# Spontaneous Phosphatidylcholine Transfer by Collision between Vesicles at High Lipid Concentration†

J. D. Jones and T. E. Thompson*
*Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908*
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ABSTRACT: The transfer kinetics of [$^3$H]-1-palmitoyl-2-oleoylphosphatidylcholine ([$^3$H]POPC) and 1-palmitoyl-2-(pyrenyldecanoyl)phosphatdylcholine (PyrPC) from POPC small unilamellar vesicles were examined at 37 °C with lipid concentrations ranging from 0.1 to 40 mM. The rate of [$^3$H]POPC transfer was determined by analyzing the movement of this lipid from charged donor to neutral acceptor vesicles. The rate of decay of the ratio of the intensity of pyrene excimer fluorescence to that from the pyrene monomer ($E/M$) upon addition of an unlabeled vesicle population to a population containing PyrPC was used to evaluate PyrPC transfer. For both lipids, the kinetic data are best described by a model which assumes that transfer occurs by vesicle collisions as well as by desorption from the bilayer. For [$^3$H]POPC, the off-rate constant is 0.014 $h^{-1}$ while the collisional rate constant is 0.0016 $mM^{-1}$ $h^{-1}$. PyrPC has an off-rate constant of 0.023 $h^{-1}$ and a collisional constant of 0.0015 $mM^{-1}$ $h^{-1}$. These numbers were calculated by assuming the rate of interbilayer transfer to be negligible relative to that of intervesicular transfer. The large transfer fluxes in the high vesicle concentration range where the collisional process dominates suggest that spontaneous transfer may be of importance in membrane biogenesis.

Spontaneous nonprotein-mediated phospholipid transfer between model membranes is a well-established phenomenon. Transfer is typically determined by one of two general methods. In the one, movement of a fluorescent phospholipid derivative (Roseman & Thompson, 1980; Nichols & Pagano, 1981, 1982; Silvius et al., 1987) or of a radiolabeled phospholipid analogue (Mclean & Phillips, 1981, 1984; Patton et al., 1985; De Cuyper et al., 1983; De Cuyper & Joniau, 1985) between vesicle populations is monitored. Alternatively, changes in light scattering or thermotropic behavior can be used to monitor lipid transfer between vesicles initially composed of distinct lipid species (Papahadjopoulos et al., 1976; Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977; Kremer et al., 1977).

The vast majority of studies of this process have demonstrated that the rate of transfer is independent of vesicle concentration under the usual experimental conditions. This result suggests that the rate-limiting step in transfer is lipid desorption from the bilayer with subsequent rapid diffusion through the aqueous phase. This transfer mechanism has been supported by numerous studies on phospholipid transfer (Roseman & Thompson, 1980; Mclean & Phillips, 1981, 1984; Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977), as well as in studies concerning transfer of cholesterol (Mclean & Phillips, 1981; Bar et al., 1986; Fugler et al., 1985) and sphingolipids (Correa-Freire et al., 1982; Frank et al., 1983; Brown et al., 1985; Masserimini & Freire, 1986; Brown & Thompson, 1987).

A much more limited number of studies support a mechanism by which lipids transfer upon vesicle collisions. The study by Kremer et al. (1977) on phospholipid transfer between model membranes supported collisionally mediated transfer. Also, a study on cholesterol transfer from vesicular stomatitis virus membranes to phospholipid vesicles (Patzer et al., 1978) as well as an investigation of cholesterol transfer from vesicles to brush border membranes (Müsch et al., 1986) suggested this mechanism.

The aim of the present study is to analyze phospholipid transfer between model membranes over a wide range of vesicle concentrations (0.1–40 mM) in order to determine if the transfer kinetics are adequately described by a single rate process. The transfer of [$^3$H]-1-palmitoyl-2-oleoylphosphatidylcholine ([$^3$H]POPC)[1] from unilamellar donor to acceptor vesicles was monitored with a charged vesicle assay. The data indicate that transfer is characterized by two rate processes; one first order and the other second order with respect to vesicle concentration. These two processes almost certainly reflect desorption from the donor vesicles and transfer due to donor–acceptor collisions, respectively. As vesicle concentration is increased, the transfer flux arising from the concentration-dependent process dominates that from desorption. Thus, the transfer mechanism is clearly not adequately described by a first-order process over this extended concentration range. However, almost all studies in the literature are in the low vesicle concentration regime where the second-order term is negligible. This explains the observation of apparent first-order kinetics in these reports.

