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The Movement of Molecules Across Lipid Membranes: A Molecular Theory

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Summary. The movement of molecules across membranes is discussed in terms of thermal fluctuations in the hydrocarbon chains of the membrane lipids. The thermal motion of the hydrocarbon chains results in the formation of conformational isomers, so-called kink-isomers of the hydrocarbon chains. "Kinks" may be pictured as mobile structural defects which represent small, mobile free volumes in the hydrocarbon phase of the membrane. The diffusion coefficient of kinks is calculated to be 10^{-5} cm^2/sec; thus kink diffusion is a fast process. Small molecules can enter into the free volumes of kinks and migrate across the membrane together with the kinks; thus kinks may be regarded as intrinsic carriers of lipid membranes. An expression is derived from this model for the flow of molecules through lipid membranes. The calculated value for the water permeability is compatible with measurements on lipid bilayers.

Biological membranes and artificial lipid membranes show a relatively high intrinsic permeability to both water and neutral "lipophilic" molecules, such as indole and others (Hanai & Haydon, 1966; Huang & Thompson, 1966; Finkelstein & Cass, 1968; Price & Thompson, 1969; Lieb & Stein, 1969; Bean, Shepherd & Chan, 1968; Stein, 1967). This process of nonspecific permeation depends on: (1) the partition coefficient of the permeating molecules between the aqueous phase and the hydrocarbon region of the membrane; and (2) the molecular weight of the permeating molecules. In previous work, the nonspecific permeation has been explained in terms of channels or pores within the membranes. However, a satisfactory molecular theory for this general type of permeation is not yet available.

Zwolinski, Eyring and Reese (1949) discussed the problem from the point of view of absolute rate theory. Their theory regards the movement of molecules within membranes as a series of successive jumps from one equilibrium position to the next. A possible energy profile for a molecule diffusing through a membrane is shown in Fig. 1. However, when this

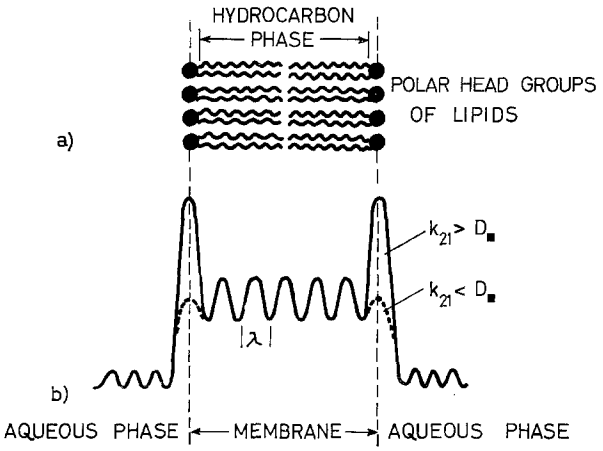

Fig. 1. (a) Bimolecular leaflet. The hydrocarbon phase is made up of fatty acid chains (CH_2-chains). (b) Possible potential energy profile for a molecule diffusing through a membrane

model is applied to biological systems, it is difficult to interpret the elementary processes and the parameters entering into the theory.

More recently, the diffusion of molecules within membranes has been discussed in connection with thermal fluctuations of the membrane-forming molecules. Lieb and Stein (1969) have shown that nonelectrolyte diffusion within biological membranes resembles diffusion within soft polymers. These authors proposed a diffusion mechanism analogous to the so-called free-volume theory of diffusion within polymers (Crank & Park, 1968). In this theory, it is assumed that transient "holes" or pockets of free volume are opened up by thermal fluctuations, which serve as the passage for diffusing molecules.

In the present paper, a detailed molecular mechanism for the diffusion within lipid membranes is proposed. This model is based on recent experimental and theoretical investigations of the mechanical relaxation of paraffins, polyethylene and other polymer materials (Pechhold, Blasenbrey & Woerner, 1963; Pechhold, Dollhopf & Engel, 1966; Pechhold & Blasenbrey, 1967; Blasenbrey & Pechhold, 1967; Pechhold, 1968). These studies strongly support the view that polymer materials, both in the crystalline and in the liquid crystalline state, contain certain types of *mobile* structural defects, so-called kinks, which result from conformational changes in the hydrocarbon chains. Kinks, if present in the hydrocarbon region of a membrane, would produce small mobile pockets of free volume of different size depending upon the type and the arrangement of the kinks. A molecule

