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# Transport of Lipids Through Water as Exchange Mechanism Between two Liposome Populations

G. Duckwitz-Peterlein and H. Moraal

Institut für Theoretische Physik der Universität zu Köln,
D-5000 Köln 41, Federal Republic of Germany

**Abstract.** A theoretical description of lipid exchange between two liposome populations as a linear transport phenomenon is presented. As a special case of the general theory in which two different lipids are simultaneously exchanged we also obtain formulae for "one-way" lipid transfer. The case in which the lipids differ only by a radioactive label is also included in a natural way. The predictions of the model are shown to be in excellent agreement with two recent experiments. This provides additional strong support for the hypothesis that lipid transfer takes place mainly through the aqueous phase.

**Key words:** Liposomes – Lipid exchange – Linear transport.

## 1. Introduction

In the last decade much experimental evidence has been accumulated concerning the exchange of lipids between different membrane structures. Phospholipid exchange between natural membranes in vitro has been shown to take place (Wirtz, 1974; McMurray and Dawson, 1969; Wojtczak et al., 1971; Akiyama and Sagakami, 1969) as well as phospholipid and cholesterol exchange between artificial liposomes and subcellular organelles (Zilversmit, 1971; Wirtz and Zilversmit, 1969; Ehnholm and Zilversmit, 1973; van den Besselaar et al., 1975). Cholesterol and phospholipids have also been transferred into viable cells upon treatment with liposomes (Inbar and Shinitzky, 1974; Grant and McConnell, 1973; Pagano et al., 1974; Papahadjopoulos et al., 1974). The mechanisms by which this transfer may take place are dependent not only on the systems studied, but also on the conditions under which the experiments are performed. Therefore, it is difficult to compare different studies concerned with liposome-liposome interactions. Under certain conditions there is evidence for liposome-cell membrane fusion (Grant and McConnell, 1973; Pagano et al., 1974; Papahadjopoulos et al., 1974) or liposome-liposome fusion (Taupin and McConnell, 1972; Paphadjopoulos et al., 1974; Prestegard and Fellmeth, 1974; Kantor and Prestegard, 1975; Lawaczek et al., 1975; Martin and MacDonald,

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1976; Marsh et al., 1976), but molecular transport of lipids through the aqueous phase (Demel et al., 1973; Pagano and Huang, 1975) has also been observed.

The most careful experiments on the interaction between two liposome populations have been performed recently by Martin and MacDonald (1976) and by Duckwitz-Peterlein et al. (1977). Both these experiments make use of optical methods to determine the transition temperature $T_t$ of a liposome dispersion. This transition may be described as a transition from a two-dimensional solid phase (hexagonally closest packed bilayer) to a two-dimensional liquid crystalline phase in which the lipid molecules can move rather freely in the plane of the bilayer (Ladbrooke and Chapman, 1969; Overath and Träuble, 1973). The temperature $T_t$ is, for identical polar head groups, characteristic of the acyl chain composition of the lipid (Ladbrooke and Chapman, 1969) and there is a rather sharp drop in turbidity on increasing the temperature through $T_t$ (Yi and MacDonald, 1973; Abramson, 1971) as well as in the intensity of 90° light scattering (Overath and Träuble, 1973). This turbidity change was employed by Martin and MacDonald (1976) to determine the transition temperatures $T_t'$ and $T_t''$ of two liposome populations as function of time. Duckwitz-Peterlein et al. (1977) used 90° light scattering for the same purpose.

Since the transition temperature depends on the acyl moieties of the lipid molecules, $T_t$ should be a function of the composition of the liposomes if two types of acyl moieties are present initially. In the above mentioned systems $T_t$ depends linearly on the mole fraction of one of the components present (Duckwitz-Peterlein et al., 1977; Lee, 1977; Duckwitz-Peterlein, unpublished results). The experiments of Martin and MacDonald (1976) and Duckwitz-Peterlein et al. (1977) therefore allow the time dependence of the composition of both liposome populations to be followed.

