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Interaction of Intestinal Brush Border Membrane Vesicles with Small Unilamellar Phospholipid Vesicles. Exchange of Lipids between Membranes Is Mediated by Collisional Contact†

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ABSTRACT: The kinetics of lipid transfer from small unilamellar vesicles as the donor to brush border vesicles as the acceptor have been investigated by following the transfer of radiolabeled or spin-labeled lipid molecules in the absence of exchange protein. The labeled lipid molecules studied were various radiolabeled and spin-labeled phophatidylcholines, radiolabeled cholesteryl oleate, and a spin-labeled cholestane. At a given temperature and brush border vesicle concentration similar pseudo-first-order rate constants (half-lifetimes) were observed for different lipid labels used. The lipid transfer is shown to be an exchange reaction leading to an equal distribution of label in donor and acceptor vesicles at equilibrium (time $t \rightarrow \infty$). The lipid exchange is a second-order reaction with rate constants being directly proportional to the brush border vesicle concentration. The results are only consistent with a collision-induced exchange of lipid molecules between small unilamellar phospholipid vesicles and brush border vesicles. Other mechanisms such as collision-induced fusion or diffusion of lipid monomers through the aqueous phase are negligible at least under our experimental conditions.

Very little is known about how the digested lipids in the lumen of small intestine are absorbed by the enterocytes and pass from there into the lymph system. If only the first step in this process, that is, the interaction of the digested lipid with the absorptive surface of the enterocyte, is considered, then there are two questions. The first concerns the form in which the dispersed and digested lipids are absorbed: it could be monomeric, micellar, and vesicular (Carey et al., 1983). Although for lipids with a relatively high water solubility (or critical micellar concentration) uptake of monomers could be efficient, it would seem that for lipids with a low water solubility (e.g., diacyl phospholipids) uptake is only likely to be efficient if they are dispersed in micelles or bilayer vesicles. The second problem is that the site of lipid absorption on the enterocyte membrane is not known. If only the brush border is considered, then absorption could occur over the total microvillar membrane or be locally restricted.

Here we present part of a systematic study of lipid uptake by the enterocyte. We first chose to investigate lipid transfer between brush border membrane and small unilamellar vesicles made of phosphatidylcholine. The results presented are not only relevant to the question of lipid uptake by the enterocyte

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but also to exchange of lipids between membranes in general.

Materials and Methods

Egg phosphatidylcholine (EPC)[1] and dioleoylphosphatidylcholine (DOPC) were purchased from Lipid Products (Surrey, U.K.). Cholesteryl [1-^{14}C]oleate (specific activity 57 Ci/mol) was obtained from New England Nuclear (Boston, MA). 1-Palmitoyl-2-palmitoyl-3-*sn*-[9,10-^{3}H]-phosphatidylcholine ([^{3}H]DPPC, specific activity 85 Ci/mol) was synthesized from 1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (palmitoyllysophosphatidylcholine) and [9,10-^{3}H]palmitic acid according to Gupta et al. (1977); ^{3}H-labeled fatty acid was purchased from Amersham, U.K. Spin-labeled 1-palmitoyl-2-(5-doxylstearoyl)-3-*sn*-phosphatidylcholine (5-doxyl-PC) and 1-palmitoyl-2-(8-doxylpalmitoyl)-3-*sn*-phosphatidylcholine (8-doxyl-PC) were prepared from 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine by R. Berchtold (Biochemisches Labor, Bern). The spin-labeled cholestane, 3-doxyl-5-cholestane, was purchased from Molecular Probes, Junction City, OR.

Preparation of Brush Border Vesicles. The source was small intestine (stored at −50 °C) from freshly killed farm rabbits. The method of preparation was essentially that of Hauser et al. (1980) which is a modification of the method of Schmitz et al. (1973) and Kessler et al. (1978): the isolation buffer was 10 mM Hepes plus NaOH to pH 7.6, 0.3 M D-mannitol, 2 mM EGTA, and 0.02% NaN_3; 10 mM $MgCl_2$ was used instead of $CaCl_2$ in the precipitation step. The brush border pellet was redispersed in 0.3 M D-mannitol, 10 mM Hepes plus Tris to pH 7.6, 5 mM EDTA, and 0.02% NaN_3.

