$$\cot \Delta_{obsd} = (p_{\parallel}p_{\perp} + q_{\parallel}q_{\perp})/(p_{\parallel}q_{\perp} - p_{\perp}q_{\parallel})$$

and

$$\cot \Delta_0 = (P_{\parallel}P_{\perp} + Q_{\parallel}Q_{\perp})/(P_{\parallel}Q_{\perp} - P_{\perp}Q_{\parallel}) \quad (3)$$

From these definitions and eq 2

$$\cot \Delta_{obsd} = [(\cot \Delta_0)/(1 - 2\alpha)][1 + C\alpha(1 - \alpha)] \quad (4)$$

where

$$C = [(P_{\parallel} - P_{\perp})^2 + (Q_{\parallel} - Q_{\perp})^2]/(P_{\parallel}P_{\perp} + Q_{\parallel}Q_{\perp}) \quad (5)$$

Figure 9 shows the value of C for a limiting anisotropy of 0.4 as a function of the parameters $\omega\tau$ (circular frequency times lifetime) and $6R\tau$(6 times rotational rate times lifetime). Only exceptionally does the value of C approach or exceed unity, remaining in general well below it. Moreover, C decreases monotonically with the limiting anisotropy. As $\alpha(1 - \alpha)$ is less than 0.1, a satisfactory correction is obtained by taking

$$\tan \Delta_0 = \tan \Delta_{obsd}/(1 - 2\alpha) \quad (6)$$

The experimental procedure for the determination of α is given by Paladini & Weber (1981) and by P. L.-G. Chong and G. Weber (unpublished results).

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Intervesicular Phospholipid Transfer. A Free-Flow Electrophoresis Study†

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ABSTRACT: Using the free-flow electrophoresis technique, the spontaneous transfer or exchange of phospholipid molecules between differently charged phospholipid vesicles has been examined. The basic experiment concerns the transfer phenomena occurring in a mixture of equal amounts of dimyristoylphosphatidylcholine (DMPC)–dimyristoylphosphatidylglycerol (DMPG) (molar ratio 9:1) vesicles and DMPC vesicles above their gel-to-liquid-crystal phase transition. At 33 °C in 5 mM 4-morpholineethanesulfonic acid buffer (pH 6.0)–10 mM potassium chloride, the two peaks in the electrophoretograms approach each other according to a first-order reaction rate, indicating that DMPG moves from one vesicle population ("donor") to the other ("acceptor"). The half-time of the overall kinetic process (41 min) is independent of total lipid concentration but increases considerably with increasing ionic strength. These findings are consistent with a transfer of phospholipid through the aqueous phase. The transfer properties of the phosphatidylglycerol molecule are strongly affected by its fatty acyl side chain composition. As compared with DMPG, the transfer of dioleoylphosphatidylglycerol occurs much faster, whereas in the case of dipalmitoylphosphatidylglycerol (DPPG) immobile electrophoretograms are found. Transfer of DMPG also depends on the relative fluidity of the donor membranes. Substituting DMPC by dioleoylphosphatidylcholine (DOPC) in the donor vesicles doubles the half-time for DMPG transfer, whereas changing DMPC in the neutral acceptor vesicles by DOPC has no effect. A different approach has been used to examine separately the individual transfer rates of DMPG and DMPC. Use was made of DMPC–DPPG (molar ratio 9:1) and DMPC vesicles, a mixture of which produces immobile electrophoretograms. Either the neutral or anionic vesicle population was labeled with either [^{3}H]DMPC or [^{3}H]DMPG. In these experiments, the half-times for transfer are calculated from the changes in radioactivity associated with the neutral or the anionic vesicle population. It appears that the transfer behavior of DMPG—in contrast to that of DMPC—is strongly affected by the charge properties of the membranes. Indirectly, we also deduce that flip-flop movements occur at a faster rate than intervesicular phospholipid transfer.

