes such as erythrocyte- or liver microsome-membranes has a value of $D_L \approx 10^{-7}$ cm^2/sec at 40 °C.

Now, take an average molecular weight of a protein of $M_P = 50{,}000$. One would thus expect a value of $D_P \approx 10^{-8}$ cm^2/sec. This value of D_P is in good agreement with the measured diffusion coefficient of rhodopsin in the rod outer segment membranes, $D_P = 5 \times 10^{-9}$ cm^2/sec (Liebmann & Entine, 1974) which justifies application of Eq. (21).

It should be emphasized that the transport in biomembranes may be even more effective. About 50% of the membrane surface is occupied by integral proteins which will be normally surrounded by a halo of rigidified (boundary) lipids. Between these rigid patches, channels of fluid lipid are spreading (e.g., Fig. 6). Naturally, the transport will be restricted to these streamlets and will thus become much more effective.

Table 8. Lateral diffusion coefficients and jump frequencies (*according to* $D_{diff} = 1/4\,\lambda^2\,\nu$) *for lipid analogues and membrane-bound antigens in lipid bilayers determined by other fluorescence techniques*

Method	Ref.	D_{diff} (cm^2/sec)	ν_j (sec^{-1})	Probe molecule	Lipid matrix	Temperature (°C)
Fluorescence photobleaching recovery (FPR)	Wu *et al.* (1977)	4×10^{-8}	0.25×10^8	N-4-Nitrobenz.-2-oxa-1,3-diazo-PE	Egg-lecithin	25
		8×10^{-8}	0.5×10^8	N-4-Nitrobenz.-2-oxa-1,3-diazo-PE	DMPC	30
		2×10^{-8}	$0{,}12 \times 10^8$	N-4-Nitrobenz.-2-oxa-1,3-diazo-PE	DMPC/Chol (1 : 1)	30
Triplet-triplet annihilation	Naqvi *et al.* (1974)	2.3×10^{-7}	1.4×10^8	PE-anthracene	DPPC	50
Fluorescence correlation spectroscopy (FCS)	Fahey *et al.* (1976)	2.4×10^{-7}	1.5×10^8	DPPE-rhodamine	Egg-lecithin	24
		1.6×10^{-7}	1.0×10^8	DPPE-rhodamine	Egg-lec./Chol. (1 : 1)	24
Fluorescence photobleaching recovery (FPR)	Wolf *et al.* (1972)	4.5×10^{-8}	0.12×10^8	2,4,6 Trinitro phenylrhodamine stearoyl dextrane (membrane-bound antigen)	Egg-lecithin	24
Excimer technique	This work	1.3×10^{-7}	0.8×10^8	Pyrene lecithin	Egg-lecithin	25
		1.6×10^{-7}	1.0×10^8	Label	DPPC	50
		0.65×10^{-7}	0.4×10^8		DMPC (estimated)	30

V. Concluding Remarks

The simple excimer technique yields reliable values of lateral diffusion coefficients. Diffusion coefficients within local fluidized areas of biological membranes may be measured by this technique. By analyzing the experiments in terms of the statistical Montroll model of 2-dimensional diffusion, the frequency of successful diffusional steps is measured. The jump frequency is a measure of lateral transport which does not involve the knowledge of lattice constants.

In Table 8 the results obtained with our excimer technique are compared with the data determined by other optical methods. The results are in good agreement. For DPPC, for example, the value of ν_j obtained with the excimer and the triplet-triplet-annihilation technique differs only by about 30 %. This demonstrates that the presently applied fluorescence techniques give reliable results.

References

Adam, G., Delbrück, M. 1968. Reduction of dimensionality in biological diffusion processes. *In:* Structural Chemistry and Molecular Biology. N. Davidson, and A. Rich, editors. p. 198. W.H. Freeman, San Francisco

Albrecht, O., Gruler, H., Sackmann, E. 1978. Polymorphism in phospholipid monolayers. *J. Phys.* **39**:301

Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E.L., Webb, W.W. 1976. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* **16**:1055

Birks, J.B., Dyson, D.J., Munroe, I.H. 1963. Excimer fluorescence: II. Life time studies of pyrene solutions. *Proc. R. Soc. London A* **275**:575

Cohen, M.H., Turnbull, D. 1959. Molecular transport in liquids and glasses. *J. Chem. Phys.* **31**:1164

Deveaux, P., McConnell, H.M. 1972. Laterale diffusion in spin-labelled phosphatidylcholine-multilayers. *J. Am. Chem. Soc.* **94**:4475

Edelmann, G.M. 1976. Surface modulation in cell recognition and cell growth. *Science* **192**:218