Preparation of Small Unilamellar Vesicles (SUV). The appropriate quantity of phosphatidylcholine plus the appropriate amount of label were dried from a chloroform–methanol solution (2:1 v/v) by rotary evaporation. The lipid film was dried under high vacuum for 30 min and then dispersed in buffer (0.3 M D-mannitol, 10 mM Hepes plus Tris to pH 7.6, 5 mM EDTA, 0.02% NaN_3). About 3-mL dispersions were sonicated with a microtip sonicator (Branson B-30) for 1 h (50% duty cycle) as described before (Brunner et al., 1978). Alternatively, radiolabeled phospholipid dispersions (1 mL) were sonicated in a bath sonicator (Laboratory Supplies, Hicksville, NY).

Incubation of Brush Border Vesicles with Labeled Small Unilamellar Phosphatidylcholine Vesicles. Prior to incubation the small unilamellar vesicles were centrifuged at 120000*g* for 14 min in order to remove large multilamellar structures, and the brush border vesicles were pelleted at 80000*g* for 10 min. A Beckman airfuge was used. Brush border vesicles and labeled SUV made of phosphatidylcholine, both suspended in the buffer (0.3 M D-mannitol, 10 mM Hepes plus Tris to pH 7.6, 5 mM EDTA, 0.02% NaN_3), were incubated at room temperature (20 ± 1 °C). At required time intervals 0.1-mL samples were removed and centrifuged at 80000*g* for 10 min. With radioactive labels, the radioactivity in the pellet or in the supernatant was measured; with spin-labels, the ESR spectra in both pellet and supernatant were recorded.

ESR spectra were recorded at 9.2 GHz with a Varian X-band spectrometer (Model E-104A) fitted with a variable temperature control. The temperature inside the 1 mm diameter sample capillary was measured by using a thermocouple; the accuracy was ±0.5 °C.

Analytical Procedures. The lipid content of the brush border vesicle suspension was determined gravimetrically after extraction into hexane–2-propanol (6:4 v/v; Radin, 1981) and evaporation of the organic solvent. Lipid phosphorus was determined according to Chen et al. (1956), and the protein content of brush border membrane was measured according to Lowry et al. (1951).

Results

Uptake by Brush Border Membrane of Spin-Labeled Phosphatidylcholine from Small Unilamellar Phosphatidylcholine Vesicles. Brush border vesicles were incubated with small unilamellar vesicles (SUV) made from a mixture of phosphatidylcholine (egg PC or dioleoyl-PC) and 5-doxyl-PC (mole ratio 10:1) for 19 h at room temperature. After the incubation, during which the spin-label was not enzymatically degraded, the SUVs were separated from brush border vesicles by centrifugation. The brush border pellet was resuspended, and ESR spectra were recorded at different temperatures. At all temperatures the spectra are characteristic of rapid but anisotropic motion with very little or no contribution from spin exchange (Figure 1A). In contrast, the ESR spectra of the spin-labeled phosphatidylcholine SUV contain a significant contribution from spin exchange (data not shown). This indicates that the ESR signal arises from 5-doxyl-PC molecules that have become integrated in the brush border membrane. This is shown more clearly in Figure 1B. Here the maximum hyperfine splitting $2T_{\parallel}$ (as derived from Figure 1A) is plotted vs. the reciprocal of the absolute temperature. The maximum hyperfine splitting is a measure of the anisotropy of motion, and as such it is sensitive to the environment that the spin-label senses in the membrane. The $2T_{\parallel}$ values of 5-doxyl-PC in the brush border membrane (solid symbols) are distinctly different from those of the same label present in bilayers of SUV made from EPC (○) or DOPC (△). The possibility that the ESR signal associated with the brush border vesicle pellet arises from contaminating SUV can therefore be ruled out. The solid line (Figure 1B) was included for comparison and represents the $2T_{\parallel}$ values of 5-doxyl-PC in brush border membranes that were incubated with a small amount of spin-labeled EPC SUV in the presence of PC exchange protein as described previously by Barsukov et al. (1980) and Mütsch et al. (1983). It is seen that there is little difference between the spin-label incorporated into brush border membrane in the presence or absence of PC exchange protein. If a fivefold excess (referred to brush border membrane lipid) of DOPC SUV labeled with 8-doxyl-PC was interacted with brush border vesicles in the absence of PC exchange protein, the results presented in Figure 1C were obtained. Over the total temperature range the $2T_{\parallel}$ values for the spin-label present in brush border membrane (solid circles) were significantly larger than those measured for the same label incorporated into bilayers of DOPC vesicles (open circles). The $2T_{\parallel}$ values (solid circles) were, however, significantly smaller than those of the solid line. The latter was obtained with brush border vesicles that were incubated with a small quantity of spin-labeled DOPC SUV in the presence of PC exchange protein (Mütsch et al., 1983). In this case apparently 8-doxyl-PC together with relatively small quantities of DOPC were incorporated in the brush border membrane, and consequently the $2T_{\parallel}$ values measured are characteristic of the lipid environment of this membrane. In contrast, when an excess of DOPC SUV was interacted with brush border membrane vesicles, sufficient DOPC appeared