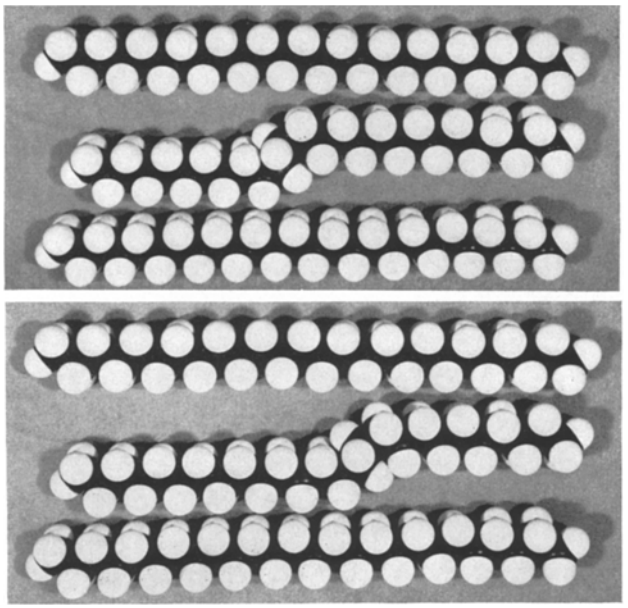

Fig. 2. A "$2g1$" kink in a CH_2-chain shown in different positions. This kink is formed by two rotations about C−C bonds which are separated by one chain unit; rotation angles $\varphi_1 = +120°$, $\varphi_2 = -120°$. By formation of one kink, the chain is shortened by one CH_2 unit length. Therefore, kinks cannot be generated and cannot disappear inside of the hydrocarbon bulk phase. They are more easily generated at the surface from where they migrate into the bulk phase

present in the aqueous phase adjacent to the membrane may jump into the free volume of a kink at the membrane surface and may then diffuse across the membrane together with the mobile kink, in a "hitch-hiking" process.

A permeation theory based on this picture requires knowledge of the molecular structure and of the process of formation and diffusion of kinks in a hydrocarbon phase. The molecular structure of the simplest so-called $2g1$ kink (*cf.* Fig. 3) in a CH_2 chain is shown in Fig. 2. This kink can be formed from a straight hydrocarbon chain by rotating about a particular C−C bond by an angle of 120° and rotating either of the two next nearest neighboring C−C bonds by −120°. By this procedure, two trans configurations in the CH_2 chain are transformed into gauche configurations.

The dependence of the potential energy on the angle of rotation about a C−C bond in such a chain has been calculated by Harris and Harris (1959) and by Volkenstein (1963) for a butane molecule as shown in Fig. 3. The energy difference ΔE between trans ($\varphi = 0°$) and gauche ($\varphi = 120°$)

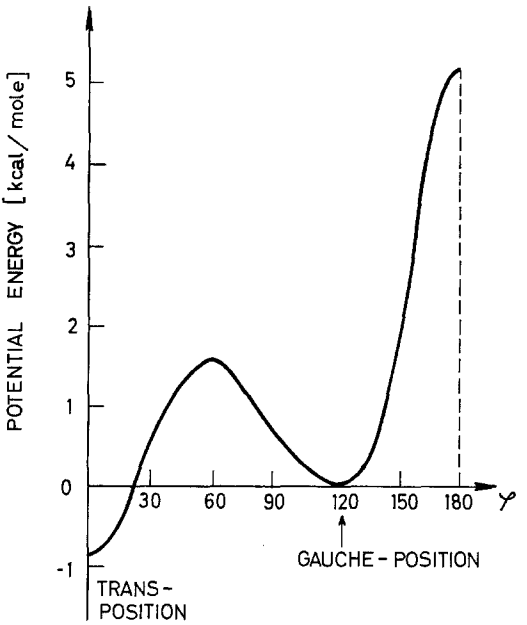

Fig. 3. Rotational potential energy for isolated butane molecule as a function of rotation angle φ about a C—C bond in the middle of the molecule (Volkenstein, 1963). Sum of exchange and Van der Waals energy. The maximum value at $\varphi = 180°$ is because of strong Van der Waals interaction between approaching H atoms. The normal trans configuration ($\varphi = 0°$) and the gauche configuration at $\varphi = 120°$ are separated by an activation barrier of about 2.4 kcal/mole. In a $2g1$ kink, two gauche configurations are separated by one trans configuration

configurations is 0.8 kcal/mole. Thus the formation of one kink increases the self-energy of an isolated chain by $2 \times 0.8 = 1.6$ kcal/mole. However, the activation energy for the transition from trans to gauche configuration is about 2.4 kcal/mole; two times this energy, i.e., 4.8 kcal/mole, must be supplied by the thermal energy to move a kink along the chain. Within a polymer material, there is a further contribution to the self-energy of a kink, owing to the distortion of the polymer chain in the neighborhood of the kink. This additional energy has been estimated by Pechhold (1968) to be about 2 kcal/mole. The equilibrium concentration of kinks is determined by the resulting change in free enthalpy ($\Delta G = \Delta H - T\Delta S$) of the whole polymer. The formation of kinks within a polymer is accompanied by a considerable increase in entropy or disorder (positive ΔS); therefore, kinks are favorable thermodynamically. Further, the free energy of formation and migration of kinks is comparable to the thermal energy at room temperature ($RT \approx$ 0.6 kcal/mole); therefore, it is to be expected that the hydrocarbon region