In order to determine if the observed concentration dependence of transfer is due to the presence of the net negative charge on the vesicles in the above assay, the rate was determined for 1-palmitoyl-2-(pyrenyldecanoyl)phosphatidylcholine (PyrPC) transferring from the equivalent POPC matrix. In this case, transfer was calculated from the theoretical dependence of the pyrene eximer/monomer ratio on the amount of lipid transferred as outlined by Roseman and Thompson (1980). The results indicated that in this system transfer is also characterized by a sum of vesicle concentration independent and dependent rate processes. If spontaneous lipid transfer between membranes in vivo is a concentration-dependent process, it may play a role for some lipids in the biogenesis of cell membranes.

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[1] Abbreviations: POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PyrPC, 1-palmitoyl-2-(pyrenyldecanoyl)phosphatidylcholine; TLC, thin-layer chromatography; EDTA, ethylenediaminetetraacetic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); DEAE, diethylaminoethyl.

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## Materials and Methods

POPC, 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG), and 1-palmitoyllysophosphatidylcholine were purchased from Avanti Polar Lipids (Birmingham, AL). PyrPC was purchased from KSV Biochemicals (Helsinki, Finland). [9,10-$^3$H(N)]Oleic acid (sp act. 56.6 mCi/mmol) was obtained from New England Nuclear (Boston, MA). Unlabeled oleic acid was purchased from Sigma. All lipids were checked for purity by TLC and stored under nitrogen at 0 °C.

4-Pyrrolidinopyridine was purchased from Aldrich and further purified by recrystallization from low-boiling petroleum ether. DEAE-Sephacel was obtained from Pharmacia (Uppsala, Sweden). *N,N'*-Dicyclohexylcarbodiimide (DCC) was purchased from Sigma, and silica gel G plates for TLC were obtained from Analtech.

*Synthesis of Labeled POPC.* [$^3$H]Oleic acid anhydride was prepared from the corresponding fatty acid with DCC as described by Selinger and Lapidot (1966). [$^3$H]POPC was synthesized by acylating lysophosphatidylcholine with the anhydride by employing 4-pyrrolidinopyridine as described in detail by Mason et al. (1981). The labeled product was purified by preparative TLC using a solvent system of $CHCl_3$/MeOH/$H_2O$ (65/25/5). The chemical and radioactive purity of the compound was >99%.

*Liposome Preparation.* All experiments were carried out with small unilamellar vesicles prepared by cosonication of the appropriate lipid mixture in 10 mM Pipes, 25 mM KCl, 0.5 mM EDTA, and 0.02% $NaN_3$ as described by Barenholz et al. (1977). The vesicles were incubated overnight at room temperature prior to experiments. Phospholipid phosphate concentrations were determined according to the method described by Bartlett (1959).

*Charged Vesicle Assay.* [$^3$H]POPC transfer was monitored with a charged vesicle assay which is based on the method originally described by Hellings et al. (1974). This assay employs two vesicle populations, negatively charged donor and neutral acceptor vesicles. Lipid transfer was determined by analyzing the movement of label from the donor to the acceptor fraction as a function of time. The POPC donor vesicles contained 15 mol % POPG, [$^3$H]POPC, and [$^{14}$C]cholesteryl oleate which served as a nonexchangeable marker. POPC vesicles were used as acceptors. In each case, a given concentration of donor and acceptor vesicles was mixed in a total volume of 1.25 mL and equilibrated at 37 °C. At appropriate time intervals, a 0.05-mL aliquot was placed on a minicolumn containing 0.8 mL of DEAE-Sephacel which had been preequilibrated with 0.3 μmol of acceptor vesicles. The neutral acceptor vesicles were recovered by elution with 1.2 mL of Pipes buffer. This procedure ensured high acceptor recoveries (91%). Donor leakage was always less than 5%. After the experiments, lipids were extracted with chloroform/methanol and analyzed by TLC. No breakdown products of any of the lipids were detected.