Spontaneous phospholipid transfer or exchange has been established by using a wide variety of techniques (Dawidowicz & Rothman, 1976; De Kruijff & Van Zoelen, 1978; McLean & Phillips, 1981; Nichols & Pagano, 1981; Roseman & Thompson, 1980). Comparing the different results, however, remains difficult. Indeed, evidence has been presented in support of two fundamentally different mechanisms: one involving transfer upon collision (Martin & MacDonald, 1976) and the other involving transfer from the monomeric (or micellar) pool of phospholipid, which is in equilibrium with the bilayer phase (McLean & Phillips, 1981; Roseman & Thompson, 1980). Also, in studies of phospholipid transfer phenomena, attention has been focused mainly on the influence of the fatty acyl chain length of the molecules, thereby neglecting the influence of double bonds and the role played by the polar head-group type. For instance Duckwitz-Peterlein et al. (1977) found that the shorter *trans*-9-hexadecenoate-containing phospholipid molecules move more rapidly than *trans*-9-octadecanoate-containing ones. Although these authors mention that their vesicle types contain about 20% acidic phospholipids, the individual contribution of these to the overall process was not checked.

Intervesicular interactions between differently charged membrane structures have been approached by using the ion-exchange chromatography technique with dicetyl phosphate or phosphatidic acid as an immobile anionic membrane component of the donor and/or acceptor vesicle population (De Kruijff & Van Zoelen, 1978; McLean & Phillips, 1981; van den Besselaar et al., 1975). Direct measurements of spontaneous phospholipid transfer of negatively charged phospholipids by this method have not been reported. In

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addition, an important disadvantage of this technique is the poor recovery of both donor and acceptor vesicles (McLean & Phillips, 1981; van den Besselaar et al., 1975).

As an innovating alternative, we introduced the use of the continuous free-flow electrophoresis in the liposome field (De Cuyper et al., 1980). We proved that, particularly in the low-charge range, small differences in anionic phospholipid content are sufficient to obtain a quantitative separation. Also, we qualitatively observed that a spontaneous phospholipid transfer occurs between mixed DMPC–DMPG[1] (molar ratio 9:1) and DMPC vesicles. We now extend these preliminary observations to other fundamental aspects of the transfer process. The kinetics, the transfer mode, the role played by the hydrophobic and hydrophilic part of the phospholipid molecule, and the influence of membrane fluidity and membrane surface charge are analyzed.

Experimental Procedures

Lipids. DMPC was purchased from Sigma (St. Louis, MO). DOPC and DOPG were obtained from Serdary (London, Canada). These lipids were used without further purification.

DMPG and DPPG were made by enzymatic exchange of the choline head group for glycerol, catalyzed by phospholipase D (Papahadjopoulos et al., 1973). A similar procedure was used for the preparation of [^{3}H]DMPG with [^{3}H]glycerol (Amersham, England). [^{3}H]DMPC was prepared from dimyristoylphosphatidylethanolamine (Calbiochem, Switzerland) by quaternarization with [^{3}H]CH_3I (Amersham) according to Stockton et al. (1974). The crude lipid preparations were purified on preparative silica gel thin-layer plates (Merck, West Germany) with methanol (for purification of the phosphatidylglycerols) or chloroform–methanol–acetic acid 96%–water (50:25:7:3 by volume) (for purification of [^{3}H]DMPC) as eluting solvents. All lipids ran as a single spot on analytical thin-layer chromatograms.

Preparation of Phospholipid Vesicles. Phospholipids were dissolved in chloroform–methanol (2:1 v/v), and the solvent was evaporated in a thermostatable sonication vial by using a stream of nitrogen. In case we used radiolabeled phospholipids, 0.05 μCi/mg of total vesicle lipid (approximately 0.05% w/w) was added. Then, the lipids were suspended in 10 mL of 5 mM MES buffer, pH 6.0, containing 10 mM potassium chloride (unless otherwise stated) and sonicated at an amplitude of 18 μm peak to peak in an ultrasonic desintegrator (150 W, MSE) for 20 min. For removal of titanium dust, the sample was centrifuged for 10 min at 10000g. Unless otherwise indicated sonication, centrifugation, and storage were performed at 33 °C. Dimensions and architecture of the vesicles were checked by Sepharose CL-2B column chromatography and electron microscopy. They nearly exclusively consist of small unilamellar vesicles, about 30 nm in diameter.