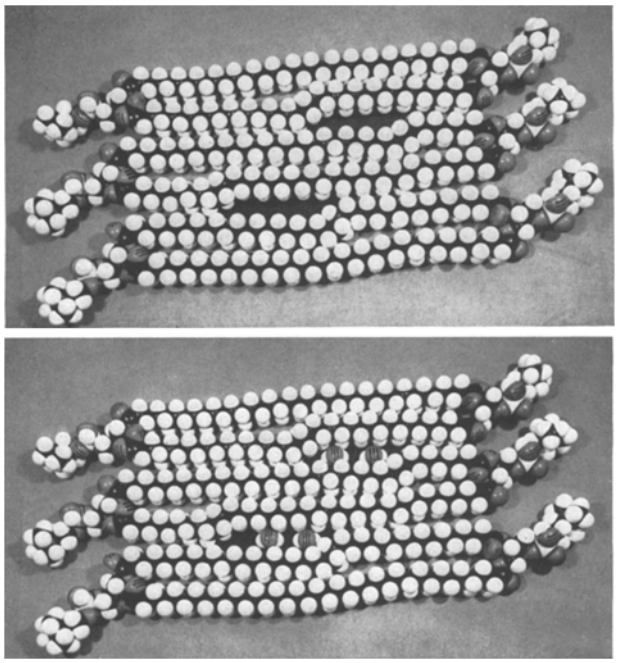

Fig. 4a and b. Diacyl-Phosphatidylcholine molecules in bilayer arrangement. (a) By combination of two $2g1$ kinks, fairly large pockets of free volume can be formed. (b) H_2O molecules or larger molecules fit into these pockets

of a membrane under equilibrium conditions contains many kinks, which are continuously moving up and down the hydrocarbon chains.

Pechhold and Blasenbrey (1967) and Pechhold (1968) have applied statistical methods to calculate absolute values of the kink concentration, expressed as the fraction ξ of CH_2 units of the polymer in gauche positions. In general, ξ increases with increasing temperature. In paraffins and linear polymers, as well as in lipids, the kink concentration undergoes two sharp increases with increasing temperature. One occurs at the transition temperature T_t *below* the melting point of the material. This temperature T_t is associated with the so-called "rotational phase transition". The second sharp increase in kink concentration with increasing temperature occurs at the melting point T_m. For $T \leqq T_t$, the ξ values range from 0.01 to 0.05, whereas for $T > T_t$, ξ ranges from 0.1 to 0.5. Most membrane-forming lipids at room temperature are above the transition temperature T_t. Thus the kink concentration of lipid membranes is probably between 0.1 and 0.5.

Fig. 4 shows the possible effect of kinks on the structure of the hydrocarbon phase within a lipid bilayer. By appropriate combination of kinks,

volumes large enough to accomodate molecules of the size of water o indole can be formed. Most of these "holes" will extend only a few chai segments and not completely across the membrane, so that in genera no pores will be formed by this mechanism. A simple consequence of thi picture is that the presence of kinks and an increase in kink concentratio should be accompanied by an increase in specific volume. In fact, Pechhol *et al.* (1966) have shown by X-ray measurements that the rotational phas transition in paraffins is accompanied by a volume change of 2 to 4%. If similar measurements are carried out with lipid membranes in aqueou solution, smaller volume changes of about 1% are found. This may be take to indicate that about 50% of the free volume of the kinks is occupie by water molecules.

A model is developed below for the nonspecific permeation throug membranes in which kinks serve as carriers for the permeating molecules. It is assumed that, at the polar surface of the membrane, complexes ar formed between the permeants and free kinks which then move across th membrane. A net flow of occupied kinks across the membrane is establishe if a difference in concentration of the permeant exists across the membrane. The net flow is calculated for a single neutral permeant, assuming that onl one type of kinks representing a fixed free volume is involved in the act o permeation. For this purpose, the coupling between the diffusional flow $\Phi_{\blacksquare}$ of occupied kinks and the formation and dissociation of kink-permeant complexes at the membrane surface must be considered. For this case (*see* Fig. 5), the two reactions at the membrane-solvent boundaries are:

$$\bullet + \square \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \blacksquare + \Phi_{\blacksquare} \qquad \text{at side (}'\text{) of membrane,} \tag{1}$$

and

$$\bullet + \square \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \blacksquare - \Phi_{\blacksquare} \qquad \text{at side (}''\text{) of membrane;} \tag{2}$$

where ● denotes the permeating molecule, □ the free kink, and ■ the occupied kink. k_{12} and k_{21} are the overall rate constants for the formation and dissociation of kink-permeant complexes. These constants are determined by the partition ratio of the permeating molecules between the aqueous phase and the hydrocarbon phase, and by the potential barrier at the interphase between membrane and solution. The dissociation equilibrium constant K is given by the ratio k_{21}/k_{12} for this reaction. K has the dimension of a concentration. A similar permeation problem has been formulated by Britton (1964).