The movement of label from donors to acceptors due to spontaneous transfer is given by

$$[^3H]_{ST} = [^3H]_t - [^3H]_0[^{14}C]_t/[^{14}C]_0 \quad (1)$$

where $[^3H]_0/[^{14}C]_0$ is the initial ratio of the two labels in the donor vesicles. This equation corrects the observed net [$^3$H]POPC transfer ($[^3H]_t$) for artifacts arising from leakage of donor vesicles or nonspontaneous transfer due to donor–acceptor fusion. The corrected percent transfer is given by normalizing the calculated spontaneous transfer for acceptor recovery:

$$X(t) = [^3H]_{ST}/0.91 \quad (2)$$

*PyrPC Transfer Studies.* All excimer/monomer intensity measurements ($E/M$) were carried out on an SLM-Aminco (Urbana, IL) Model 4800 spectrofluorometer. Temperature was regulated to within ±0.1 °C. The excitation wavelength was 346 nm (2-nm slit width). Monomer and excimer emission were monitored at 378 nm (1-nm slit width) and 470 nm (1-nm slit width), respectively, with two monochrometers. All samples were flushed and sealed under argon in gas-tight cuvettes to eliminate artifacts arising from either oxygen quenching or lipid peroxidation.

The procedure for experimental determination of PyrPC transfer between vesicles was as follows. A given concentration of POPC vesicles containing 4 mol % PyrPC was equilibrated at 37 °C. After initial measurements were taken, unlabeled acceptor vesicles were added to give a total volume of 3 mL, and the decrease in $E/M$ was monitored as a function of time. Pure donor vesicles were used as a control. Detailed vesicle compositions will be described when individual results are presented. Light scattering was negligible in all cases. Analysis of lipids following experiments yielded no evidence of breakdown products.

The principles underlying the use of pyrene-labeled lipids in transfer studies have been described by Roseman and Thompson (1980) and Correa-Freire et al. (1982). This approach is based on methods originally developed by Doody et al. (1978), Charlton et al. (1976, 1978), and Sengupta et al. (1976). When an excited pyrene molecule collides with a ground-state pyrene, a complex called an excimer is formed which emits light at a longer wavelength than the monomer. Excimer formation is a concentration-dependent reaction, and thus a higher local pyrene concentration is reflected in a higher $E/M$ value. This property is exploited to measure transfer of PyrPC from a PyrPC-labeled vesicle population to an unlabeled population.

The derivation of equations relating the observed $E/M$ at a given time point to the amount of transfer has been given by Roseman and Thompson (1980). The relevant equation is

$$\frac{E}{M} = \frac{C_D^2(C_{D0} - C_D) + RC_D^2C_h + (C_{D0} - C_D)^2(C_D + C_h)}{C_hC_D(C_{D0} - C_D) + RC_h^2C_D + RC_h(C_{D0} - C_D)(C_D + C_h)} \times (E/M)_{max} \quad (3)$$

where $C_{D0}$ is the initial probe concentration in the donor vesicles, $C_D$ is the concentration at time $t$, and $R$ is the acceptor/donor ratio. $(E/M)_{max}$ is the ratio of the excimer quantum yield as $C_D \rightarrow \infty$ and the monomer quantum yield as $C_D \rightarrow 0$. $C_h$ is the half-value concentration.

$C_D$ values were calculated from computer simulations of eq 3. $C_h$ was obtained from

$$C/M = \frac{C}{LK_2C_hN_{M,max}} + \frac{1}{LK_2N_{M,max}} \quad (4)$$

as $C \rightarrow 0$, where $C$ is the mole ratio of probe to phospholipid, $L$ is the molar concentration of phospholipid, $N_{M,max}$ is the maximum monomer quantum yield as $C \rightarrow 0$, and $K_2$ is a proportionality constant. This equation as given in Roseman and Thompson (1980) contains an error. The correct formulation can be found in Correa-Freire et al. (1982). A plot of $C/M$ vs $C$ yields a slope of $1/LK_2C_hN_{M,max}$ and an intercept of $1/LK_2N_{M,max}$. For this system, $C_h = 0.0129$. $(E/M)_{max}$ is obtained by substituting $C_h$ into

$$\frac{E}{M} = \frac{C}{C_h}(E/M)_{max} \quad (5)$$

$(E/M)_{max}$ was found to be 0.0345.

*Electron Microscopy.* Negative stain electron micrographs were obtained on a Hitatchi HU12A electron microscope. Vesicles were stained with 2% uranyl formate.

*Photon Correlation Spectroscopy.* Quasi-elastic light scattering measurements were carried out on a Nicomp Model HN5-90 instrument equipped with a Model 170 autocorrelator.

