Glucocerebroside Transfer between Phosphatidylcholine Bilayers†

Maria C. Correa-Freire, Y. Barenholz,‡ and T. E. Thompson*

ABSTRACT: We have studied the kinetics of transfer of glucocerebroside between phospholipid bilayers by using pyrene and ^{3}H-labeled glucocerebroside incorporated into dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) bilayers. Pyrene-labeled glucocerebroside (PyrCer) molecules are able to form an excited complex (eximer, E) between a PyrCer in the ground state and an excited monomer (M). When vesicles containing a known amount of PyrCer (donors) are incubated with unlabeled vesicles (acceptors), transfer of PyrCer from donor to acceptor populations is reflected in a decrease of the observed E/M intensity ratio. The results obtained from these studies show that the half-time of transfer from donor DMPC–PyrCer vesicles to acceptor DMPC vesicles is greater than 30 days at 37 °C. This very slow transfer of glucocerebroside was confirmed by using tritiated glucocerebroside incorporated into small unilamellar DPPC donor vesicles incubated with large unilamellar DPPC acceptor vesicles above the phase transition. Separation of the two vesicle populations by molecular sieve chromatography at 45 °C shows a half-time for transfer of approximately 32 days. We conclude that, in contrast to the results obtained for phosphatidylcholines [Roseman, M., & Thompson, T. E. (1980) *Biochemistry 19*, 439], glucocerebroside does not rapidly transfer between bilayers under these conditions.

It has been argued that phospholipid transfer from other membranes or lipoproteins could help preserve the marked compositional asymmetry between the inner and outer monolayers of the plasma membrane. This transfer has been shown to occur primarily by two routes: transfer aided by phospholipid exchange proteins (Crain & Zilversmit, 1980a,b) and spontaneous phospholipid transfer from one bilayer to another. Spontaneous transfer of phospholipid molecules has been studied by a variety of nonfluorescent methods (Martin & MacDonald, 1976; Papahadjopoulus et al., 1976; Kremer et al., 1977). These results show a net phospholipid flux between bilayers with distribution half-times varying between 2 and 24 h, depending on the particular system and the temperature. Half-times of exchange as short as this, shorter than the life span of a mammalian cell, have important biological implications since they make possible phospholipid redistribution between different membranes. This is particularly important in the case of glycosphingolipids, which are thought to be involved in cell–cell identification and recognition (Horowitz, 1978; Moss & Vaughn, 1979; Roseman, 1974). Evidence of a similar rapid exchange of glycosphingolipids between bilayers would imply that the role of these lipids as specific cell surface markers would have to be reassessed.

With this information in mind, we decided to determine directly the rate of spontaneous transfer of glucocerebroside between bilayers. For this purpose we have used 1-*O*-(β-D-glucopyranosyl)-*N*-[10-(1-pyrenyl)decanoyl]-D-*erythro*-sphingosine (PyrCer)[1] as a cerebroside analogue incorporated into dimyristoylphosphatidylcholine donor vesicles. The protocol used to study the kinetics of transfer was developed by Doody et al. (1978, 1980), Charlton et al. (1976), Sengupta et al. (1976), and Roseman & Thompson (1980) for pyrene-labeled lipids. It is based on the ability of pyrene molecules to form an excited complex (eximer, E) between a pyrene molecule in the ground state (M) and an excited monomer (M*) (Förster, 1969):

$$M + M^* = (MM)^* = E \quad (1)$$

The excited monomer M* has a characteristic emission peak at 376 nm, while the eximer emits at 460 nm. From eq 1 it is clear that the presence of the eximer depends on the local concentration of pyrene molecules. When a population of donor vesicles containing a known amount of PyrCer is incubated with a population of unlabeled acceptor vesicles, any transfer of PyrCer from the donor to the acceptor vesicles will be reflected in the observed intensity ratio, E/M. When the decrease of the E/M is monitored, it is possible to follow the time course of exchange.