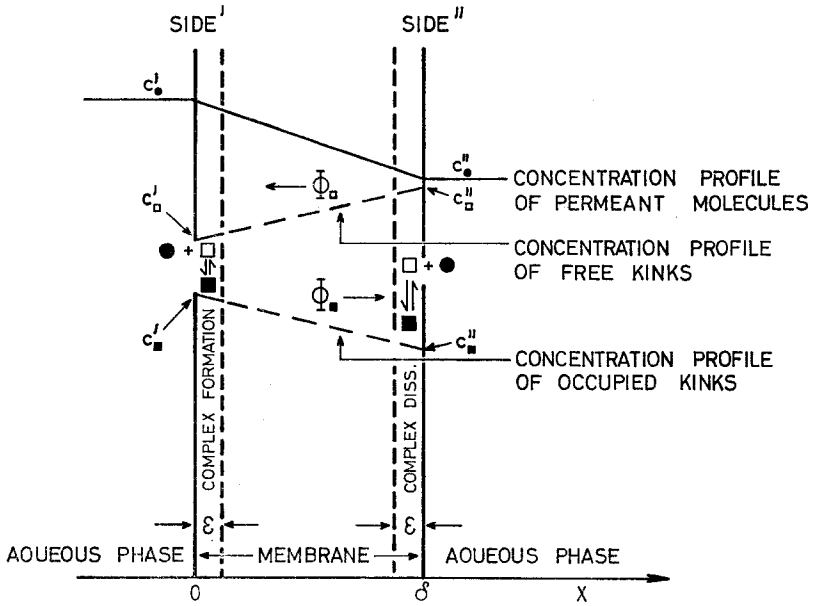

Fig. 5. Model used for calculation of kink-mediated flow of molecules across membrane. Symbols: ● permeant; □ free kink; ■ occupied kink; Φ flows. Permeant molecules jump on left side into free volumes of kinks and are carried across membrane (flow $\Phi_{\blacksquare}$) together with the kinks. The formation and dissociation of permeant-kink complexes take place within a surface sheet of thickness ε

The following notations are used:

$c'_{\bullet}$, $c''_{\bullet}$ = concentrations of permeant;
$c'_{\square}$, $c''_{\square}$ = concentrations of free kinks;
$c'_{\blacksquare}$, $c''_{\blacksquare}$ = concentrations of occupied kinks;
} on side (′) and side (″) of the membrane

δ = membrane thickness; and

$D_{\blacksquare}$ = diffusion coefficient of occupied kinks.

The steady state net flow of occupied kinks across the membrane is expressed as:

$$\Phi_{\blacksquare} = \frac{D_{\blacksquare}}{\delta}(c'_{\blacksquare} - c''_{\blacksquare}). \tag{3}$$

The rate expressions for reactions (1) and (2) are given by

$$c'_{\bullet}\, c'_{\square}\, \varepsilon k_{12} = c'_{\blacksquare}\, \varepsilon k_{21} + D_{\blacksquare} \frac{c'_{\blacksquare} - c''_{\blacksquare}}{\delta} \tag{4}$$

and

$$c''_{\bullet}\, c''_{\square}\, \varepsilon k_{12} = c''_{\blacksquare}\, \varepsilon k_{21} - D_{\blacksquare} \frac{c'_{\blacksquare} - c''_{\blacksquare}}{\delta}, \tag{5}$$

where ε denotes the thickness of the surface sheet of the membrane withi which complex formation and dissociation take place (*see* Fig. 5). The valu of ε is determined by the dimensions of the free volume of a kink.

Subtraction of Eq. (5) from Eq. (4) yields

$$\Phi_{\blacksquare} = D_{\blacksquare} \frac{c'_{\blacksquare} - c''_{\blacksquare}}{\delta} = \frac{D_{\blacksquare}}{\delta} \frac{k_{12}}{k_{21} + \frac{2D_{\blacksquare}}{\delta\varepsilon}} (c'_{\square}\, c'_{\bullet} - c''_{\square}\, c''_{\bullet}). \tag{6}$$

The net flow $\Phi_{\blacksquare}$ can be expressed as a function of the permeant concen trations $c'_{\bullet}$ and $c''_{\bullet}$, and of the total kink concentration c_k by eliminatin $c'_{\square}$ and $c''_{\square}$ from Eq. (6). For this purpose, the following assumptions ar made:

Assumption (1). The back flow of free kinks is assumed equal to the ne flow of occupied kinks:

$$\Phi_{\square} = \Phi_{\blacksquare}$$

or

$$\frac{D_{\square}}{\delta}(c''_{\square} - c'_{\square}) = \frac{D_{\blacksquare}}{\delta}(c'_{\blacksquare} - c''_{\blacksquare}). \tag{7}$$