[1] Abbreviations: cmc, critical micellar concentration; DOPC, 1,2-dioleoylphosphatidylcholine; DPPC, 1,2-dipalmitoylphosphatidylcholine; 5-doxyl-PC, 1-palmitoyl-2-(5-doxylstearoyl)-3-*sn*-phosphatidylcholine; 8-doxyl-PC, 1-palmitoyl-2-(8-doxylpalmitoyl)-3-*sn*-phosphatidylcholine; EPC, egg yolk phosphatidylcholine; PC, phosphatidylcholine; SUV, small unilamellar vesicles; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid.

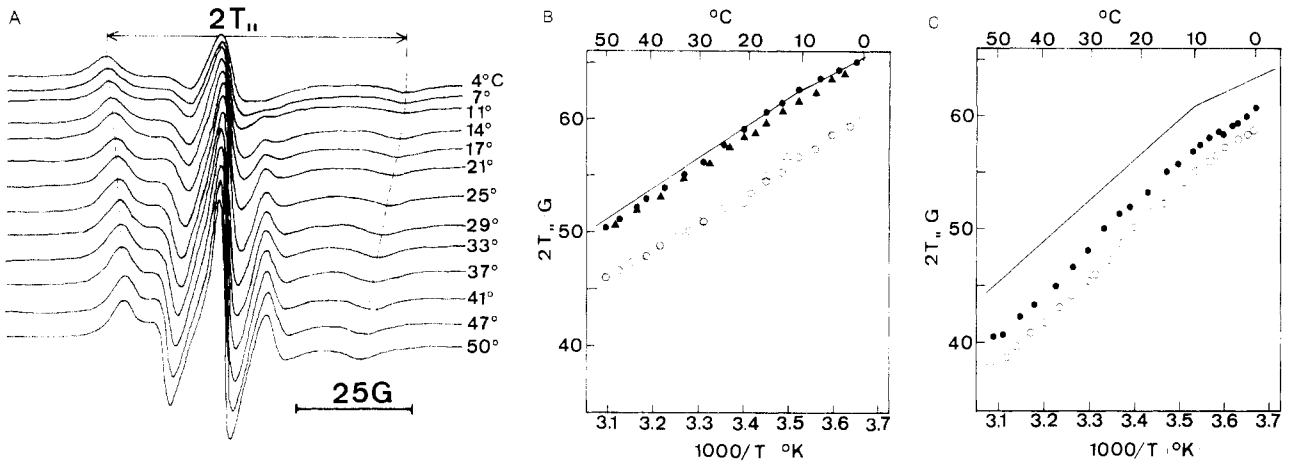

FIGURE 1: (A) Temperature dependence of the ESR spectra of 1-palmitoyl-2-(5-doxylstearoyl)-3-*sn*-phosphatidylcholine (5-doxyl-PC) incorporated into brush border vesicle membranes. The ESR spectra were normalized to a receiver gain of 3.2×10^3; the microwave power was less than 20 mW, the modulation amplitude of the 100-kHz field modulation was 1 G or less, the scan time was 8 min, and the time constant was 0.064. Brush border vesicles (10 mg of lipid/mL) were dispersed in 10 mM Hepes plus Tris to pH 7.6, 0.3 M D-mannitol, 5 mM EDTA, and 0.02 % NaN_3 and incubated with SUV made of EPC or DOPC (10 mg/mL) containing 5-doxyl-PC (lipid:spin-label mole ratio = 10) for 19 h at room temperature. No PC exchange protein was used. The brush border vesicles were separated from SUV by centrifuging a 0.1-mL sample at 80000*g* for 10 min, and the pellet of brush border vesicles was resuspended in the above buffer. (B) Maximum hyperfine splitting $2T_{\parallel}$ (G) as a function of reciprocal absolute temperature. The closed symbols are the $2T_{\parallel}$ values of 5-doxyl-PC in brush border vesicle membranes; the open symbols are those of the same spin-label in SUV made of EPC (○) or DOPC (△) at a lipid:spin-label mole ratio of 140. Brush border vesicle membranes were labeled by incubation with SUV made of EPC (●) or DOPC (▲) and containing 5-doxyl-PC [details as in the legend to (A)]. The solid line represents $2T_{\parallel}$ values of 5-doxyl-PC in brush border membrane; however, in this case the label was transferred from SUV of EPC to the brush border membrane with PC exchange protein according to Barsukov et al. (1980). (C) Maximum hyperfine splitting $2T_{\parallel}$ (G) as a function of reciprocal absolute temperature. The $2T_{\parallel}$ values (closed circles) are for 1-palmitoyl-2-(8-doxylpalmitoyl)-3-*sn*-phosphatidylcholine (8-doxyl-PC) in brush border membrane vesicles; the $2T$ values (open circles) are for the same spin-label in SUV made of DOPC at a lipid:spin-label mole ratio of 65. Brush border vesicle membranes (2.5 mg of total lipid/mL) were labeled by incubation with an about fivefold excess of SUV (14 mg/mL) made of DOPC in the absence of PC exchange protein. The solid line represents the $2T_{\parallel}$ values of 8-doxyl-PC in brush border membrane; in this case the spin-label was transferred from SUV of DOPC to brush border membrane with PC exchange protein according to Barsukov et al. (1980).

to be incorporated even in the absence of PC exchange protein so that the membrane fluidity and hence the $2T_{\parallel}$ values significantly decreased (Figure 1C). Furthermore, brush border vesicles were incubated with SUV made from EPC, and the resulting mixture was subjected to density gradient centrifugation on a continuous NaBr density gradient. Part of the liposomal phospholipid was eluted together with brush border membrane protein, indicating that phospholipid was incorporated in this membrane. This and the evidence presented in Figure 1 taken together strongly suggest that phosphatidylcholine is indeed transferred from SUV and inserted into the brush border membrane to a significant extent even in the absence of PC exchange protein.

Kinetics of the Lipid Transfer between Small Unilamellar Phosphatidylcholine and Brush Border Membrane Vesicles. The kinetic analysis of the lipid transfer from small unilamellar phosphatidylcholine vesicles as donor to brush border membrane vesicles as acceptor was carried out according to established methods (McKay, 1938; McLean & Phillips, 1981). The rate of exchange of cholesteryl [1-^{14}C]oleate between SUV made of EPC and brush border vesicles at 0 and 20 °C is shown in Figure 2. Plotting the data in this figure according to a pseudo-first-order reaction (eq 1) gave linear relationships

$$-\ln\left(1 - \frac{x}{x_\infty}\right) = k_1\left(\frac{a+b}{a}\right)t \qquad (1)$$

$$k_1 = \text{slope}\left(\frac{a}{a+b}\right) \qquad (2)$$

(data not shown). x is the fractional transfer of label at time t and x_∞ is the fractional transfer at equilibrium ($t \rightarrow \infty$). Assuming that there is true mass exchange between donor and acceptor vesicles, then $x_\infty = a/(a+b)$, where a and b are the acceptor and donor concentration, respectively. If the fraction