In order to determine whether the results observed with PyrCer were characteristic of the cerebroside molecule itself or whether they reflected perturbing properties of the label, we also studied the spontaneous exchange of tritiated glucocerebroside ([^{3}H]GlcCer)[1] between bilayers. The experiments described below clearly show that the transfer of glucocerebroside between bilayers of phosphatidylcholine is a very slow process, much slower than the transfer of phosphatidylcholine itself. A preliminary report of this work has appeared elsewhere (Correa-Freire et al., 1981).

Experimental Procedures

Materials. Dimyristoyl- and dipalmitoylphosphatidylcholines (DMPC and DPPC, respectively)[1] were purchased from Avanti Biochemicals, Inc. The lipids were checked for purity by thin-layer chromatography and stored at −20 °C until used.

Pyrenyl glucocerebroside was synthesized by a modification of the procedure described by Hammarström (1971) developed by S. Gatt and T. Dinur (personal communication). Ten micromoles of D-*erythro*-sphingosinyl glucoside, 15 μmol of 10-(1-pyrenyl)decanoic acid, and 15 mg of *N*,*N*-dicyclohexylcarbodiimide were pooled in a screw cap test tube and dried under vacuum overnight. To start the synthesis, 2 mL of the reaction solution, consisting of 2.5 mL of CH_2Cl_2, 2.5

† From the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22908. *Received August 11, 1981.* This work was supported by U.S. Public Health Service Grants GM-14628, GM-23573, and HL-17576 and U.S.–Israel BSF Grant 1688.

‡ Also at the Department of Biochemistry, the Hebrew University, Hadassah Medical School, Jerusalem, Israel.

[1] Abbreviations: GlcCer, glucocerebroside; pyrenyl glucocerebroside or PyrCer, 1-*O*-(β-D-glucopyranosyl)-*N*-[10-(1-pyrenyl)decanoyl]-D-*erythro*-sphingosine; [^{3}H]GlcCer, [^{3}H]glucocerebroside; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PDA, pyrenedecanoic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/82/0421-1244$01.25/0

mL of CH_3CN, and 0.5 mL of CH_3OH, was added to the dry reagent. The sealed test tube was stirred overnight in a 45 °C oil bath. Special precautions were taken to avoid water contamination of the reagents. Large HR silica gel plates, 0.25 mm thick, were used to purify the sample. After the reaction mixture was spotted onto the plates, these were chromatographed by using a 85:15:1.5 chloroform–methanol–H_2O solvent. Appropriate cerebrosides were used as markers. The fluorescent sample was then scraped from the plate and placed in a small narrow glass column. Extraction was accomplished by eluting the gel with pure methanol until no fluorescence remained. 10-(1-Pyrenyl)decanoic acid (PDA)[1] was obtained from Molecular Probes. The purified PyrCer was stored in a 2:1 ratio of spectral chloroform to methanol and kept at −20 °C. The purity of the sample was better than 99% as judged by thin-layer chromatography. The PyrCer was rechromatographed, however, before each use to assure purity. After transfer experiments 12 h in length, no impurities derived from PyrCer breakdown could be detected. The absorption and emission spectra of PyrCer were identical with those of pyrenedecanoic acid.

Glucocerebroside (GlcCer)[1] was extracted from a biopsy of the spleen of a patient with Gaucher disease. It was purified by silicic acid chromatography and the fatty acid composition determined by gas–liquid chromatography, as previously described (Correa-Freire et al., 1979). [^{3}H]GlcCer[1] was labeled and purified as described by Schwarzmann (1978).

Preparation of Liposomes. Single-walled vesicles were prepared by cosonication of PyrCer and DMPC in 0.05 M KCl–0.01 M Tris-HCl–10^{-4} M EDTA (pH 7.5) under N_2 by the method of Barenholz et al. (1977). For the PyrCer transfer experiments, the following donor and acceptor populations were used as donor vesicles: DMPC:PyrCer (90:10) and DMPC:PyrCer:PDA (90:9:1). The acceptor populations were pure DMPC vesicles and DMPC:GlcCer (90:10) vesicles. Vesicle preparations were annealed separately at 37 °C for 12 h prior to use. At the end of the experiments, no multilamellar vesicles could be detected.