## Kinetic Analysis

The kinetic model used is a modification of Thilo's (1977) application of the analysis proposed by Nakagawa (1974) to describe monomer association and dissociation from micelles in surfactant solutions.

*First-Order Model.* In this model, lipids are assumed to transfer between vesicles through the aqueous phase via desorption from the bilayer. All vesicles are assumed to have equivalent surface areas. A similar treatment for two-component vesicles can be found in Nichols and Pagano (1981).

Consider one lipid type found initially in one vesicle population (donors) transferring into a second population (acceptors):

$$[N_d] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [N_m] \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [N_a] \quad (6)$$

where $[N_d]$ = concentration of lipid in donor vesicles, $[N_a]$ = concentration of lipid in acceptor vesicles, and $[N_m]$ = concentration of lipid in aqueous phase. The rate of dissociation of molecules from the donor vesicles can be expressed as

$$d[N_d]/dt = -k_1[N_d] + k_2[N_m][D] \quad (7)$$

where [D] is the concentration of donor vesicles. The rate of transfer into the aqueous phase is

$$d[N_m]/dt = k_1[N_d] + k_4[N_a] - k_2[N_m][D] - k_3[N_m][A] \quad (8)$$

where [A] is the acceptor vesicle concentration. The aqueous phase is assumed saturated with lipid and thus $d[N_m]/dt = 0$ over the course of an experiment:

$$[N_m] = \frac{k_1[N_d] + k_4[N_a]}{k_2[D] + k_3[A]} \quad (9)$$

Substitution of eq 9 into eq 7 and rearrangement of terms yields the general equation

$$\frac{d[N_d]}{dt} = \frac{k_1k_2[N_d][D] + k_2k_4[N_a][D]}{k_2[D] + k_3[A]} - k_1[N_d] \quad (10)$$

Net lipid transfer from donors is assumed to be compensated for by back-transfer from acceptors. Thus, [D] and [A] are treated as constants in the subsequent formulations.

*Initial Rate Approximation.* In all experiments, only the initial rate of transfer is measured. Thus, $[N_a]$ is set = 0:

$$\frac{d[N_d]}{dt} = \frac{k_1k_2[N_d][D]}{k_2[D] + k_3[A]} - k_1[N_d] \quad (11)$$

Integration of this equation subject to $[N_d]_{t=0} = [N_d]_0$ yields

$$[N_d]_t = [N_d]_0 \exp\left[\left(\frac{k_1k_2[D]}{k_2[D] + k_3[A]} - k_1\right)t\right] \quad (12)$$

where $[N_d]_0$ = initial concentration of donor lipid. In our case data are always expressed as fractional transfer of lipid from donor vesicles. Thus, $[N_d]_0 = 1$. The fraction of lipid remaining in the donors at time $t$ is given by $[N_d]_t = 1 - X(t)/X(\infty)$ where $X(t)$ is the fraction transferred at time $t$ and $X(\infty)$ is the fraction available for transfer. A recent study on transbilayer migration of phospholipids in POPC small unilamellar vesicles showed that the rate of flip-flop for a series of fluorescently labeled phosphatidylcholines was negligible in comparison to their off-rates (Homan & Pownall, 1988). Thus, we used a value of 0.67 for $X(\infty)$, which was obtained by assuming the lipids are symmetrically disposed on the surface of small unilamellar vesicles.

The slope of a log plot of $[N_d]_t$ vs $t$ in eq 12 is given by

$$\text{slope} = \frac{k_1k_2[D]}{k_2[D] + k_3[A]} - k_1 \quad (13)$$

In our case, all transfer occurs between vesicle populations of similar composition and geometric conformation. Thus, the donor and acceptor on-rates are assumed approximately equal ($k_2 = k_3$), yielding

$$\text{slope} = \frac{k_1[D]}{[D] + [A]} - k_1 \quad (14)$$

and $k_1 = -\text{slope}[([D] + [A])/[A]]$. The term $([D] + [A])/[A]$ corrects for the back-transfer from the aqueous phase to the donors. This formulation yields the value of $k_1$ which would be obtained under conditions of infinite acceptor concentration. The first-order half-time is given by $t_{1/2} = (\ln 2)/k_1$.