Assumption (2). The diffusion coefficients of free and occupied kink are assumed to be equal:

$$D_{\square} = D_{\blacksquare}. \tag{8}$$

From Eqs. (7) and (8) we obtain:

$$c''_{\square} - c'_{\square} = c'_{\blacksquare} - c''_{\blacksquare}. \tag{9}$$

Assumption (3). The total kink concentration c_k is assumed to be con stant. Thus,

$$\frac{1}{\delta}\int_0^{\delta} \{c_{\blacksquare}(x) + c_{\square}(x)\}\, dx = c_k$$

or

$$c_k = \tfrac{1}{2}(c'_{\blacksquare} + c''_{\blacksquare}) + \tfrac{1}{2}(c'_{\square} + c''_{\square}). \tag{10}$$

The concentrations $c'_{\square}$ and $c''_{\square}$ may be eliminated from Eq. (6) as follows

Combine Eqs. (6) and (7) and let $D_{\square} = D_{\blacksquare}$ [assumption (2)]; this yield

$$c'_{\square} = c''_{\square} \frac{1 + c''_{\bullet}\left(\dfrac{k_{12}}{k_{21} + \dfrac{2D_{\blacksquare}}{\varepsilon\delta}}\right)}{1 + c'_{\bullet}\left(\dfrac{k_{12}}{k_{21} + \dfrac{2D_{\blacksquare}}{\varepsilon\delta}}\right)}. \tag{11}$$

From Eqs. (3), (5), (9) and (10), $c''_{\square}$ is expressed as

$$c''_{\square}=\frac{c_k-\frac{\Phi_{\blacksquare}}{\varepsilon k_{21}}}{1+c''_{\bullet}/K}. \tag{12}$$

Inserting $c'_{\square}$ and $c''_{\square}$ according to Eqs. (11) and (12) in Eq. (6), and setting the flow of permeant $\Phi_{\bullet}$ equal to $\Phi_{\blacksquare}\times n$, where n denotes the number of molecules per kink, yields the following expression for the flow of permeant:

$$\Phi_{\bullet}=\frac{D_{\blacksquare}}{\delta}\Delta c_{\bullet}\,c_k\frac{nK}{\left(K+c''_{\bullet}+\frac{2D_{\blacksquare}K}{\varepsilon\delta k_{21}}\right)(K+c''_{\bullet})+\left(K+c''_{\bullet}+\frac{D_{\blacksquare}K}{\varepsilon\delta k_{21}}\right)\Delta c_{\bullet}} \tag{13}$$

where $\Delta c_{\bullet}=c'_{\bullet}-c''_{\bullet}$ denotes the concentration difference of the permeant across the membrane.

Eq. (13) states that the kink-mediated flow of molecules across the membrane is essentially the product of three terms: (1) the expression $\frac{D_{\blacksquare}}{\delta}\Delta c_{\bullet}$, in which the concentration gradient $\Delta c_{\bullet}/\delta$ of the permeant across the membrane and the diffusion coefficient $D_{\blacksquare}$ of occupied kinks are combined into Fick's diffusion law; (2) the total kink concentration c_k; and (3) a factor which includes the complex formation and dissociation process at the membrane-solvent surface and other characteristic features of the kink-mediated transport. According to Eq. (13), the flow $\Phi_{\bullet}$ saturates with increasing concentration difference $\Delta c_{\bullet}$ of the permeant across the membrane. The maximum value of flow, $\Phi^{\max}_{\bullet}$, is given by

$$\Phi^{\max}_{\bullet}=\frac{D_{\blacksquare}Knc_k}{\delta}\frac{1}{K+c''_{\bullet}+\frac{D_{\blacksquare}K}{\varepsilon\delta k_{21}}}, \tag{14}$$

and depends upon the concentration of the permeant in the aqueous phase.

Eq. (13) is considered for two limiting cases:

$$\text{(a)}\ \frac{2D_{\blacksquare}}{\varepsilon\delta}\gg k_{21},\quad\text{and}\quad\text{(b)}\ \frac{2D_{\blacksquare}}{\varepsilon\delta}\ll k_{21}.$$

In case (a), the diffusion is fast compared to the dissociation of the kink-substrate complex. Equilibrium does not exist between kinks and permeant on the membrane surface. The dissociation rate constant k_{21} is rate limiting.