The [^{3}H]GlcCer transfer experiments used large unilamellar DPPC liposomes as the acceptor population and DPPC:[^{3}H]GlcCer (90:10) vesicles as the donor population. Large unilamellar DPPC vesicles (approximately 700 Å in diameter) were prepared by fusion as described by Schullery et al. (1980). DPPC:[^{3}H]GlcCer vesicles were prepared in an analogous fashion to the DMPC:PyrCer vesicles described above. Only labeled GlcCer was used in order to have vesicles with the highest possible specific activity. These vesicles were maintained above the DPPC gel–liquid phase transition at 45 °C at all times.

The PyrCer-containing multilamellar liposomes for the calorimetric and fluorescence studies were prepared as described by Lentz et al. (1976a,b) in 50 mM KCl containing 15% sucrose to avoid sedimentation during the experiment.

Fluorescence Measurements for Transfer Experiments. Fluorescence measurements were carried out on an Perkin-Elmer MPF-3 spectrofluorometer. The temperature was controlled by a Lauda Mk-2 thermoregulated bath, and the temperature in the cuvettes was monitored by a thermistor probe connected to a digital ohmmeter. The quartz cuvettes were sealed at all times with Teflon stoppers. The PyrCer emission spectra were recorded in the scanning range 280–600 nm. The excitation wavelength was kept constant at 345 nm. Uncorrected spectra were used for our calculations.

The procedure for the experimental determination of PyrCer transfer between bilayers was as follows. Each sample contained appropriate amounts of donor (D) and acceptor vesicles (A) to attain the desired concentration ratio (R). The final sample volume was 4 mL, with a total lipid concentration between 0.005 and 0.113 mM. The eximer intensity at 460 nm and the monomer intensity at 376 nm were measured prior to and immediately after mixing vesicle populations. The relative intensity of each peak was obtained by measuring its height, and the total sample intensity was determined by cutting and weighing each complete profile. Subsequent intensity determinations were made at different time intervals. Pure donor vesicles were used as controls, and light scattering was negligible. Experimental conditions such as vesicle composition will be described in detail when the individual experimental results are presented.

The derivation of the equations relating the experimental E/M ratio to the concentration of probe remaining in the donor vesicles during the transfer experiments have been presented by Roseman & Thompson (1980). The expression of the E/M fluorescence intensity ratio for this system is

$$\frac{E}{M} = \frac{C_D^2(C_{D_0} - C_D) + RC_D^2C_h + (C_{D_0} - C_D)^2(C_D + C_h)}{C_hC_D(C_{D_0} - C_D) + RC_h^2C_D + RC_h(C_{D_0} - C_D)(C_D + C_h)} \times \frac{K_1}{K_2}\frac{N_{E_{max}}}{N_{M_{max}}} \quad (2)$$

where C_{D_0} is the initial probe concentration in the donor vesicles, C_D is the probe concentration in the vesicles at any time t, and R is the ratio of acceptor to donor vesicles. C_h is the half-value concentration, and K_1 and K_2 are proportionality constants. $N_{E_{max}}$ and $N_{M_{max}}$ are the maximum eximer quantum yield as $C \rightarrow \infty$ and monomer quantities yield as $C \rightarrow 0$, respectively.

C_D values were extrapolated from computer simulated curves of E/M vs. C_D by using eq 2. C_h was obtained from

$$C/M = \frac{C}{LK_2C_hN_{M_{max}}} + \frac{1}{LK_2N_{M_{max}}} \quad (3)$$

as $C \rightarrow 0$. C is the ratio of PyrCer to DMPC concentrations, and L is the molar phospholipid concentration. Equation 11 in the paper presented by Roseman & Thompson (1980) appeared with an error. The correct formulation of this equation is given above in eq 3. Our value of C_h is 0.0117 ± 0.001.

E_{max}/M_{max} was obtained by substituting experimental values of C_h into

$$\frac{E}{M} = \frac{C}{C_h}\frac{E_{max}}{M_{max}} \quad (4)$$

Here E_{max} represents the maximum eximer fluoresence intensity as $C \rightarrow \infty$, and M_{max} is the maximum monomer fluorescence intensity as $C \rightarrow 0$. $E_{max}/M_{max} = 0.0322 \pm 0.005$ for this system.