*Second-Order Model.* In this model, lipids are assumed to transfer upon collision of donor and acceptor vesicles in addition to transfer via the aqueous phase. Thus, an additional rate term, proportional to both donor lipid and acceptor vesicle concentration, is added to eq 11. As in the previous case, $[N_a]$ is set = 0:

$$\frac{d[N_d]}{dt} = \frac{k_1k_2[N_d][D]}{k_2[D] + k_3[A]} - k_1[N_d] - k_c[N_d][A] \quad (15)$$

Integration of this equation as in the previous case yields

$$[N_d]_t = [N_d]_0 \exp\left[\left(\frac{k_1k_2[D]}{k_2[D] + k_3[A]} - k_1 - k_c[A]\right)t\right] \quad (16)$$

Thus, the slope of a log plot of transfer vs time equals

$$\frac{k_1[D]}{[D] + [A]} - k_1 - k_c[A] \quad (17)$$

In the case of $[A] \gg [D]$, the term $k_1[D]/([D] + [A])$ can be ignored. Therefore, a plot of –slope vs [A] yields an intercept of $k_1$ and a slope of $k_c$.

Kinetic data were fitted by an iterative nonlinear least-squares analysis (Johnson & Frasier, 1985) on a Control Data Corp. Cyber 730 computer.

*Predictions of Models.* (1) A log plot of transfer vs time is predicted to be linear in both the first- and second-order cases. This result is due to the fact that the donor and acceptor vesicle concentrations are constants and therefore $[N_d]$ is the only variable in the equations. (2) In the second-order model, although the net flux of lipid is a function of donor lipid and acceptor vesicle concentration, the half-time of transfer is dependent only on acceptor concentration. This results from the fact that the rate of transfer from a given donor vesicle is dependent only on collision with acceptor vesicles. Increased donor concentration results in increased lipid flux, but the half-time of transfer from each donor vesicle and thus the overall half-time are unaffected.

## Results and Discussion

*[$^3H$]POPC Transfer at Low Vesicle Concentrations.* Figure 1 shows a log plot of the fraction the [$^3$H]POPC remaining

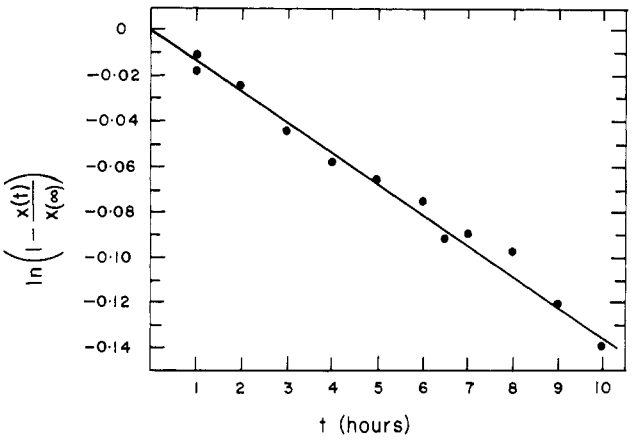


FIGURE 1: [$^3$H]POPC transfer at 37 °C according to eq 12. Donor and acceptor concentrations are 0.01 and 0.2 mM, respectively.

Table I: Apparent First-Order Half-Time for [$^3$H]POPC Transfer at 37 °C as a Function of Acceptor Concentration for the Indicated Donor Concentration

| [A] | $k_1$ ($h^{-1}$)[a] | $t_{1/2}$ (h) |
|---|---|---|
| | 0.01 mM Donors | |
| 0.1 | 0.015 ± 0.001 | 46 ± 4 |
| 0.2 | 0.013 ± 0.001 | 53 ± 4 |
| 0.4 | 0.016 ± 0.002 | 43 ± 7 |
| 0.8 | 0.016 ± 0.001 | 43 ± 4 |
| | 0.05 mM Donors | |
| 0.5 | 0.014 ± 0.002 | 50 ± 6 |
| 1.0 | 0.014 ± 0.003 | 50 ± 10 |
| 2.0 | 0.013 ± 0.002 | 53 ± 6 |
| | Average Values | |
| | 0.014 ± 0.001 | 50 ± 3 |

[a] Each value represents the average obtained from triplicate experiments consisting of at least eight time points. [A] denotes acceptor lipid concentration.

in the donor vesicles as a function of time for 0.01 mM donors and 0.2 mM acceptors. Table I gives the apparent first-order rate constants and half-times for transfer from 0.01 and 0.05 mM donors at the indicated acceptor concentrations. These values are not statistically different ($p < 0.05$). Thus, within this concentration range, the data are adequately described by the first-order model for desorption-limited transfer through the aqueous phase. The average value obtained for the half-time ($t_{1/2}$ = 50 ± 3 h) is in general agreement with that reported by Mclean and Phillips (1984) for POPC transfer from an egg PC matrix. These authors also observed the half-time to be independent of vesicle concentration over a similar concentration regime.