In case (b), the diffusion is slow compared to the kink-permeant dissociatio allowing equilibrium between permeant and carrier to be establishe ($\bullet + \square \rightleftharpoons \blacksquare$). The diffusion coefficient $D_\blacksquare$ of the kinks is rate limiting

(a) *Kink-Substrate Complex Dissociation is Rate Limiting.* Condition (a together with $\Delta c_\bullet \ll c_\bullet$ leads to

$$\Phi_\bullet = \frac{D_\blacksquare}{\delta}\left(\frac{n c_k}{K + c''_\bullet}\right)\frac{K}{\frac{D_\blacksquare}{\delta \varepsilon k_{12}} + c''_\bullet}\Delta c_\bullet, \tag{15.1}$$

which for $\frac{D_\blacksquare}{\delta \varepsilon k_{12}} \gg c''_\bullet$ simplifies to

$$\boxed{\Phi_\bullet = k_{21}\varepsilon\left(\frac{n c_k}{K + c''_\bullet}\right)\Delta c_\bullet}. \tag{15.2}$$

(b) *Diffusion within the Membrane is Rate Limiting.* Condition (b leads to

$$\Phi_\bullet = \frac{D_\blacksquare}{\delta}\left(\frac{n c_k}{K + c''_\bullet}\right)\frac{K}{K + c'_\bullet}\Delta c_\bullet, \tag{16.1}$$

which for $c'_\bullet \ll K$ simplifies to

$$\boxed{\Phi_\bullet = \frac{D_\blacksquare}{\delta}\left(\frac{n c_k}{K + c''_\bullet}\right)\Delta c_\bullet}. \tag{16.2}$$

The term $n c_k/(K + c''_\bullet)$ in these equations can be identified with the equilib rium partition coefficient K_p of the permeant between the aqueous phas and the membrane. For the case of equilibrium, reaction (1) reads: $\bullet + \square \rightleftharpoons \blacksquare$ The corresponding rate equation is $c_\bullet c_\square k_{12} = c_\blacksquare k_{21}$. With $c_\square = c_k - c_\blacksquare$ and $K = k_{21}/k_{12}$, we obtain:

$$\frac{n c_k}{K + c_\bullet} = \frac{n c_\blacksquare}{c_\bullet} = K_p. \tag{17}$$

The term $n c_\blacksquare/c_\bullet$ is the concentration ratio of the permeant between the membrane phase and the aqueous phase. This value thus represents the partition coefficient K_p of the system.

Eqs. (15.2) and (16.2) are formally identical. However, in case (a) the rate limiting factor is $k_{21}\varepsilon$, whereas in case (b) the ratio $D_\blacksquare/\delta$ between the diffusion coefficient of the kinks and the membrane thickness is rate limiting. The diffusion coefficient $D_\blacksquare$ of the kinks can be regarded as an intrinsic property of the hydrocarbon phase of the membrane, whereas

k_{21} depends both on the polar head groups of the membrane and on the affinity of the permeating molecule to the hydrocarbon phase of the membrane. The apparent activation energies are different for the two cases; they are determined by the partition coefficient K_p and by the factors k_{21} and $D_{\blacksquare}$, respectively. A plot of ln $\Phi_{\bullet}$/T against 1/T will give a straight line only if $K \gg c_{\bullet}$. In the general case, described by Eq. (13), a plot of ln $\Phi_{\bullet}$/T against 1/T will be curved, since the expression for $\Phi_{\bullet}$ contains sums of exponential functions.

In order to test our model against permeability data from lipid bilayers, the absolute values for the diffusion coefficient $D_{\blacksquare}$ and the concentration c_k of kinks must be known. The diffusion coefficient $D_{\blacksquare}$ for kinks can be expressed as:

$$D_{\blacksquare} = p\lambda^2 = \nu \cdot e^{\frac{\Delta S}{R}} \cdot e^{-\frac{Q}{RT}} \lambda^2, \tag{18}$$

where λ refers to the distance between two successive equilibrium positions of a kink moving along a CH_2 chain (*see* Fig. 1); thus $\lambda = 1.27$ Å. The term $p = \nu \cdot e^{\frac{\Delta S}{R}} \cdot e^{-\frac{Q}{RT}}$ denotes the probability for a jump per second. ν is the frequency of the thermal vibrations and can be assumed equal to the frequency ν_r of the CH_2 rocking vibrations; from infrared spectroscopy data of paraffins and lipids, ν_r is known to be 2×10^{13} sec^{-1} (Chapman, 1965). The activation energy Q for the movement of a kink is given by two times the value of the energy barrier for the trans → gauche conformational change (*see* Fig. 3); thus $Q = 4.8$ kcal/mole.

The entropy factor $e^{\frac{\Delta S}{R}}$ has a value of about 10 (Pechhold, 1968). This leads to

$$D_{\blacksquare} = 1 \times 10^{-5} \text{ cm}^2/\text{sec}. \tag{19}$$

We note that $D_{\blacksquare}$ has the same order of magnitude as the diffusion coefficient for molecules such as ethanol or phenol in water. Thus the diffusion of kinks within a hydrocarbon phase is a very fast process.