The mechanism of fusion appears to be ruled out by the experiments reported in [6 and 19], since two distinct and sharp transitions are observed for all times for which the compositions are not yet indistinguishable. There remain two other mechanisms: a) exchange of lipid molecules on collision of liposomes and b) exchange of lipid molecules via the aqueous phase. In case a) one expects the rate of lipid transfer to be quadratic in the liposome concentration; instead, this rate is found to be independent of liposome concentration (Duckwitz-Peterlein et al., 1977). This difficulty may, of course, be overcome if one assumes that the collision rate is not the rate-determining step of the exchange process. Such a highly artificial explanation is made very unlikely by the excellent agreement between the theory based on possibility b) presented in this article and the experimental data of [6 and 19].

The theoretical description of the exchange of lipid molecules via the aqueous phase is analogous to a recent treatment of membrane transport processes (Duckwitz and Moraal, 1977) and is detailed in the next section. In section 3 we relate this description to the more familiar phenomenological linear transport laws of irreversible thermodynamics. Section 4 deals with the comparison of the experimental results of [6 and 19] with the predictions of the theory and a discussion of the results is given in section 5.

## 2. Theoretical Description of Transport of Lipids Through the Aqueous Phase

We consider two populations of liposomes, denoted by a prime (′) and a double prime (″), respectively, consisting of two types of lipid molecules $L_1$ and $L_2$. Since the solubility of lipids in water is very small, we may neglect the number of lipid molecules present in the aqueous phase at all times. We denote by $n_1'(t)$ $[n_2'(t)]$ and $n_1''(t)$ $[n_2''(t)]$ the number of moles of $L_1$ $[L_2]$ in the two populations at time $t$. The mole fractions $x'(t)$ and $x''(t)$ of lipid $L_1$ are then

$$x'(t) = n_1'(t)\,[n_1'(t) + n_2'(t)]^{-1}\,,$$

$$x''(t) = n_1''(t)\,[n_1''(t) + n_2''(t)]^{-1}\,. \tag{1}$$

Further our assumption of small solubility implies the constancy in time of the total number of lipids $L_i$ in the two phases:

$$n_1'(t) + n_1''(t) = N_1\,, \qquad n_2'(t) + n_2''(t) = N_2\,. \tag{2}$$

Equations (1) and (2) may be solved for the number of moles $n_i^{(\prime,\prime\prime)}(t)$ as

$$\frac{n_1'}{N_1 + N_2} = x'(x_\infty - x'')\,(x' - x'')^{-1}\,,$$

$$\frac{n_2'}{N_1 + N_2} = (1 - x')\,(x_\infty - x'')\,(x' - x'')^{-1}\,,$$

$$\frac{n_1''}{N_1 + N_2} = x''(x' - x_\infty)\,(x' - x'')^{-1}\,,$$

$$\frac{n_2''}{N_1 + N_2} = (1 - x'')\,(x' - x_\infty)\,(x' - x'')^{-1} \tag{3}$$

where $x_\infty$ is the equilibrium mole fraction:

$$x_\infty = \frac{N_1}{N_1 + N_2}\,. \tag{4}$$

We assume here that the two lipids are completely miscible (or nearly so), so that $x_\infty$ is the true equilibrium mole fraction.

The normalized fluxes $\phi_{1,2}$ of lipids $L_{1,2}$ are then given as

$$\phi_1 = -\frac{dn_1'}{dt}\left[\frac{1}{N_1 + N_2}\right] = -\frac{dP}{dt} - x_\infty \frac{dQ}{dt}\,,$$

$$\phi_2 = -\frac{dn_2'}{dt}\left[\frac{1}{N_1 + N_2}\right] = -\frac{dP}{dt} - (1 - x_\infty)\frac{dQ}{dt}\,, \tag{5}$$

where we introduced the abbreviations

$$P = yz(y + z)^{-1}, \qquad Q = z(y + z)^{-1}; \tag{6}$$

$$y = x' - x_\infty, \qquad z = x_\infty - x''. \tag{7}$$

The variables $y$ and $z$ are chosen in such a way as to be positive for all times for the initial conditions $x'(0) > x''(0)$.

Since the only quantity which distinguishes the two liposome populations is the mole fraction, the $\phi_i$ must be functions of these mole fractions, $\phi_i = \phi_i(x', x'')$.