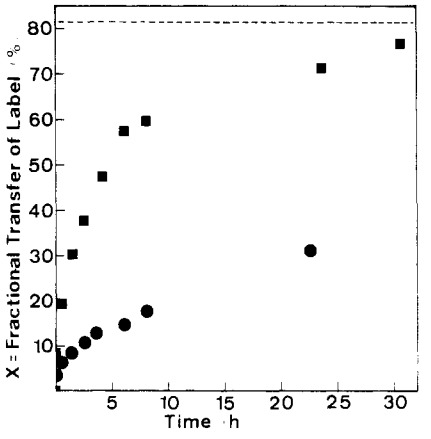

FIGURE 2: Time course of the fractional transfer of labeled [^{14}C]-cholesteryl oleate from SUV made of EPC to brush border vesicles at 0 (●) and 20 °C (■). The concentration of EPC vesicles (donor) was 2.5 mg/mL (=3.33 mM) and that of the brush border vesicles as the acceptor was 11 mg of total membrane lipid/mL. The expected equilibrium transfer x_∞ of label is therefore $x_\infty = a/(a+b) = 81\%$ as indicated by the dotted line; x_∞ represents equal distribution of cholesteryl oleate in donor and acceptor vesicles.

x of label retained in the donor vesicles was measured as a function of time, linearization of the data was carried out according to eq 3 where x_0 at $t = 0$ is 1 or 100 depending on

$$-\ln\left(\frac{x - x_\infty}{x_0 - x_\infty}\right) = k_1\left(\frac{a+b}{a}\right)t \qquad (3)$$

$$k_1 = \text{slope}\left(\frac{a}{a+b}\right) \qquad (4)$$

whether the counts retained in the donor are expressed as fractional counts or as percent. It is reasonable to assume that

Table I: Pseudo-First-Order Rate Constants k_1 and Half-Times $t_{1/2}$ for Lipid Transfer from SUV Made of EPC to Brush Border Membrane Vesicles

lipid transferred	wt ratio, BBV lipid/ SUV lipid	temp (°C)	k_1[a] (h^{-1})	$t_{1/2}$ (h)
[^{14}C]-cholesteryl oleate	4.5	20	0.17 ± 0.011	4.1 ± 0.28
[^{14}C]-cholesteryl oleate	4.5	4	0.022 ± 0.001	31.5 ± 1.4
[^{14}C]-cholesteryl oleate	20	20	0.51 ± 0.018	1.37 ± 0.05
5-doxyl-PC	20	20	0.55 ± 0.03	1.27 ± 0.07
3-doxyl-cholestane	20	20	0.63 ± 0.05	1.1 ± 0.09
[^{3}H]DPPC	10	20	0.28 ± 0.02	2.47 ± 0.18
[^{3}H]DPPC	1.25	20	0.039 ± 0.003	17.8 ± 1.4
[^{3}H]DPPC[b]	1.0	20	0.033 ± 0.008	21 ± 5
[^{3}H]DPPC	27	30	1.11 ± 0.1	0.62 ± 0.056

[a] k_1 values were derived from the least-squares fit of the data plotted according to eq 1 and 3 and are expressed as the mean ± SD. The error in the k_1 determination was 5–10% except for the back-reaction where the error was 24%. [b] Lipid transfer in the back-reaction from brush border vesicles to SUV made of EPC.

the acceptor concentration is identical with that of the total lipid pool of the brush border membrane (as will be discussed). In the experiment shown in Figure 2 the concentration was 11 mg of total membrane lipid/mL. The donor concentration was 2.5 mg of EPC/mL, and hence the fractional transfer at equilibrium is expected to be $x_\infty = 81\%$. As seen in Figure 2, this equilibrium value is nearly reached in a first-order process after 30 h at 20 °C. The data in Figures 2 and 3A support the assumption that there is lipid exchange rather than net transfer. The slope of the linear plot $-\ln(1 - x/x_\infty)$ vs. t (data not shown) was determined by a least-squares fit and is related to the pseudo-first-order rate constant k_1 by eq 2. This assumes that there is back-transfer of the label from brush border membrane to SUV of EPC. That this is indeed the case is shown below. Half-times were calculated from $t_{1/2} = (\ln 2)/k_1$. The pseudo-first-order rate constants k_1 and half-times $t_{1/2}$ thus derived were $k_1 = 0.17 \pm 0.011$ h^{-1} ($t_{1/2} = 4.1 \pm 0.28$ h) and $k_1 = 0.022 \pm 0.001$ h^{-1} ($t_{1/2} = 31.5 \pm 1.4$ h) for 20 and 0 °C, respectively. The pseudo-first-order rate constants for the transfer of radiolabeled cholesteryl oleate, DPPC, and spin-labeled lipids such as 5-doxyl-PC and the cholestane spin-label are summarized in Table I. Several points are immediately obvious from this table: (I) both the radiotracer method and ESR spin-labeling gave consistent results; (II) the k_1 values derived for the transfer of different lipid molecules are in good agreement within the error of the measurement; (III) for a particular lipid the rate of transfer depends on the brush border vesicle (acceptor) concentration. A double-label experiment was carried out where the EPC donor vesicles contained both [^{3}H]DPPC and [^{14}C]cholesteryl oleate. As expected from the results presented in Table I both labels were transferred stoichiometrically to the brush border membrane.