Results and Discussion

Concentration Dependence of Pyrenyl Cerebroside E/M Intensity Ratio. In order to determine a suitable PyrCer concentration range to use in our experiments, we studied the dependence of the E/M ratio as a function of the PyrCer concentration in DMPC and DPPC unilamellar vesicles. Figure 1 presents the results of these studies. It is apparent that there is a linear dependence of E/M on the PyrCer microscopic concentration up to 10 mol %. This linear relation has been interpreted as indicative of a diffusion-controlled process (Galla & Sackmann, 1974). Concentrations of PyrCer

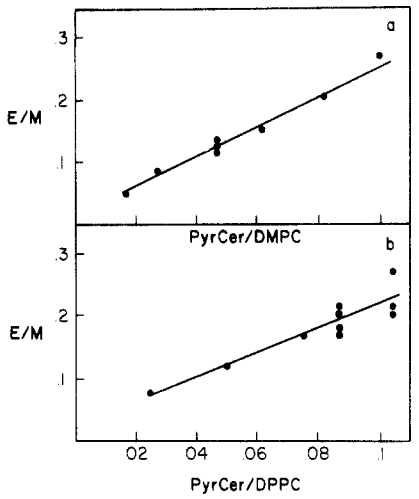

FIGURE 1: Dependence of the E/M ratio on the pyrenyl cerebroside concentration: (a) PyrCer in DMPC vesicles at 37 °C and (b) PyrCer in DPPC vesicles at 45 °C.

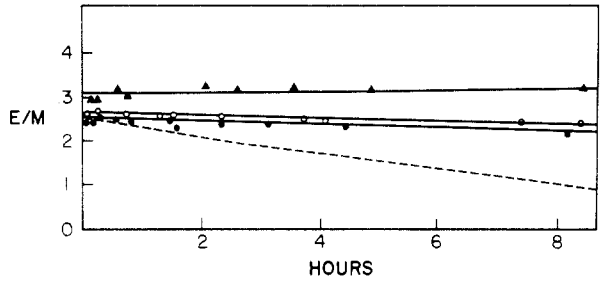

FIGURE 2: Kinetics of PyrCer transfer. Time dependence of the E/M ratio for (▲) control vesicles (90:10) DMPC:PyrCer, (90:10) DMPC:PyrCer donor vesicles incubated with a 5-fold excess of (○) (90:10) DMPC:GlcCer, and (●) pure DMPC vesicles. (---) Pyrene-PC transfer results obtained by Roseman & Thompson (1980); the E/M scale was normalized to make their results comparable to ours.

lower than 2 mol % were so dilute that the eximer emission could not be measured accurately. Higher PyrCer concentrations were not used in order to avoid possible structural perturbations of the system and to aovid complications in comparing our results with those of Roseman & Thompson (1980). Unless otherwise specified, the PyrCer to DMPC mole ratio used for the transfer experiments was 0.1.

Pyrenyl Cerebroside Transfer Experiments. Figure 2 shows the kinetics of PyrCer transfer obtained for (90:10) DMPC:PyrCer donor vesicles with pure DMPC or (90:10) DMPC:GlcCer acceptor vesicles in a 5:1 acceptor/donor ratio. There was no change in the E/M ratio of a control vesicle dispersion which contained no added acceptor vesicles throughout the duration of the experiments. The samples containing acceptor vesicles showed only a very slow decay in the E/M ratio as a function of time.

After 8 h 97.5% of the label present at time zero remained in the donor vesicles. Since the difference between this value and 100% is at the limit of detectability, the half-time for PyrCer transfer in this experiment is >6.7 days. It should be noted, however, that a very small but rapid decrease in E/M was observed immediately following the mixing of the donor and acceptor populations. This instantaneous decay is probably due to small amounts of pyrene fatty acid or pyrene derivatives produced during sonication. The amount of label exchanged during this period was less than 2%. The curves obtained for both acceptor vesicle types are identical within experimental error. For comparison we have also plotted the results obtained by Roseman & Thompson (1980) using a pyrene derivative