Free-Flow Electrophoresis. The free-flow electrophoresis experiments, according to Hannig & Heidrich (1977), are performed on a Desaga FF-48 apparatus (Heidelberg, GFR) essentially as described (De Cuyper et al., 1980). Briefly, the vesicle material is injected continuously (rate = 0.74 mL h^{-1}) into a thin laminar flow sheet (rate = 225 mL h^{-1}) of a 5 mM MES–10 mM potassium chloride separation buffer (pH 6.0). The electrode buffer has the same composition but is 10-fold concentrated. Perpendicular to the downward flow of separation buffer an electric field of 60 V/cm is set up, which gives rise in this medium and at 33 °C to a current of 150 mA. In these conditions, the injected material stays for about 2 min in the electric field. Coating of the glass walls of the separation chamber with albumin, which is usually recommended to compensate for electroendosmotic effects (Hannig & Heidrich, 1977), is omitted in order to avoid undesirable lipid–protein interactions. At the bottom of the separation chamber the buffer stream is collected by means of a multichannel peristaltic pump into 48 fractions. After each run (20 min) the fractions were analyzed for light scattering at 400 nm on an Aminco Bowman spectrophotofluorometer. Occasionally, they were also analyzed for radioactivity in a Packard Tricarb 2425 liquid scintillation counter.

Kinetic Analysis. In our previous report (De Cuyper et al., 1980) we showed that a curved relationship exists between the fractional amount of DMPG (from 0 to 100%) present in mixed DMPC–DMPG vesicles and the migration distance in the electric field, applied in the free-flow electrophoresis separation chamber. In the range between 0 and 10% DMPG, the data points can be fitted by linear regression to two straight lines: one between 0 and 5% (regression coefficient 0.99_1) and the other between 5 and 10% DMPG (regression coefficient 0.99_7). Changes in acceptor respectively donor vesicles are restricted within these two zones (see Results). Using this procedure, we can follow transfer of DMPG simply by measuring changes in the migration distance of the vesicles. The kinetics were expressed by the first-order rate equation

$$\log [(F_t - F_{eq})/(F_0 - F_{eq})] = -k_1 t/2.303$$

where F refers to the fraction number of the vesicle before transfer starts (F_0), at any time during the transfer process (F_t), and at equilibrium (F_{eq}). Identical rate constants were calculated irrespective as to whether the decrease in migration distance of DMPG-giving donor vesicles or the increase in migration distance of the DMPG-accepting vesicles was followed.

A different procedure was used to measure the transfer behavior of traces of radiolabeled phospholipids, namely, by making use of a vesicle system, the electrophoretogram of which is constant in the course of time. In this case, the fractional transfer was determined from the decrease in radioactivity associated with the vesicles that were originally doped with the labeled phospholipid or—with identical results—from the time-dependent increase in radioactivity in the vesicles accepting the labeled molecule. Here, too, the transfer behavior can be described by the first-order rate expression

$$\log [(R_t - R_{eq})/(R_0 - R_{eq})] = -k_1 t/2.303$$

where R_0 refers to the amount of radioactivity associated with the vesicle which either accepts or gives the labeled phospholipid before transfer starts, at any time during the transfer process (R_t), and at equilibrium (R_{eq}).

Results

Evidence for Spontaneous Phospholipid Transfer. In our experimental conditions for free-flow electrophoresis, DMPC and DMPC–DMPG (9:1) vesicles are separated by approximately 15 fractions (Figure 1). In an equimolar mixture of both, the two peaks merge as a function of time, indicating that intervesicular interaction occurs. Using markers for both the bilayer matrix and the internal volume of the vesicles, we have already been able to exclude the possibility that fusion

[1] Abbreviations: DOPC, dioleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; MES, 4-morpholineethanesulfonic acid; T_t, phase transition temperature; cbc, critical bilayer concentration; $t_{1/2}$, half-time.