The kink concentration c_k, which is defined by the fraction ξ of CH_2 groups in gauche position, can be assumed equal to the corresponding value calculated for paraffins in the liquid crystalline state; thus $\xi \approx 0.1$ (Pechhold, 1968). Taking $\delta = 50$ Å for the membrane thickness and assuming that each hydrocarbon chain of the lipids is 20 CH_2-groups in length and has a cross-sectional area of 20 [Å]^2 (Van Deenen, Houtsmuller, de Haas & Mulder, 1962), one finds for the concentration of kinks

$$c_k = \xi \times 8.5 \times 10^{-2} \tag{20}$$

kinks in mole per cm^3 of hydrocarbon phase.

Except with respect to water, the data for the permeation of molecule through lipid bilayer membranes are rather meager. The values for the water permeability coefficient P [cm/sec] obtained by several investigators using various kinds of phospholipids range from 10^{-2} to 5×10^{-4} cm/sec; however, most values are concentrated at 2×10^{-3} cm/sec (Hanai & Haydon 1966; Huang & Thompson, 1966; Cass & Finkelstein, 1967; Finkelstein & Cass, 1968).

For a quantitative comparison with the theory, the permeability coefficient P_{theor} is calculated from Eq. (16.1), assuming that the permeation rate is determined by the diffusion through the bulk membrane. In order to estimate the value of the equilibrium dissociation constant $K = k_{21}/k_{12}$ in Eq. (16.1), we identify the partition coefficient $K_p = n c_k/(K + c''_{\bullet})$ with the partition coefficient $K_p = 0.64 \times 10^{-4}$ between water and hexadecane as measured by Schatzberg (1963, 1965). Using c_k according to Eq. (20) and taking $\xi = 0.1$, one calculates the value of K as 1.3×10^2 mole/cm^3 thus $K \gg c$. Using this together with $D_{\blacksquare}$ as given by expression Eq. (19) we obtain $P_{\text{theor}} = 1.3 \times 10^{-3}$ cm/sec. This value can well be in error by at least one order of magnitude, mainly for two reasons. (1) The used value for the kink concentration c_k has been adapted from calculations for hydrocarbon bulk material. Even in this case, c_k could be five times larger than assumed. (2) The partition coefficient between water and hexadecane may be different from the required partition coefficient of the water-phospholipid system. Thus the good agreement between the estimated and the measured values ($P_{\text{exp}} = 2 \times 10^{-3}$ cm/sec) may be somewhat fortuitous.

The proof of the validity of the proposed kink-mediated permeation mechanism requires, of course, a systematic investigation of the characteristic functional dependencies expressed by Eq. (13), for example, the dependence of the flow $\Phi_{\bullet}$ on permeant concentration, or the predicted saturation behavior of $\Phi_{\bullet}$ with increasing concentration difference $\Delta c_{\bullet}$. A further test could be provided by measuring the temperature dependence of the flow $\Phi_{\bullet}$ in membranes which undergo a phase transition with increasing temperature. At the phase transition, the kink concentration is expected to increase sharply which should result in a comparable increase of permeability.

Our discussion until now has referred to fully saturated hydrocarbon chains. However, most naturally occurring lipids are partially unsaturated, the double bonds being of the cis-type. These molecules will fit into a regular arrangement only if kinks are formed near the double bond. Thus it is expected that the kink concentration and consequently the permeability increases with the increasing degree of unsaturation. In fact, Finkelstein and Cass (1968) reported that membranes formed from egg lecithin completely

saturated by hydrogenation have a P value of 1.7×10^{-3} cm/sec compared to $P = 4.2 \times 10^{-3}$ cm/sec for the partially unsaturated egg lecithin.

Another phenomenon of interest is the decrease of water permeability with increasing cholesterol concentration. Finkelstein and Cass (1968) observed a decrease in water permeability from 4.2×10^{-3} to 0.75×10^{-3} cm/sec as the cholesterol-phospholipid molar ratio increased from 0:1 to 8:1. This effect can be attributed to the decrease in kink concentration accompanying the decrease in hydrocarbon contents.

The foregoing treatment can be generalized in several respects.

(1) The diffusion coefficient of the free kinks can be different from the diffusion coefficient of occupied kinks ($D_\square \neq D_\blacksquare$). The following expression for the permeant flow $\Phi_\bullet$ is obtained for this case:

$$\Phi_\bullet = \frac{D_\blacksquare}{\delta} \Delta c_\bullet c_k \cdot \frac{nK}{\left(K + c''_\bullet \frac{D_\blacksquare}{D_\square} + \frac{2D_\blacksquare K}{\varepsilon \delta k_{21}}\right)(KK' + c''_\bullet) + \left(KK' + c''_\bullet \frac{D_\blacksquare}{D_\square} \frac{D_\blacksquare K}{\varepsilon \delta k_{21}}\right) \cdot \Delta c_\bullet} \tag{21}$$

where

$$K' = \frac{1}{2}\left(\frac{D_\square}{D_\blacksquare} + 1\right) - \frac{1}{2}\left(\frac{D_\square}{D_\blacksquare} - 1\right) \frac{1 + c''_\bullet \dfrac{k_{12}}{\left(k_{21} + \dfrac{2D_\blacksquare}{\varepsilon \delta}\right)} \dfrac{D_\blacksquare}{D_\square}}{1 + c'_\bullet \dfrac{k_{12}}{\left(k_{21} + \dfrac{2D_\blacksquare}{\varepsilon \delta}\right)} \dfrac{D_\blacksquare}{D_\square}}$$

For $D_\blacksquare / D_\square \to 1$, Eq. (21) goes over into Eq. (13).