Alternatively, we can write $\phi_i = \phi_i(x' - x'', x' + x'')$ and expand $\phi_i$ in a Taylor series in $x' - x''$. Since the fluxes must be odd functions of $x' - x''$, i.e., change sign upon interchange of the labels (′) and (″) of the liposome populations, such an equation has the form:

$$\phi_i = A_i(x' - x'') + C_i(x' - x'')^3 + 0\,[(x' - x'')^5], \tag{8}$$

where $A_i$ and $C_i$ are functions of $x' + x''$ only. Close to equilibrium, we may set $A_i = A_i(x' + x'') \approx A_i\,(2\,x_\infty)$ and neglect terms of higher than first order in $x' - x''$:

$$\phi_i \approx (-1)^{i+1} p_i(x' - x'') = (-1)^{i+1} p_i(y + z) \tag{9}$$

where we have set $(-1)^{i+1} p_i = A_i$ in order to have the $p_i$ both positive. We expect these $p_i$ to be independent of the liposome concentration since the rate of exchange can only depend on the details of the interaction of the liposome surface with the solution which under all circumstances may be assumed to be saturated with lipids.

In using this kind of model for membrane transport it was found (Duckwitz and Moraal, 1977) that Equation (9) is valid up to quite large values of $x' - x''$. This is due to the absence of a term proportional to $(x' - x'')^2$ in Equation (8) and to the fact that $x' + x''$ does not deviate too far from $2\,x_\infty$ in most cases. We, therefore, assume Equation (9) to hold for the case studied here for all values of $x'$ and $x''$.

Since both fluxes $\phi_i$ are proportional to $x' - x''$, Equations (5) imply

$$\frac{dP}{dt} = \alpha(y + z); \qquad \frac{dQ}{dt} = -\beta(y + z), \tag{10}$$

with $\alpha$ and $\beta$ related to $p_{1,2}$ by

$$p_1 = -\alpha + x_\infty\beta, \qquad p_2 = -\alpha - (1 - x_\infty)\beta. \tag{11}$$

Insertion of Equation (6) into Equation (10) yields a set of two coupled differential equations for $y$ and $z$. These have been solved in [5] with the result that $y$ and $z$ may be expressed in terms of a function $h(t)$ by

$$y = -\frac{\alpha}{\beta}\frac{h}{h - \beta C}, \qquad z = \frac{\alpha}{\beta}\frac{h}{(1 - \beta C) + h} \tag{12}$$

and $h(t)$ is implicitly given by

$$F(h) = \frac{1}{2}h^2 + (1 - 2\beta C)h + [(\beta C)^2 - \beta C] \ln |h| = -\alpha t + D . \tag{13}$$

Here $C$ and $D$ are integration constants depending on the initial conditions.

It follows from Equation (12) upon elimination of $h$ that a plot of $y/z$ versus $y$ should give a straight line:

$$\frac{y}{z} = \frac{1}{\alpha C} y + \left(\frac{1}{\beta C} - 1\right) . \tag{14}$$

If this is indeed found, the parameters of this straight line furnish the values of $\alpha C$ and $\beta C$ and, therefore, also of $\alpha/\beta$. These parameters may be used to calculate $h$ from $y$ by inverting the first of Equation (12):

$$h = (\beta C) y \left(y + \frac{\alpha}{\beta}\right)^{-1} \tag{15}$$

and $F(h)$ of Equation (13) can be calculated and plotted against the time. If again a straight line is obtained, $\alpha$ follows from the slope and, since $\alpha/\beta$ is already known, $\beta$ and the permeabilities $p_{1,2}$ can be calculated. It should be stressed that the straight line *tests* are of more importance than the actual values of $p_{1,2}$ obtained since these tests, if found to be conclusive, are strong evidence in favours of the correctness of the transport phenomenon proposed here.

A special case deserves a more explicit treatment. Assume lipid $L_1$ does not diffuse at all and let the initial conditions be $x'(0) = 1$ and $x''(0) = 0$, i.e., the primed population consists initially of a non diffusing lipid $L_1$ and the double primed population consists of pure diffusing lipid $L_2$. Under these circumstances $x''$ cannot change in time, i.e., we have $z = x_\infty - x'' = x_\infty$. Also, $p_1 = 0$ implies $\alpha = \beta x_\infty$ by the first of Equation (11). The second of Equation (12) can only yield $z = x_\infty$ if $\beta C = 1$. Therefore, the first of Equation (12) becomes