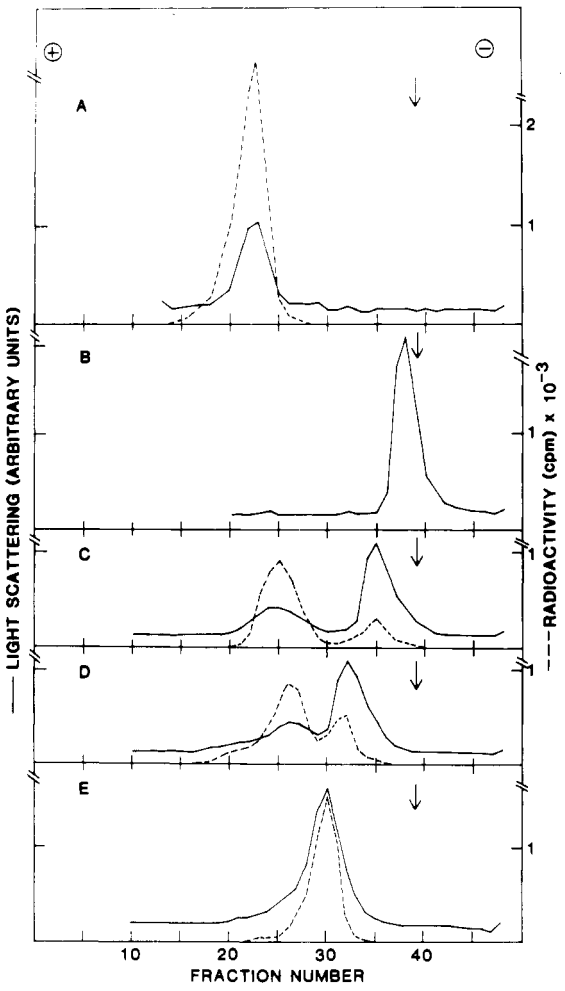

FIGURE 1: Measurements of DMPG transfer from changes in migration distance. Electrophoretograms of (A) DMPC–DMPG (9:1), (B) DMPC vesicles, and an equimolar mixture of both populations as a function of incubation times of (C) 45 min (D) and 130 min and (E) at equilibrium (240 min). Incubation and analysis is performed at 33 °C. The final phospholipid concentration equals 2 mg mL^{-1}. Light scattering is shown in arbitrary instrumental units. [^{3}H]DMPG is followed by liquid scintillation counting. The arrow indicates that injection into the separation chamber of the free-flow electrophoresis apparatus has occurred above fraction 39. Other experimental details are given under Experimental procedures.

or aggregation processes are responsible for this behavior (De Cuyper et al., 1980). In the present study, using radiolabeled DMPG, we demonstrate that the time-dependent changes in the electrophoretograms are associated with a net transfer of DMPG from the DMPC–DMPG (9:1) (designated as donors) to the neutral DMPC vesicles (designated as acceptors). The rate by which the two vesicle populations approach each other can be described by a simple first-order rate expression over at least 85% of the reaction course [see Figure 3 curve (+)]. At 33 °C in 5 mM MES–10 mM KCl, pH 6.0, a half-time of 41 min is calculated.

Dependence on Temperature. The temperature dependence of the spontaneous transfer process in the above-mentioned system is examined by means of an Arrhenius plot (not shown). Between 24 and 41 °C a straight line is obtained. From the slope an activation energy of 123 kJ mol^{-1} is calculated. Contrarily at 4 °C, which is below the gel-to-liquid-crystal phase transition temperature of both DMPC and DMPG (24 °C; Dangreau et al., 1979), the two original peaks remain at the same position as their light scattering signal slowly decreases (not shown). Concomitantly, a third peak with intermediate electrophoretic mobility arises, the light scattering

Table I: Transfer Rates of DMPG, DOPG, and DPPG between Equimolar Amounts of Phospholipid Vesicles, Differing in Charge and Degree of Fluidity[a]

	vesicle composition		
part	donor (9:1)	acceptor	rate (min)
A	DMPC–DOPG	DMPC	$t_\infty < 15$
		DOPC	$t_\infty < 15$
	DMPC–DMPG	DMPC	$t_{1/2} = 41$
		DOPC	$t_{1/2} = 50$
	DMPC–DPPG	DMPC	no transfer measurable
		DOPC	no transfer measurable
B	DOPC–DOPG	DMPC	$t_\infty < 15$
		DOPC	$t_\infty < 15$
	DOPC–DMPG	DMPC	$t_{1/2} = 86$ min
		DOPC	$t_{1/2} = 86$ min
	DOPC–DPPG	DMPC	no transfer measurable
		DOPC	no transfer measurable

[a] The final lipid concentration equals 2 mg mL^{-1}. Experiments are performed in 5 mM MES buffer (pH 6.0)–10 mM potassium chloride at 33 °C.

of which increases, indicating that fusion and/or aggregation phenomena dominate.

Dependence on Total Phospholipid Concentration. In equimolar mixtures of donor and acceptor vesicles the half-time of lipid transfer is independent on total lipid concentration in the range studied (from 0.5 to 4.0 mg mL^{-1}).