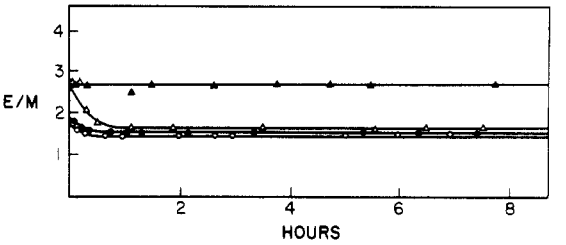

FIGURE 3: Kinetics of PyrCer transfer. Time dependence of the E/M ratio for (▲) (90:9:1) DMPC:PyrCer:PDA control vesicles and (90:9:1) DMPC:PyrCer:PDA donor vesicles incubated with (△) 5-fold excess, (●) 10-fold excess, and (○) 20-fold excess of (90:10) DMPC:GlcCer acceptor vesicles.

of phosphatidylcholine. As can be seen, the rates of phospholipid exchange are considerably faster than those obtained for cerebroside exchange.

In order to investigate further the rapid decrease in E/M at very short time periods, we incorporated pyrenedecanoic acid (PDA)[1] along with PyrCer into donor vesicles in a ratio of 90:9:1 DMPC:PyrCer:PDA. Varying concentrations of (90:10) DMPC:GlcCer acceptor vesicles were used to obtain 5:1, 10:1, and 20:1 acceptor/donor ratios. Figure 3 shows the results of these experiments. As expected, there was no change in the E/M ratio of the control sample. However, there was a rapid decay in the E/M ratio for samples containing acceptor vesicles. This decay was concentration dependent, suggesting that transfer occurred through a collisional process. Furthermore, the amount of label transferred from the donor population during this time was 10%, suggesting that PDA transfer was responsible for this decrease in E/M intensity. The E/M ratio remained relatively constant thereafter. The half-time of transfer for this latter phase was greater than 30 days. These results are in agreement with those obtained by Doody et al. (1980). These experiments suggest that it is very likely that the rapid initial decrease in E/M is due to small amounts of PDA contaminating our PyrCer preparations. We conclude therefore that the rate of transfer of PyrCer from one bilayer to another is very slow in contrast to the relatively more rapid rate of phosphatidylcholine transfer.

In order to determine any perturbing effects of the pyrene moiety on the properties of our system, we systematically studied (1) the effect of oxygen on the optical properties of the system, (2) the thermotropic behavior of DPPC:PyrCer liposomes, and (3) the transfer of [^{3}H]GlcCer instead of PyrCer from donor to acceptor vesicle populations.

Oxygen Quenching of Pyrenyl Cerebroside. The quenching of pyrene emission by oxygen has been previously demonstrated (Parker & Hatchard, 1961). Figure 4 shows the results of a simple experiment done to determine the extent of O_2 quenching over an 8-h period. No special precautions were taken to exclude oxygen from the system; sonication, however, was carried out, as usual, under a N_2 atmosphere. After preparation the vesicles were placed in stoppered cuvettes, and the emission spectra were determined as a function of time. Figure 4a shows the change in relative intensities of the monomer (376 nm) and eximer (460 nm) emissions. It is clear that the intensities of both peaks are reduced as oxygen enters into the system. However, after 4 h, oxygen has equilibrated, and the monomer and eximer intensities remain constant. The E/M ratio, however, remained constant throughout the experiment as shown in Figure 4b. Identical results were observed for DPPC:PyrCer vesicles. Since the E/M ratio rather than absolute intensities are required in this study, it is clear oxygen quenching presents no problem except to reduce intensity.

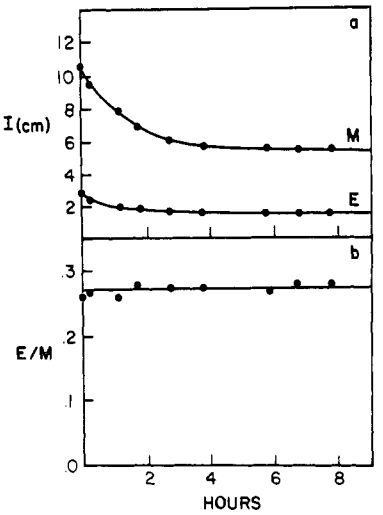

FIGURE 4: Time dependence of the monomer and eximer fluorescent emission intensities upon oxygen quenching: (a) decrease in intensity of the monomer (M) and eximer (E) peaks and (b) E/M intensity ratio as a function of time.