The observed independence of the rate of transfer on vesicle concentration over this range supports the validity of the assumption that the donor and acceptor vesicles have approximately equivalent rate constants for lipid absorption from the aqueous phase. If the on-rate for the donor vesicles is significantly greater than that for the acceptors, as occurs for transfer between liquid-crytalline donors and gel-phase acceptors, a dependence of half-time on acceptor concentration will be observed for transfer via the aqueous phase [see Nichols and Pagano (1981) for a detailed discussion]. However, this condition does not operate in our system in which the only difference between the donor and acceptor vesicles is the presence of 15 mol % POPG in the donors. Earlier studies from this laboratory on cholesterol transfer showed that equivalent results were obtained if the charge was placed on either the donors or the acceptors. This indicates a negligible effect of the charged lipid on the transfer process. This point

Table II: Apparent First-Order Half-Time (h) for [$^3$H]POPC Transfer at 37 °C as a Function of Donor Concentration for the Indicated Acceptor Concentrations

| | [A] (mM)[a] | | | | |
|---|---|---|---|---|---|
| [D] (mM) | 2.5 | 5.0 | 10.0 | 20.0 | 40.0 |
| 0.125 | 46 ± 3[b] | 33 ± 2 | 20 ± 2 | | |
| 0.25 | 42 ± 3 | 34 ± 2 | 26 ± 2 | 18 ± 2 | |
| 0.5 | | 31 ± 4 | 26 ± 2 | 14 ± 1 | 7 ± 1 |
| 1.0 | | | 18 ± 2 | 13 ± 1 | 8 ± 1 |

[a] [A] and [D] are acceptor and donor lipid concentrations, respectively. [b] Each value represents averages obtained from at least triplicate experiments consisting of at least eight data points.

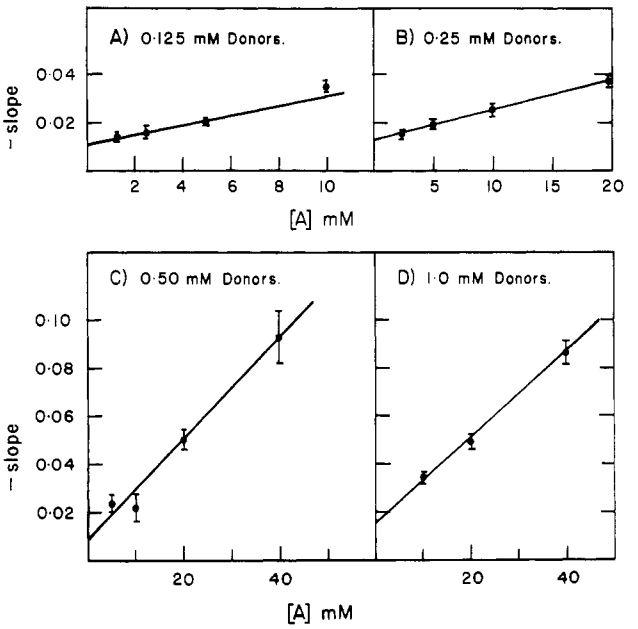


FIGURE 2: Graphs of the slope of log transfer of [$^3$H]POPC at 37 °C vs time against acceptor concentration for the indicated donor concentrations according to eq 17.

will be discussed in more detail in the section on PyrPC transfer.

*[$^3$H]POPC Transfer at High Vesicle Concentrations.* Table II gives the apparent first-order half-time of transfer for varying donor concentrations as a function of acceptor concentration. In the region of 5 mM acceptor concentration, the half-time begins to show a clear dependence on acceptor concentration. As predicted by the second-order model, the half-time of transfer exhibits no statistically significant dependence on donor concentration at a constant acceptor concentration ($p < 0.05$). At all concentration values, a linear plot of log transfer vs time was observed.