Dependence on Ionic Strength. Next, we incubated the equimolar mixture of DMPC and DMPC–DMPG vesicles in a 5- or 10-fold concentrated MES–potassium chloride buffer and injected it in the separation chamber filled with the regular buffer (5 mM MES–10 mM potassium chloride). The rate of transfer is decreased by a factor of 3.5 and 5.0, respectively. In spite of differences in osmotic pressure between the incubation mixture and the carrier buffer in the electrophoresis apparatus, the integrity of the vesicle structures was preserved as indicated by electron microscopy, Sepharose CL-4B column chromatography, and measurements of pyranine release (Clement & Gould, 1981) which we used to qualitatively monitor the internal volume of the anionic donor vesicles (De Cuyper et al., 1980).

Dependence on the Physical State of Donor and Acceptor Membrane. Changing the physical characteristics of the acceptor vesicles by replacing DMPC by the more fluid DOPC does not alter the transfer rate (Table I). Alternatively, if the main component (DMPC) of the donor vesicles is replaced by DOPC, the transfer rate of DMPG is decreased by a factor of 2 [$t_{1/2}$ = 41–50 min with DMPC–DMPG (Table IA) vs. $t_{1/2}$ = 86 min with DOPC–DMPG donor vesicles (Table IB)].

Dependence on the Fatty Acyl Side Chain Type of the Anionic Phospholipid. Extremely large differences in transfer rates are found by changing the hydrophobic moiety of the phosphatidylglycerol molecule to be transferred at 33 °C (Table I). Irrespective of the phosphatidylcholine type of the fluid donor and acceptor vesicle population, transfer of DOPG occurs too fast to be measured by our approach. In contrast, DPPG is not transferred, even after an incubation time of 2 days, resulting in completely immobile electrophoretograms.

Influence of the Membrane Surface Charge on the Transfer Properties of DMPC and DMPG. The immobile electrophoretograms, obtained with the system consisting of equal amounts of DMPC–DPPG (9:1) and DMPC vesicles, were further exploited to evaluate separately the individual transfer rates of DMPC and DMPG. First, we built in a trace of [^{3}H]DMPG into the DMPC–DPPG vesicles and followed its transfer behavior by monitoring the time-dependent changes in the amount of radioactivity associated with both vesicle

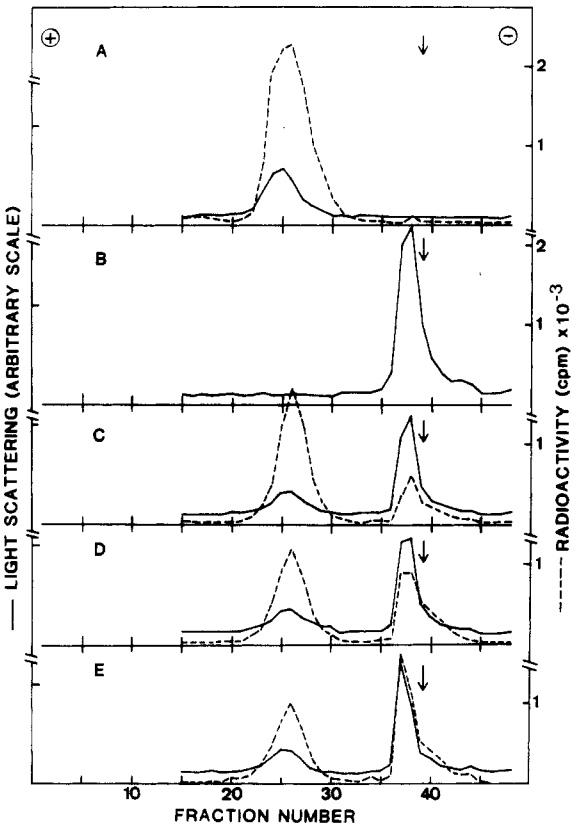

FIGURE 2: Measurements of DMPG transfer in immobile electrophoretograms. Electrophoretic distribution curves of (A) DMPC–DPPG (9:1) vesicles doped with a trace of [^{3}H]DMPG and (B) DMPC vesicles. The electrophoretograms of an equimolar mixture of both after (C) 60, (D) 140, and (E) 230 min are also shown. Other details are described in the legend to Figure 1.