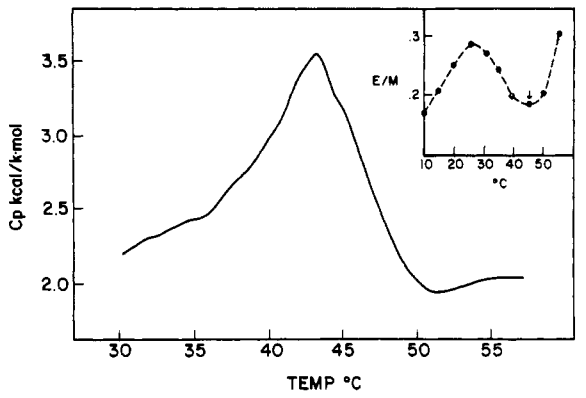

FIGURE 5: Calorimetric scan of (90:10) DPPC:PyrCer multilamellar liposomes. (Insert) E/M intensity ratio as a function of temperature.

Thermotropic Behavior of DPPC:PyrCer Liposomes. In previous studies we have extensively characterized the thermotropic behavior of DPPC and DPPC:GlcCer multilamellar liposomes (Correa-Freire et al., 1979). Shown in Figure 5 is the heat capacity curve of DPPC:PyrCer (90:10) multilamellar liposomes as a function of temperature. The heat capacity profile for this sample is somewhat broader than that observed for DPPC:GlcCer liposomes of a similar molar ratio [see Figure 1 in Correa-Freire et al. (1979) for comparison] and shows a maximum of 43.6 °C, which is slightly higher than that of DPPC:GlcCer at this Glc:Cer concentration. These changes are relatively small, suggesting that the presence of the pyrene moiety on the cerebroside molecule does not alter significantly its behavior in comparison to the behavior of GlcCer in the same system.

The lipid phase transition can also be monitored by measuring E/M as a function of temperature. This method is based on the observation that the formation of eximers in fluid membranes is a diffusion-controlled process (Galla & Sackmann, 1974). Above the phase transition temperature there is a linear decrease in E/M with decreasing temperature. At T_m this trend is reversed; the cause of the increase in E/M is not completely clear, but it is probably due to lateral phase separation of the probe which increases its local concentration, thus promoting greater eximer formation. These observations are presented more clearly in the insert of Figure 5. This figure shows the temperature dependence of E/M on temperature in DPPC:PyrCer (190:10) multilamellar liposomes. The transition temperature, as determined by a minimum in E/M denoted by the arrow, is 44 °C. This result is in good agreement with the value determined calorimetrically. The final decrease in E/M observed below 25 °C has no clear explanation, though it has been consistently found by Galla & Sackmann (1974) and was also present in all samples we examined. This decrease could be a result of pyrene self-quenching at high local concentrations caused by lateral phase separation. These results as well as the differential scanning calorimetric data clearly establish the fact that PyrCer is present only in DPPC bilayers and is not present as a macroscopic pure phase. This conclusion is consistent with the thermotropic behavior of GlcCer–DPPC mixtures previously reported (Correa-Freire et al., 1979).