(2) The net back flow of free kinks can be different from the net flow of occupied kinks ($\Phi_\square \neq \Phi_\blacksquare$). In this case, it must be assumed that kinks are continuously generated on one side of the membrane and disappear on the other side of the membrane. It is possible that the permeant molecules have an influence on the kink-formation frequency.

(3) It is conceivable that, in the general case, different types of kinks with different concentrations, different free volumes and different diffusion coefficients participate in the transport of permeants. This leads to a selectivity between permeant molecules of different mass and volume.

(4) The possible dependence of the kink concentration c_k on the length of the hydrocarbon chains should be taken into account. It has been shown by Blasenbrey and Pechhold (1967) in a theoretical study that the kink concentration in paraffin lamellae increases with decreasing chain length.

However, this effect is expected to be less important for lipid lamellæ because the hydrocarbon chains are anchored pairwise to the glycerol par of the lipid molecules.

(5) In the foregoing derivation, the kinks have been considered to b independent of one another. It is possible for high values of the kink co centration that the interaction between the kinks may play a role, but th importance of this effect cannot be estimated before experimental valu of the kink concentration in lipid membranes are available. Measuremen of membrane thickness or volume could be used for the determinatio of kink concentrations. The presence of kinks reduces the length of th hydrocarbon chains compared to the fully stretched molecules and it lea to a volume increase of the membrane hydrocarbon phase. Thus it shoul be possible to estimate the kink concentration from X-ray studies of th membrane thickness and from measurements of the membrane volume i comparison with closely packed hydrocarbon crystals. Some qualitativ evidence exists for a substantial kink concentration in phospholipid mem branes. The thickness of the hydrocarbon phase of lipid leaflets determine by X-ray studies is substantially lower than twice the length of the full extended hydrocarbon chains (Luzatti, 1968). A sharp increase of the specifi volume of aqueous dipalmitoylphosphatidylcholine dispersions has bee observed by the author at the temperature of the phase transition fro the crystalline to the liquid crystalline state at 40 °C. The increase in kin concentration at the phase transition can be estimated from this resul A report of this work is in preparation.

Final Remarks

In the model proposed here, the hydrocarbon phase of the lipid membran is considered as a defective *ordered* structure. The kink mechanism wi not be applicable when the degree of disorder is so high that the hydrocarbo phase resembles a fluid more than an ordered structure. It has been suggeste (Chapman, 1965) that the hydrocarbon chains in all membranes and bilaye of biological significance are in a liquid crystalline state, or at a temperatur above T_t, characteristic of the transition from the crystalline to the liqui crystalline state (for hydrated dipalmitoylphosphatidylcholine, $T_t \approx 40$ °C The degree of configurational freedom of the hydrocarbon chains in th liquid crystalline state is not well known. Phillips, Williams and Chapma (1969) concluded from a comparison of thermodynamic parameters that th chain fluidity in the liquid crystalline state of lipids is about half that foun

in liquid *n*-alkanes. Further information bearing on this question may be deduced from X-ray investigations. For $T < T_t$, a sharp diffraction maximum near 4.2 Å is observed (Luzatti, 1968; Engelman, 1970), characteristic of a well-ordered structure. Above T_t, a broader maximum near 4.6 Å is observed, indicative of a "more fluid" state. A comparison of X-ray studies with monolayer experiments on lipids in the liquid crystalline state has been performed by Lecuyer and Dervichian (1969). These authors conclude that, although in the fluid state, the hydrocarbon chains have a limited degree of freedom, corresponding to that of a condensed monolayer.

The kink model in the form proposed here is meant to describe the permeation of small neutral molecules with an effective volume equal to or somewhat larger than the volume of a CH_2 monomer unit. Much smaller neutral permeants may find enough free volume between the hydrocarbon chains to permeate the membrane in a hopping mechanism similar to the mechanism of diffusion of interstitial atoms within solids. The proposed kink mechanism cannot be applied to the diffusion of relatively large molecules, such as cyclic antibiotics. The presence of such large molecules within the hydrocarbon phase of a membrane would require large distortions of the hydrocarbon chains. Such distortions can, however, be described in terms of formation of a combination of kinks in neighboring chains. It is conceivable that the cooperative displacement of these kinks represents the rate-limiting mechanism for the diffusion of very large molecules within the hydrocarbon phase of the membrane.

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