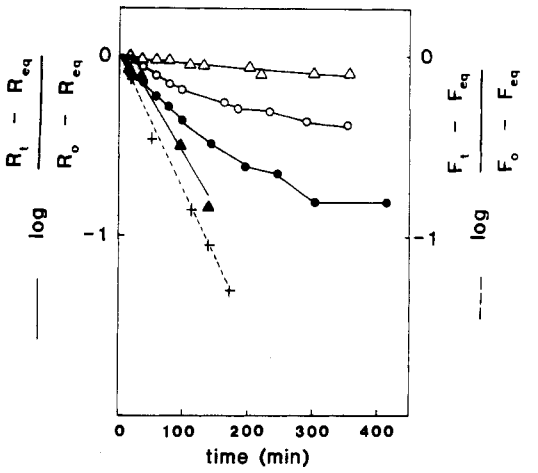

FIGURE 3: First-order kinetic plots for phospholipid transfer between equimolar amounts of differently charged phospholipid vesicles Kinetics are followed by measuring changes in the content of radiolabeled phospholipid, associated with the vesicles. R_0, amount of radioactivity associated with the vesicle population which either accepts or gives the radiolabeled phospholipid before transfer starts; R_t, at any time during the reaction course; R_{eq}, at equilibrium. At equilibrium, the labeled phospholipid is equally distributed over the two vesicle populations. (▲) Transfer of [^{3}H]DMPG from DMPC–DPPG (9:1) to DMPC vesicles (cf. Figure 2); (△) transfer of [^{3}H]DMPG from DMPC to DMPC–DPPG (9:1) vesicles; (●) transfer of [^{3}H]DMPC from DMPC–DPPG (9:1) to DMPC vesicles [for calculation of the half-time value (see Results), the first six time points are taken]; (○) Transfer of [^{3}H]DMPC from DMPC to DMPC–DPPG (9:1) vesicles [for calculation of the half-time value (see Results), the first six time points are taken]. For comparison, the first-order kinetic plot derived from the time-dependent changes in migration distances of an equimolar mixture of DMPC and DMPC–DMPG (9:1) vesicles (cf. Figure 1) is also shown (+). F_0, number of the fraction tube in which the vesicle is collected before transfer starts; F_t, at any time during the transfer process; F_{eq}, at equilibrium.

populations (Figure 2). At the end of the reaction period a 50:50 distribution of the labeled DMPG over the two vesicle types is found. Furthermore, the straight line in the kinetic plot [Figure 3, curve (▲)] shows that first-order reaction conditions are maintained during the whole reaction time. From the slope a half-time of 50 min is calculated. In contrast, if the transfer of DMPG is followed in the opposite direction (by incorporating [^{3}H]DMPG in the DMPC vesicles), the half-time is considerably increased ($t_{1/2} \cong 900$ min) [Figure 3, curve (△)]. This points to a tremendous effect of the surface charge of both membrane structures on the mobility of a charged phospholipid component.

A similar procedure is used to assess the transfer rate of the uncharged DMPC which also equally distributes over the two vesicles types at the end of the reaction period. However, much less difference between the transfer properties of DMPC is found ($t_{1/2} = 90$ min for the transfer of [^{3}H]DMPC from DMPC–DPPG to DMPC vesicles and $t_{1/2} = 130$ min for its transfer in the reverse direction), indicating a much smaller effect of membrane surface charge. It is also noteworthy that the first-order graphs, representing the kinetics of DMPC transfer [Figure 3, curves (●) and (○)] in both systems, are curved.

Discussion

In studying spontaneous phospholipid transfer phenomena, we have decided in favor of well-defined artifical small unilamellar vesicles, containing phospholipids with well-characterized polar head-group types and fatty acyl side chains. Our basic experiment concerns the phospholipid transfer between DMPC and DMPC–DMPG (molar ratio 9:1) vesicles above their gel-to-liquid-crystal phase transition temperature. From Figure 3 it is clear that both DMPG and DMPC are actively involved in this transfer process. Whereas we can deduce from Figure 1 that DMPG undergoes a net mass transfer, in the case of DMPC most probably an exchange process rather than a net transfer occurs. The half-times for DMPC transfer at 33 °C [90 min [Figure 3, curve (●)]; 130 min [Figure 3, curve (○)]] are in excellent agreement with the half-time value of 80 min for spontaneous DMPC transfer at 37 °C between two populations of DMPC vesicles containing 1.75 and 15% phosphatidic acid (De Kruijff & Van Zoelen, 1978).