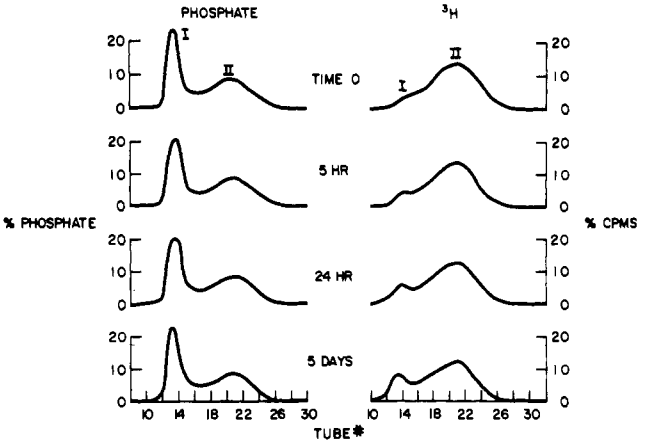

FIGURE 6: Sepharose CL-4B elution profiles of the DPL + (90:10) DPL:[^{3}H]GlcCer incubation mixture from [^{3}H]GlcCer transfer experiments as a function of time at 45 °C. Peak I corresponds to the larger fused DPL acceptor vesicle population and peak II to the smaller unilamellar donor population, as determined by phosphate and radioactivity assays. Recovery of lipid phosphorus and radioactivity after Sepharose chromatography was greater than 95%.

Net Transfer of [^{3}H]GlcCer between Phospholipid Bilayers. Unlike the fluorescent cerebroside analogue, [^{3}H]GlcCer has an identical structure to the original glucocerebroside, except for the ^{3}H atoms incorporated in the ceramide portion of the molecule. Its macroscopic behavior is also identical with that of the original molecule, yet it has the distinct advantage of being easily detectable. The experimental protocol for these studies was as follows: large unilamellar DPPC acceptor vesicles were incubated with small sonicated DPPC:[^{3}H]-GlcCer (90:10) donor vesicles at a 1:1 donor to acceptor ratio. Incubations were carried out at 45 °C. Aliquots from the incubation mixture were taken at predetermined time points. The sample aliquots were then chromatographed on a 45 °C Sepharose CL-4B column calibrated with small and large unilamellar liposomes. Fractions of 1.7 mL were collected. The 45 °C column kept the vesicles above their phase transition in order to minimize vesicle fusion (Schullery et al., 1980).

The donor and acceptor vesicle populations were easily separated by molecular sieve chromatography on the basis of their difference in size. The results of this experiment are shown in Figure 6. Two distinct peaks were found: peak I, corresponding to the larger acceptor liposomes, and peak II, corresponding to the smaller tritiated donor vesicles. In the event of net glucocerebroside exchange, radioactive label was expected to be associated with the acceptor population. There was in fact an increase in the amount of tritium associated with the acceptor population as a function of time, which, however, could be completely accounted for by fusion of donor vesicles with acceptor or other donor vesicles. Fusion was determined by monitoring the increase in the phosphate con-

centration of the larger population as a function of time. The extent of fusion for the first 24 h was less than 3%, and results corrected for this value showed negligible [^{3}H]GlcCer exchange during this time. At longer times the instability of small vesicles presented a problem. The elution profile of the sample incubated for 5 days showed peak II closer to the void volume due to larger vesicle sizes. This increase in size, presumably due to donor–donor vesicle fusion, makes net glucocerebroside transfer impossible to determine. If fusion is not taken into consideration, the glucocerebroside transfer in this latter case is less than 8%, which gives an apparent half-time of transfer of approximately 32 days. On the basis of this analysis, we conclude that the half-time for glucocerebroside transfer is greater than 32 days. This conclusion is consistent with the lower limit to the half-time of 30 days obtained by using PyrCer.

Recent work in this laboratory on phosphatidylcholines and sphingomyelins indicates that the very long half-time for glucocerebroside transfer is consistent with transfer from gel phase bilayers (A. Frank, Y. Barenholz, and T. E. Thompson, unpublished results). It is possible, therefore, that at the temperatures and concentrations examined in this study, glucocerebroside is present in a discontinuous gel-like phase distributed in a continuous liquid-crystalline phase consisting of DMPC or DPPC.