$$y = -\frac{x_\infty h}{(h-1)} , \tag{16}$$

while Equation (13) reduces to

$$F(h) = \frac{1}{2}h^2 - h = -\alpha t + D . \tag{17}$$

Now the initial condition $y(0) = 1 - x_\infty$ implies for $h$ the initial value $h(0) = 1 - x_\infty$ also and this in turn shows that

$$D = \frac{1}{2}(1 - x_\infty)^2 - (1 - x_\infty) . \tag{18}$$

The solution of Equation (17) satisfying the initial condition is then

$$h(t) = 1 - \sqrt{x_\infty^2 - 2\alpha t} . \tag{19}$$

Inserting this into Equation (16) and using the facts that $x' = y + x_\infty$ and, from the second of Equation (11), $p_2 = -\alpha/x_\infty$ yields finally for $x'(t)$ the expression

$$x'(t) = \left[1 + \frac{2\,p_2 t}{x_\infty}\right]^{-1/2}, \tag{20}$$

or, expressed differently,

$$\left[\frac{1}{x'}\right]^2 = 1 + \frac{2\,p_2}{x_\infty} t\,. \tag{21}$$

Equation (21) shows that for the special case considered here, i.e., for $p_1 = 0$ and $x'(0) = 1$, $x''(0) = 0$, a plot of $(1/x')^2$ versus time should give a straight line. It is to be noted that Equation (21) cannot hold for all times; as $t$ approaches the time $t_c$ defined by

$$\left[\frac{1}{x_\infty}\right]^2 = 1 + \frac{2\,p_2}{x_\infty} t_c\,, \tag{22}$$

the number of molecules in the ($''$)-phase becomes comparable to the number of molecules in the aqueous phase and the description given here of the diffusion phenomenon breaks down. We expect, however, that Equation (21) will be valid for all times $t < t_c$, i.e., that $x'$ will be very close to $x_\infty$ before the description breaks down.

The most interesting feature of the previous special case is the strongly nonexponential form of the decay. There is another special case in which the decay of the $x'$ and $x''$ is purely exponential. This is obtained only if $p_1 = p_2$ for all values of $x_\infty$ implying $\beta = 0$ and

$$p_1 = p_2 = -\alpha\,. \tag{23}$$

This case may be realized if both liposome populations consist of identical lipids, but one of the populations is labelled by means of a radioactive isotope.

Equation (10) are now easily solved to give exponential solutions for $x = x'$ or $x''$:

$$x = x_\infty + (x(0) - x_\infty) \exp - \alpha' t\,, \tag{24}$$

where $\alpha'$ is proportional to $p = p_1 = p_2$.

This result is, of course, identical with earlier results on the kinetics of isotope exchange reactions (McKay, 1938) and have been used (van den Besselaar et al., 1975) to study lipid exchange in the presence of phospholipid exchange protein (Wirtz and Zilversmit, 1969; Ehnholm and Zilversmit, 1973; van den Besselaar et al., 1975). We will not consider this special case further in this article.

## 3. The Connection to Irreversible Thermodynamics

In the previous section we derived the linear transport Equations (9) from a consideration of the functional form of the fluxes and from the fact that the fluxes can only depend on $x'$ and $x''$ since these are the only parameters distinguishing the liposomes. On the other hand, it is well-known from nonequilibrium thermodynamics that close to equilibrium the fluxes have to be linear functions of the differences of the chemical potential of the components in the two phases (de Groot and Mazur, 1962; Schlögl, 1964):

$$\phi_1 = L_{11}\Delta\mu_1 + L_{12}\Delta\mu_2\,, \qquad \phi_2 = L_{21}\Delta\mu_1 + L_{22}\Delta\mu_2\,. \tag{25}$$

Here $\Delta\mu_i$ is the difference in chemical potential of lipid $L_i$ between the liposome populations and the $L_{ij}$ are phenomenological coefficients satisfying $L_{ij} = L_{ji}$ (Onsager relation), $L_{ii} \geqslant 0$ and $L_{11}L_{22} - L_{12}^2 \geqslant 0$. It has been shown by a number of authors (Duckwitz and Moraal, 1977; Krämer and Sauer, 1967) that if the pressures of two phases are equal, then the $\Delta\mu_i$ are proportional to $x' - x''$ and Equations (25) and (9) are equivalent with the identifications:

$$p_1 = k_B T\, q \left[\frac{L_{11}}{x_\infty} - \frac{L_{12}}{1 - x_\infty}\right], \qquad p_2 = k_B T\, q \left[-\frac{L_{12}}{x_\infty} + \frac{L_{22}}{1 - x_\infty}\right], \tag{26}$$

where $k_B$ is Boltzmann's constant, $T$ the absolute temperature and $q$ is a factor dependent on the lipid-lipid interactions in the membrane (Duckwitz and Moraal, 1977). Since this factor is unknown in practice, Equation (26) are most useful in ratio form:

$$\frac{p_1}{p_2} = \frac{(1 - x_\infty)L_{11} - x_\infty L_{12}}{(x_\infty - 1)L_{12} + x_\infty L_{22}}\,. \tag{27}$$

The equivalence shown above is valid only if the pressures, in the sense of surface tension for the two-dimensional systems studied here, in the two liposome populations are equal. In general, however, these surface tensions will also depend on the composition of the liposomes in an unknown way. It should be stressed that this does *not* invalidate the assumptions made in the previous section since there we only used the fact that the liposomes experience an identical aqueous environment, but an interpretation of permeability ratios $p_1/p_2$ in terms of phenomenological coefficients $L_{ij}$ may not be possible if the measurements are not performed in the neighbourhood of equilibrium.

## 4. Experimental Verifications

### *1. The Experiment of Martin and MacDonald*

As already stated in the introduction, Martin and MacDonald (1976) used the change in turbidity at $T_t$ of a dispersion of liposomes to study lipid transfer. They

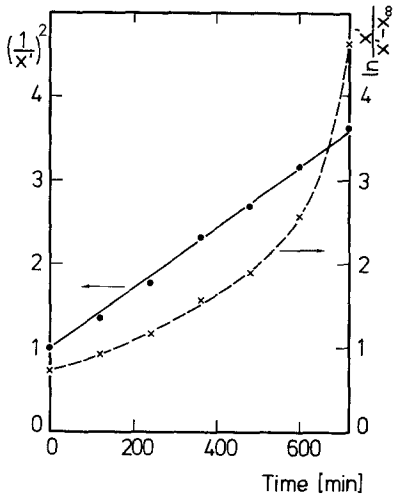


**Fig. 1.** Experimental verification of Equation (21): a plot of $(1/x')^2$ versus time. The experimental points (●) are calculated from the work of Martin and MacDonald (1976) as described in the text. The straight line is a computer-generated least-squares-fit, the slope of which gives the permeability $p_2 = 9.4 \times 10^{-4}$ min$^{-1}$. Also shown are values (+) of ln $x'/(x' - x_\infty)$ which should be a linear function of the time in Thilo's (1977) theory

prepared a mixture of equal concentrations (by weight) of dimyristoyllecithin (DML) and dipalmitoyllecithin (DPL) liposomes, so that $x_\infty = 0.52$ since DPL has a higher molecular weight than DML, and found that DML is not transported, i.e., that $T_t''$ does not change. We, therefore, have an instance of the special case $p_1 = 0$, $x'(0) = 1$, $x''(0) = 0$ studied in section 2 and Equation (22) is expected to be obeyed. Calculation of the $x'(t)$-values from the $T_t$-values reported in [19] assuming $x'$ to be a linear function of $T_t'$ as discussed in the introduction, results in Figure 1, where $(1/x')^2$ is plotted versus time. An excellent straight line relationship is found, confirming the molecular nature of the transport process. From the slope of the straight line in Figure 1 and from Equation (22) one can calculate $p_2$ to have the value $9.4 \times 10^{-4}$ min$^{-1}$. As remarked before, this absolute value is not too important since $p_2$ depends in an unknown manner on the arbitrary value of $x_\infty$.

## 2. *The Experiment of Duckwitz-Peterlein et al.*

In [6], 90° light scattering was used to follow the exchange of phospholipids between liposomes. These liposomes were obtained from total phospholipid extracts of *Escherichia coli* mutants. The initial compositions of these liposomes are given in Table 1. Since the acyl moiety of a phospholipid determines not only the transition temperature (Ladbrooke and Chapman, 1969) but also the transport properties (see also the discussion in section 5), the measurements actually determine the exchange of the *trans*-$\Delta^9$-18 : 1 (component 1) and *trans*-$\Delta^9$-16 : 1 (component 2) lipids. This implies that $x'$ and $x''$ as calculated from the $T_t'$ and $T_t''$ values are actually "partial mole fractions" defined still by Equation (1) but with the $n_1'$, $n_2'$ and $n_1''$, $n_2''$ the number of moles of *trans*-$\Delta^9$-18 : 1 type and *trans*-$\Delta^9$-16 : 1 type lipids. This does, of course, not influence the rest of the theoretical development in section 2.