In our model, which is not complicated by fusion or aggregation, the mechanism of spontaneous phospholipid transfer can be supposed to involve a collision of particles or, alternatively, to occur by means of single phospholipid molecules or small micelles which diffuse in the aqueous phase between donor and acceptor vesicle. A distinction between these two models can be done on the basis of their kinetic consequences. The first-order characteristics observed for the transfer process between *equimolar* amounts of donors and acceptors already point to an aqueous transfer mode. Conclusive evidence for the fact that the frequency of collision between *equimolar* amounts of donors and acceptors is not rate determining is further proved by the invariance of the transfer rate constant at different total phospholipid concentrations (Duckwitz-Peterlein et al., 1977; McLean & Phillips, 1981; Doody et al., 1980; Nichols & Pagano, 1981; Roseman & Thompson, 1980). Also, our view that the "aqueous transfer model" is valid is further strengthened by the observation that upon increasing the ionic strength the transfer rate decreases. By extrapolation

of the effect of salts on the critical micellar concentration of anionic surfactants (Tanford, 1980), we would indeed expect a lower solubility of DMPG at higher salt concentrations. Contrarily, in the case of a collision-dependent process, it is expected that transfer would be enhanced by increasing salt concentration.

Below the gel-to-liquid-crystalline phase transition temperature of donor and/or acceptor vesicles, possible transfer processes are obscured of even overrun by fusion and/or aggregation phenomena (Schmidt et al., 1981). To avoid these complications we have only considered transfer processes between fluid vesicles. On the basis of DPH fluorescence polarization measurements, Helmkamp (1980) claims that DOPC vesicles are more fluid than DMPC ones at 37 °C. If in the donor vesicles which we used DMPC is replaced by DOPC as the main phospholipid component, the overall transfer rate of DMPG is halved (Table I). In contrast, between DOPC and DMPC acceptor membranes no difference in transfer rate is found, indicating that the relative fluidity of the donor vesicles is much more important than that of the acceptor vesicles.

As compared with the moderate influence of the degree of fluidity of the donor vesicles, the fatty acid composition of the phospholipid molecule to be transferred is of considerably more importance. Indeed, irrespective of the degree of fluidity of donor and acceptor vesicles the following sequence in transfer rate is found at 33 °C: DOPG > DMPG > DPPG. In the literature differences in transfer rates of phospholipids are often correlated with their water solubilities [the so-called critical bilayer concentration (cbc) (Nichols & Pagano, 1981)]. For a single vesicle population the cbc value equals the ratio of the off and on rate constants, which characterize the process by which a lipid molecule respectively escapes or is adsorbed by the vesicle. In heterogeneous mixtures of vesicles—as in our case—we expect that the cbc is a complex quantitity. First, it depends on inherent characteristics (fatty acyl characteristics, polar head group) of the lipid molecule to be transferred. Second, it may also depend on the physical properties (degree of fluidity, surface charge) of donor and acceptor vesicles, which—in addition—continuously change during the course of transfer. Finally, a wide variety of external conditions (e.g., ionic quality of the medium, temperature, etc.) may also be of importance.

The cbc values for the phospholipids of major interest are very small and therefore difficult to measure experimentally. However, from thermodynamical considerations, Martin & MacDonald (1976) calculated that DMPC is about 30 times more soluble than DPPC (cbc of DPPC = 4.6×10^{-10} M in water at 20 °C). They also correlated this difference with a completely unidirectional diffusion of DMPC molecules from DMPC to DPPC vesicles. In the case of phosphatidylglycerols, a similar behavior is expected and may explain the moderate transfer rate which we observed with DMPG in contrast with the immobile DPPG. Also, Papahadjopoulos et al. (1976) deduced from differential scanning calorimetry experiments that, during incubation of DMPG and DPPG vesicles at 45 °C, the latter population is continuously enriched with the more soluble DMPG.

The fast transfer rate of DOPG is difficult to explain. Neither oxidation products, as proved by the method of Klein (1970), nor lysophospholipids, as controlled by thin-layer chromatography, were found. On the other hand, the impact of the presence of a double bond in the fatty acyl side chains (e.g., in DOPG) on the conformation and water solubility of a monomeric phosphatidylglycerol molecule remains ambiguous. Whereas Chapman (1973) assumes that unsaturation does not affect water solubility, Tanford (1980) calculates that the presence of double bonds in alkenyl carboxylates clearly decreases the hydrophobic effect. Whether this supposed increase in solubility can be responsible for the fast transfer rate of DOPG is unknown.