Glycosphingolipids have been shown to be localized on the external surfaces of the plasma membranes of mammalian cells, where they are believed to be involved in cell–cell identification and recognition (Horowitz, 1978; Roseman, 1974) and where they act as receptors (Moss & Vaughn, 1979). It is clear that if these molecules provide a means of distinguishing one cell type from another, they must remain associated with the outside surface of the plasma membrane of the cell of their origin. To be exchangeable between the outer surfaces of different cells with the relatively rapid half-time exhibited by phosphatidylcholine (Roseman & Thompson, 1980) would be inconsistent with their function as cell surface markers and receptors. A half-time of transfer of glucocerebroside in excess of 30 days is very long compared to the time frame for the biosynthesis of membrane components and the biogenesis of membrane structures comprised of these components. This result, obtained for a simple glycosphingolipid, is therefore compatible with the putative role of this class of molecules as stable cell surface markers and receptors in mammalian cells. It should be noted, however, that cytosolic transfer proteins which promote the intermembrane transfer of neutral glycosphingolipids have recently been described (Mertz & Radin, 1980; Bloj & Zilversmit, 1981).

Acknowledgments

We thank Dr. Shimon Gatt and Tama Dinur for their help in the synthesis and purification of fluorescently labeled cerebrosides and Dr. P. Felgner for his help in preparing and running the Sepharose 4B column for the [^{3}H]GlcCer transfer experiments.

References

Barenholz, Y., Gibbes, D., Litman, B. J., Goll, J., & Thompson, T. E. (1977) *Biochemistry 16*, 2806.

Bloj, B., & Zilversmit, D. (1981) *J. Biol. Chem. 256*, 5988.

Charlton, S. C., Olson, J. S., Wong, K.-Y., Pownall, H. J., Louie, D. D., & Smith, L. C. (1976) *J. Biol. Chem. 251*, 7952.

Correa-Freire, M. C., Freire, E., Barenholz, Y., Biltonen, R. L., & Thompson, T. E. (1979) *Biochemistry 18*, 422.

Correa-Freire, M. C., Barenholz, Y., & Thompson, T. E. (1981) *Biophys. J. 33*, 112a.

Crain, R. C., & Zilversmit, D. B. (1980a) *Biochemistry 19*, 1433.

Crain, R. C., & Zilversmit, D. B. (1980b) *Biochemistry 19*, 1440.

Doody, M. C., Pownall, H. J., & Smith, L. C. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1832.

Doody, M. C., Pownall, H. J., Kao, Y. J., & Smith, L. C. (1980) *Biochemistry 19*, 108.

Förster, T. (1969) *Angew. Chem. 81*, 361.

Galla, H. J., & Sackmann, E. (1974) *Biochim. Biophys. Acta 339*, 103.

Hammarström, S. (1971) *J. Lipid Res. 12*, 760.

Horowitz, M. I. (1978) in *The Glycoconjugates II* (Horowitz, M. I., & Pigman, W., Eds.) p 387, Academic Press, New York.

Kremer, J. M. H., Kaps-Werkhoven, M. M., Pathmamanoharan, C., Gijzeman, O. L. J., & Wiersema, P. H. (1977) *Biochim. Biophys. Acta 471*, 177.

Lentz, B. R., Barenholz, Y., & Thompson, T. E. (1976a) *Biochemistry 15*, 4521.

Lentz, B. R., Barenholz, Y., & Thompson, T. E. (1976b) *Biochemistry 15*, 4529.

Martin, F. J., & MacDonald, R. C. (1976) *Biochemistry 15*, 321.

Metz, R. J., & Radin, N. S. (1980) *J. Biol. Chem. 255*, 9463.

Moss, J., & Vaughn, M. (1979) *Annu. Rev. Biochem. 48*, 581.

Papahadjopoulus, D., Hui, S., Voil, W. J., & Poste, G. (1976) *Biochim. Biophys. Acta 448*, 245.

Parker, C. A., & Hatchard, C. G. (1961) *Nature (London) 190*, 165.

Roseman, S. (1974) in *The Cell Surface in Development* (Moscona, A. R., Ed.) p 225, Wiley, New York.

Roseman, M., & Thompson, T. E. (1980) *Biochemistry 19*, 439.

Schullery, S. E., Schmidt, C. F., Felgner, P., Tillack, T. W., & Thompson, T. E. (1980) *Biochemistry 19*, 3919.

Schwarzmann, G. (1978) *Biochim. Biophys. Acta 529*, 106.

Sengupta, P., Sackmann, E., Kuhnle, W., & Schultz, H. P. (1976) *Biochim. Biophys. Acta 436*, 869.