Figure 2 illustrates the deconvolution of the apparent half-time values in Table II with eq 17. The correlation coefficient of plots of −slope log transfer vs time against [A] were in each case greater than 0.98. The $k_1$ derived from the intercept of the plots in Figure 2 is 0.012 ± 0.002 $h^{-1}$. This gives a desorption-limited half-time of 58 ± 8 h. This value agrees within experimental error with the value obtained with dilute vesicle concentrations. The slope of the plots in Figure 2 yields a $k_c$ of 0.0018 ± 0.0003 $mM^{-1}$ $h^{-1}$. A better estimate of $k_c$ is obtained by substitution of the $k_1$ value obtained at low vesicle concentration into eq 17. This procedure yields a $k_c$ value of 0.0016 ± 0.0003 $mM^{-1}$ $h^{-1}$.

These results clearly indicate that at high vesicle concentration the flux arising from the collisional mechanism dominates that due to desorption and aqueous-phase diffusion. Thus, first-order kinetics are only adequate to describe the

transfer data at vesicle concentrations where the collisional contribution is negligible (<3 mM lipid).

*Collisional Transfer vs Fusion.* The observed acceptor concentration dependence of transfer in this concentration range can be explained by a bivesicle donor–acceptor fusion process as well as lipid transfer upon vesicle collision. Transfer by fusion is ruled out in our experiments by the behavior of the nonexchangeable marker in the donor vesicles. If donor–acceptor fusion were significant over the time course of an experiment, the resulting fusion products would be evidenced by either an increase in the appearance of $^{14}C$ in the acceptor fraction over time or a decrease in acceptor recovery. No change in acceptor recovery was evident in the time frame of any of the experiments. The amount of $^{14}C$ eluting in the acceptor fraction never increased by more than 1% (compared to the 10% increase in $^{3}H$) during the course of an experiment regardless of the concentration regime studied. The transfer value was corrected for this slight increase as outlined under Materials and Methods.

The possibility of artifacts arising from fusion or aggregation of the vesicles was also examined by photon correlation spectroscopy and negative stain electron microscopy (data not shown). No evidence of an increase in the average size of the vesicles (20-nm diameter) was detected by either method. These results also rule out the occurrence of fusion during the course of an experiment.

Collisional lipid transfer which becomes negligible at low vesicle concentrations may in part explain conflicing results obtained in different laboratories. The studies previously cited which supported the off-rate limited mechanism of transfer between model membranes were typically carried out at relatively dilute vesicle concentrations. The light scattering study by Kremer et al. (1977) on transfer between dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine in ethanol-injection vesicles was also done at low vesicle concentration (<1 mg/mL). However, the experiments in which second-order kinetics were observed were carried out at 50 °C. No transfer was observed at room temperature. It is possible that collisional transfer could become significant at this temperature for this system, although the possibility of vesicle fusion could not be ruled out in these experiments.

*PyrPC Transfer.* In order to determine if collisional transfer is a peculiarity of the experimental system using the charged vesicle assay, PyrPC transfer from POPC was examined. This method permits transfer to be determined without separation of donor from acceptor vesicles. Thus, no charged lipid is necessary. Alternatively, a charged lipid can be placed in both donors and acceptors to determine the effect of net vesicle charge on the transfer process.

All experiments were carried out with donor vesicles containing 4 mol % PyrPC. Use of this low mole percent PyrPC ensures a negligible contribution to the $E/M$ decay results from net transfer of POPC from acceptors to donors due to the concentration gradient imposed by the presence of PyrPC in the donor vesicles [see Roseman and Thompson (1980) for a detailed discussion]. The increase in the $E/M$ value was observed to be linear with respect to concentration from 1 to 4% PyrPC which indicates the probe is randomly distributed in the bilayer in this concentration range.

Table III gives the calculated first-order half-time for the transfer of 4 mol % PyrPC from POPC under conditions of dilute vesicle concentration. Donor concentration was 0.05 mM, and acceptor concentration was 2.0 mM. Further dilution of the vesicles yielded no statistically significant change in the observed half-time. Thus, at this vesicle concentration, the data are well fit by the first-order model.