The polar head group of the phospholipid molecule also seems to exert a marked effect on the transfer rate characteristics. For instance, in 5 mM MES–10 mM potassium chloride, DMPG moves 2–3 times faster than DMPC from DMPC–DPPG (9:1) to DMPC vesicles [Figure 3, curves (▲) and (●)]. A possible explanation for this difference in transfer rate is that, at relatively low ionic strength, the critical micellar concentration values for charged amphiphiles are higher than the values for an amphiphile with the same alkyl chain but containing a zwitterionic head group (Tanford, 1980). Also, Doody et al. (1980) found that the spontaneous transfer between DMPC vesicles of 9-(3-pyrenyl)nonanoic acid in its carboxylate form occurs faster than in its noncharged form.

However, Tanford's (1980) studies cannot entirely explain our observations. Indeed, the transfer of the same DMPG molecule in the opposite direction [from DMPC to DMPC–DPPG (9:1) vesicles] occurs at a considerably slower rate [Figure 3, curve (△)], indicating that the surface charge of the membranes tremendously affects the transfer capacity of the anionic DMPG. Our experiments do not allow to present a detailed picture, showing the crucial step responsible for this difference. For reason of electrostatic repulsions, either the tendency of DMPG to escape from a DMPC–DPPG vesicle may be higher than its tendency to desorb from a neutral DMPC vesicle or the incorporation of a monomeric DMPG molecule may occur faster in DMPC membranes than in DMPC–DPPG vesicles. Anyhow, the extremely large difference in transfer rate observed leads us to tentatively conclude that DMPG will move in a unidirectional way in case the acceptor membrane (e.g., DMPC vesicles) is less negatively charged than the donor membrane [e.g., DMPC–DPPG (9:1) and probably also DMPC–DMPG (9:1) vesicles]. Further support for the virtual absence of a considerable back exchange of DMPG is given by the fact that the kinetics in Figures 1 and 2 remain first order over the first 85% of the reaction course [Figure 3, curves (+) and (▲)] in equimolar mixtures of donor and acceptor vesicles such as used in our experiments. This special property of our system contrasts with results of different authors, who need to work either with an excess of acceptor vesicles (McLean & Phillips, 1981; Nichols & Pagano, 1981) or with initial velocities [Backer & Dawidowicz, 1981; also our experiments in the case of DMPC transfer [Figures 3, curves (●) and (○)]] to quantitatively treat their kinetics with first-order mathematics.

A fundamental question in understanding spontaneous intervesicular transfer concerns the transbilayer movements of the lipids between the two leaflets of the bilayer. In general, flip-flop movements have been described to be slow processes (Kornberg & McConnell, 1971), but in systems that are one-sidedly perturbed fast rates are occasionally measured. For instance, De Kruijff & Baken (1978) proved that phosphatidic acid, produced during phospholipase D action on phosphatidylcholine vesicles, moves from the outer to the inner bilayer shell with a half-time of only 30 min. In our experiments a similar imbalance in charge distribution, as a result of intervesicular lipid transfer, probably enhances flip-flop movements of DMPG. Indeed the rate of transmembrane movement must be at least as fast as the intermembrane transfer process, since changes in the amount of charged

DMPG in the outer bilayer shell [illustrated in Figures 1 and 3, curve (+)] parallel the changes in the total amount of DMPG transferred [illustrated in Figures 2 and 3, curve (▲)]. Also, at the end of the reaction course DMPG is evenly distributed over the two vesicle types (Figure 2).

Summary and Conclusions

The spontaneous transfer of a phospholipid molecule between fluid vesicles is known to be largely dependent upon its fatty acyl side chain characteristics. An important new finding deduced from this work is the fact that for the anionic DMPG—in contrast to the zwitterionic DMPC—the kinetics of transfer are strictly governed by the electrostatic properties of donor and acceptor vesicles. Our observations are done by free-flow electrophoresis. We have presented this technique as a tool that can be successfully applied as an alternative for ion-exchange chromatography in studies of intermembranous interactions between vesicles.

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