The time course of $T_t$ was followed in [6] for three different mixtures of liposomes corresponding to $x_\infty = \frac{1}{3}$, $\frac{1}{2}$ and $\frac{2}{3}$, respectively. The $y$ and $z$ values were calculated from the measured $T_t$-values for these cases. In Figure 2 a check of

**Table 1.** Composition of liposomes

| Mole-% | 12 : 0[b] | 14 : 0 | *trans*-$\Delta^9$-16 : 1 | 16 : 0 | *trans*-$\Delta^9$-18 : 1 |
|---|---|---|---|---|---|
| P-lipids[a] | — | 4.3 | 82.8 | 12.8 | — |
| E-lipids[a] | 1.6 | 14.0 | 2.6 | 13.5 | 68.3 |

[a] Lipids are 82 mole-% phosphatidylethanolamin, 11 mole-% cardiolipin, 7 mole-% phosphatidyl glycerol

[b] 12 : 0, dodecanoic acid; 14 : 0, tetradecanoic acid; *trans*-$\Delta^9$-16 : 1, *trans*-$\Delta^9$-hexadecanoic acid; 16 : 0, hexadecanoic acid; *trans*-$\Delta^9$-18 : 1, *trans*-$\Delta^9$-octadecanoic acid

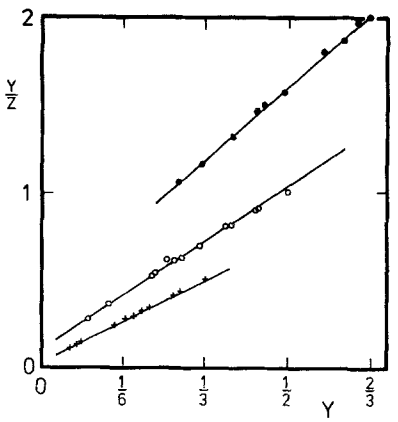


**Fig. 2.** Experimental verification of Equation (14): plots of $y/z$ versus $y$ for three values of $x_\infty$. The data are from Duckwitz-Peterlein et al. (1977), the straight lines are least-squares-fits. (●): $x_\infty = 1/3$; (○): $x_\infty = 1/2$; (+): $x_\infty = 2/3$

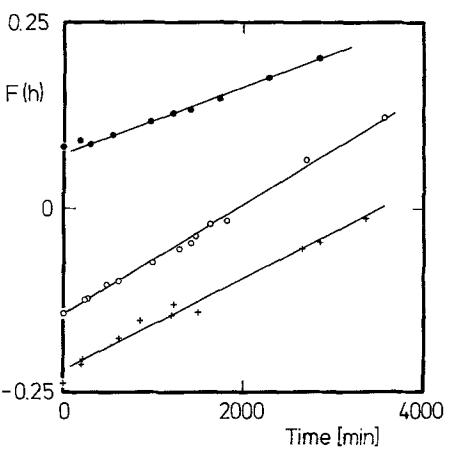


**Fig. 3.** Experimental verification of Equation (13): plots of $F(h)$ versus time for three values of $x_\infty$. The data are calculated from [6] and the parameters of the least-squares-fits of Figure 2 as described in the text. The straight lines are again least-squares-fits. (●): $x_\infty = 1/3$; (○): $x_\infty = 1/2$; (+): $x_\infty = 2/3$

Equation (14) is presented by plotting $y/z$ versus $y$ for the three cases. A straight line relationship is clearly confirmed by this figure. The parameters of the straight lines have then been used as described in section 2 to calculate the $F(h)$ of Equation (13). Plots of $F(h)$ versus time are shown in Figure 3 and again show clearly the presence of a linear relationship. Therefore, the molecular transfer mechanism is strongly supported by the results of [6]. The calculated permeabilities $p_1$ and $p_2$ for the three values are given in Table 2.