Table III: Half-Times of PyrPC Transfer from POPC at 37 °C[a]

| donors | acceptors | $t_{1/2}$ (h)[b] |
|---|---|---|
| 0.05 mM D | 2 mM A | 30 ± 3 |
| 0.05 mM PGD[c] | 2 mM PGA | 35 ● 3 |
| 0.5 mM D | 20 mM A | 13 ± 1 |
| 0.5 mM PGD | 20 mM PGA | 24 ± 2 |
| 0.5 mM PGD | 20 mM A | 15 ± 1 |

[a] D = POPC donor vesicles with 4 mol % PyrPC. A = POPC acceptor vesicles. PGD = POPC donor vesicles with 4 mol % PyrPC + 15 mol % POPG. PGA = POPC acceptor vesicles with 15 mol % POPG. [b] Each value represents averages obtained from triplicate experiments consisting f at least 20 data points. [c] Addition of POPG to the donor vesicles did not affect the values obtained for $C_h$ and $(E/M)_{max}$. Thus, the values given under Materials and Methods were used for all calculations.

The apparent first-order half-time for transfer of 4 mol % PyrPC from 0.5 mM donors to 20 mM acceptors is also given in Table III. The half-time obtained in this case suggests collisional transfer at this concentration. Substitution of the $k_1$ value determined at dilute vesicle concentration into eq 17 yields a $k_c$ of 0.0015 ± 0.0003 $mM^{-1}$ $h^{-1}$.

The observed first-order half-time for the transfer of PyrPC is slightly faster than that of [$^{3}H$]POPC from POPC. In the PyrPC transfer assay there is no internal control for fusion as in the charged vesicle assay. Thus, although no evidence of an increase in average particle size was detected by photon correlation spectroscopy in any of the experiments (data not shown), it is possible that a slight systematic error due to vesicle fusion could contribute to our results. Therefore, the half-time values should most properly be considered as lower limits. However, it is quite possible that perturbation of the bilayer packing arrangement by the bulky pyrene moiety could result in faster off-rates. The value for the collisional rate constant obtained by substitution of the $k_1$ value is roughly in agreement with the value obtained for [$^{3}H$]POPC. The ratio of off-rate and collisional constant obtained by substitution agrees within experimental error for the two lipids (9 ± 4 mM and 15 ± 4 mM for [$^{3}H$]POPC and PyrPC, respectively).

*PyrPC Transfer in the Presence of Net Negative Charge.* Collisional transfer between vesicles would be expected to be reduced if both donors and acceptors are equivalently charged. Table III gives the results of transfer with 15 mol % POPG in both donor and acceptor vesicles. The increase in the $t_{1/2}$ value (24 h as opposed to 13 h without charge) indicates that the charged lipid largely inhibits the collisional process, although its contribution remains significant. Control experiments outlined in Table III indicate the negligible effect of the charged lipid on the transfer process when POPG is incorporated only in the donor vesicles, since the half-times obtained for neutral and 15 mol % POPG donors are not statistically different.

*Mechanism of Collisional Transfer.* A model for collisional lipid transfer has been proposed by Gurd (1960). This model envisions transfer occurring as a result of transient mixing of the vesicle contents and subsequent lipid diffusion within the complex. The rate-limiting step in this case could be either collisional frequency or the rate of diffusion of molecules within the collisional complex. If collisional frequency is rate limiting, all lipid species in the bilayer are predicted to transfer at equal rates. This mechanism is clearly precluded by our results since no significant cholesteryl oleate transfer is evident. Thus, only a dramatic difference in the diffusional rates of POPC and cholesteryl oleate within a collisional complex could be invoked to explain the operation of this mechanism in our case.

Collisional lipid transfer may also arise via interaction of transition-state monomers with acceptor vesicles. Lipid molecules which are perpendicularly displaced from but still associated with the bilayer surface are in the transition state (Nichols, 1985). In this model, interaction of monomers with the acceptor bilayer surface decreases the effective free energy of activation for transfer. It should be noted that only close apposition of vesicles may be required for "collisional" transfer. In any case, further work on parameters such as temperature and vesicle composition dependence of collisional transfer is necessary to determine the molecular mechanism by which vesicle interactions lead to lipid transfer.

Spontaneous transfer due to lipid desorption from bilayers has long been believed to be too slow, and hence the fluxes too small, to be an important process in the biogenesis of cell membranes. However, the process we have described that is dependent on vesicle concentration can give rise to lipid fluxes at moderate vesicle concentrations that are substantially larger than the flux due to simple lipid desorption. The higher bilayer concentrations used in our experiments are approaching the range of intracellular membrane concentration found in many cell types. It thus seems possible that in vivo spontaneous transfer of phosphatidylcholines may in fact play a role in membrane biogenesis. Future work defining the details of the mechanism of collisional transfer is necessary, however, before more quantitative statements regarding the contribution of this process to membrane assembly can be made.

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