Fahey, P.F., Koppel, D.E., Barak, L.S., Wolf, D.E., Elson, E.L., Webb, W.W. 1976. Lateral diffusion in planar lipid bilayers. *Science* **195**:305

Förster, Th. 1969. Excimere. *Angew. Chem.* **81**:364

Förster, Th., Seidel, H.P. 1965. Untersuchungen zum Konzentrationsumschlag der Fluoreszenz des Pyrens. *Z. Phys. Chem. N.F.* **45**:58

Galla, H.-J., Sackmann, E. 1974. Lateral mobility of pyrene in model membranes of phosphlipids with different chain length. *Ber. Bunsenges. Phys. Chem.* **78**:949

Galla, H.-J., Sackmann, E. 1974. Lateral diffusion in the hydrophobic region of membranes: Use of pyrene excimers as optical probes. *Biochim. Biophys. Acta* **339**:103

Galla, H.-J., Sackmann, E. 1975. Chemically induced phase separation in mixed vesicles containing phosphatidic acid: An optical study. *J. Am. Chem. Soc.* **97**:4114

Galla, H.-J., Theilen, U., Hartmann, W. 1979. Transversal mobility in bilayer membranes: Use of pyrene lecithin as optical probe. *Chem. Phys. Lipids* **23**:239

Kapitza, H., Sackmann, E. 1979. Local measurement of lateral motion on erythrocyte membranes by photobleaching technique. *Biochim. Biophys. Acta (in press)*

Keith, A.D., Snipes, W. 1973. Viscosity of cellular protoplasma. *Science* **183**:666

Liebmann, P.A., Entine, G. 1974. Lateral diffusion of visual pigment in photoreceptor disk membranes. *Science* **185**:457

Luisetti, J. 1979. Aggregation und Diffusion in künstlichen und natürlichen Membranen. Ph. D. Thesis. University of Ulm, Ulm

Luisetti, J., Möhwald, H., Galla, H.-J. 1979. Monitoring the location profile of fluorophores in phosphatidylcholine bilayers. *Biochim. Biophys. Acta* **552**:519

Marsh, D. 1974. An interaction spin label study of lateral expansion in DPL-cholesterol-bilayers. *Biochim. Biophys. Acta* **363**:373

Montroll, E.W. 1969. Random walks on lattices. *J. Math. Phys.* **10**:753

Naqvi, K.R., Behr, J.-P., Chapman, D. 1974. Methods for probing lateral diffusion of membrane components: Triplet-triplet-annihilation of triplet-triplet-energy-transfer. *Chem. Phys. Lett.* **26**:440

Pearlstein, R.M. 1968. Donor fluorescence as a probe of energy transfer. *Photochem. Photobiol.* **8**:341

Peters, R., Peters, J., Tews, K.H., Bähr, W. 1974. A microfluorometric study of translational diffusion in erythrocyte membranes. *Biochim. Biophys. Acta* **367**:282

Rauen, R.M. 1974. Biochemisches Taschenbuch. p. 92. Springer Verlag, Berlin–Heidelberg–New York

Sackmann, E. 1976. On the application of excimers as optical probes in membrane research. *Z. Phys. Chem. N.F.* **101**:391

Sackmann, E., Träuble, H. 1972. Studies of the crystalline-liquid crystalline phase transition of lipid model membranes. *J. Am. Chem. Soc.* **94**:4482

Sackmann, E., Träuble, H., Galla, H.-J., Overath, P. 1973. Lateral diffusion, protein mobility and phase transitions in *escherichia coli* membranes: A spin label study. *Biochemistry* **12**:5360

Scandella, C.J., Devaux, P., McConnell, H.M. 1972. Rapid lateral diffusion of phospholipids in rabbit sarcoplasmic reticulum. *Proc. Nat. Acad. Sci USA* **69**:2056

Stier, A., Sackmann, E. 1973. Spinlabels as enzyme substrates, heterogeneous lipid distribution in liver microsomal membranes. *Biochim. Biophys. Acta* **311**:400

Taylor, R.B., Duffus, W.P.H., Raff, M.L., Petris, S. de 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature New Biol.* **233**:225

Träuble, H. 1971. The movement of molecules across lipid membranes: A molecular theory. *J. Membrane Biol.* **4**:193

Wolf, D.E., Schlesinger, J., Elson, E.L., Webb, W.W., Blumenthal, R., Henkart, P. 1972. Diffusion and patching of macromolecules on planar lipid bilayer membranes. *Biochemistry* **16**:3476

Wu, E.-S., Jacobson, K., Papahadjopoulos, D. 1977. Lateral diffusion in phospholipid multilayers measured by fluorescence recovery after photobleaching. *Biochemistry* **13**:3936