**Table 2.** Permeabilities of *trans*-$\Delta^9$-18 : 1 and *trans*-$\Delta^9$-16 : 1 lipids

| $x_\infty$ | $p_1$ ($10^{-5}$ min$^{-1}$) | $p_2$ ($10^{-5}$ min$^{-1}$) | $p_1/p_2$ experimental | $p_1/p_2$ theoretical[a] |
|---|---|---|---|---|
| 1/3 | 1.7 | 9.1 | 0.19 | 0.20 |
| 1/2 | 1.5 | 13.3 | 0.11 | 0.10 |
| 2/3 | 0.4 | 8.9 | 0.05 | 0.05 |

[a] Calculated from Equation (27) with the values $L_{11}/L_{22} = 0.1$ and $L_{12}/L_{22} = 0$

## 5. Discussion

If the exchange of lipid molecules between liposomes of different composition takes place through the aqueous phase, we would expect that the rate of transport will be a decreasing function of the acyl chain lenght, since the longer this hydrophobic part of the molecule is, the larger is the free energy change necessary to accomplish the transfer of a lipid from a nonpolar environment to the aqueous phase. This is indeed borne out clearly by the experiment of Martin and MacDonald (1976), who found that practically only DML with its shorter acyl chains was transported and not DPL. This fact has encouraged us to treat the less simple substances used by Duckwith-Peterlein et al. (1977): Table 1 clearly shows the compositions of the two liposome populations to differ most in lipids with short acyl chains (predominantly 14 : 1 lipids) in addition to the *trans*-$\Delta^9$-18 : 1 and *trans*-$\Delta^9$-16 : 1 lipids. Now since we expect these to be equilibrated faster than the 16 : 1 and 18 : 1 lipids, we may assume that after preincubation (see [6]) the liposome populations differ *only* in *trans*-$\Delta^9$-18 : 1 and *trans*-$\Delta^9$-16 : 1 lipid content and this justifies the procedure of section 4.2.

In Table 2 we have also given the experimental values of $p_1/p_2$; if we assume the surface tensions of the two liposome populations to be equal, we can use Equation (27) to fit these three points. As shown in Table 2 the fit is very good for $L_{11}/L_{22} = 0.1$ and $L_{12}/L_{22} = 0$, showing that the fluxes are not coupled ($L_{12} = 0$) and that the shorter acyl chain lipids diffuse about ten times as fast as the longer acyl chain ones.

There exists also a kinetic model (Thilo, 1977) for the exchange of lipid molecules via the aqueous phase. It may easily be shown that this model corresponds to the following assumptions on the fluxes:

$$\phi_i = (-1)^{i+1} k_i \frac{yz}{(y+z)}, \tag{28}$$

to be compared with Equation (9). Clearly, this is *not* equivalent to the expression (9) known to be valid close to equilibrium (section 3), unless $y$ and $z$ are proportional, i.e., in the isotope exchange case discussed in section 2. It may, however, be shown that Equation (28) leads to equations for $y$ and $z$, which are formally identical with Equation (12), except that $h$ is not given by Equation (13), but by a simple exponential:

$$h = h(0) \exp(-\mu t) . \tag{29}$$

Therefore, Equation (14) is valid in Thilo's theory also, but the time-dependence of $h$ is different. In particular, for the experiment of Martin and MacDonald (1976) treated in section 4.1, Equation (29) implies, since $h = y/(y + x_\infty)$ in this case,

$$\ln [(x' - x_\infty)/x'] = \ln (1 - x_\infty) - \mu t . \tag{30}$$

A plot of the left-hand-side of Equation (30) versus $t$ is also given in Figure 1 for this experiment. Clearly, no linear relationship exists so that Thilo's theory must be termed inappropriate on theoretical as well as on experimental grounds.

We now can summarize our conclusions on the reported experiments (Martin and MacDonald, 1976; Duckwitz-Peterlein et al., 1977) as follows:

a) The transport of lipid molecules between two liposome populations takes place through the aqueous phase.

b) The number of lipid molecules in the aqueous phase is negligible compared to the number of molecules in liposomes.

c) The number of molecules present in the aqueous phase and the transport rate both decrease strongly on increasing the acyl chain length of the lipids.

A more systematic study of these points for different lipids is certainly still necessary before the biological implications of the above can be assessed.

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