In order to prove that there is indeed lipid exchange rather than net lipid transfer from one membrane to the other, brush border vesicles were labeled with [^{3}H]DPPC as previously described for cholesteryl oleate. [^{3}H]DPPC-labeled brush border vesicles were incubated at room temperature with SUV made of EPC. The time course of the transfer of radiolabel from brush border vesicles to SUV, i.e., the back-reaction, was measured and evaluated as a pseudo-first-order reaction using eq 3 and 4. The results are included in Table I and prove the point that [^{3}H]DPPC is exchanged between brush border vesicles and SUV made of EPC. This finding is the justification for using eq 1–4 in the kinetic analysis.

Equations 1–4 are by definition first-order equations and do not contain information concerning the real order of the reaction (McKay, 1938). To derive the order of the lipid exchange reaction, the rate of [^{3}H]DPPC exchange was studied between EPC–SUV as the donor and increasing concentrations of brush border vesicles as the acceptor (Figure 3A). The radioactivity remaining in the donor vesicles was measured at timed intervals after removal of brush border vesicles by centrifugation. Each curve in Figure 3A was evaluated as a pseudo-first-order reaction using eq 3 and 4. As shown in Figure 3B the k_1 values thus obtained are directly proportional to the brush border vesicle (acceptor) concentration. The exchange of [^{3}H]DPPC between SUV and brush border vesicles is therefore a second-order reaction depending on the concentration of brush border vesicles. This is also true for the exchange of [^{14}C]cholesteryl oleate and the spin-labeled molecules (Figure 3B). The linear relationship (Figure 3B) confirms the conclusion drawn from Table I that the k_1 values for lipid transfer are by and large independent of the label used. That the lipid exchange is a second-order reaction can also be demonstrated by evaluating the experiments in Figure 3A according to eq 5 where v_0 is the initial exchange rate, C_A and

$$v_0 = -\left(\frac{dC_D}{dt}\right)_{t=0} = \left(\frac{dC_A}{dt}\right)_{t=0} = k_2 C_A^m C_D^n \quad (5)$$

C_D are the concentrations of acceptor and donor vesicles, respectively, k_2 is the second-order rate constant, and the exponents m and n are the order of reaction with respect to acceptor and donor, respectively. The slope of a logarithmic plot of initial exchange rate v_0 vs. acceptor concentration C_A is m, the values obtained for the four different lipid labels varied between 0.75 and 1 (data not shown). Similar exchange experiments, in which the acceptor vesicle concentration was kept constant while the donor vesicle concentration was varied over about a 20-fold range, gave values for the slope n between 0.8 and 1 (data not shown). The overall order of the reaction is given by the sum of the exponents yielding values of 1.6–2.

The temperature dependence of [^{3}H]DPPC exchange between SUV made of EPC and brush border membrane vesicles is given in Figure 4. The Arrhenius plot shown in this figure is best fitted by a single straight line. From the slope an apparent activation energy E for the exchange process between 0 and 33 °C is derived, $\Delta E = 57 \pm 2$ kJ mol^{-1}.

Discussion

The results presented in Figure 1 are evidence that the labeled PC originally present in SUV is transferred to the brush border membrane and indeed incorporated in the lipid bilayer of this membrane (Mütsch et al., 1983). There are two observations that rule out the possibility that the label is still present in SUV and that these vesicles are either bound to brush border vesicles or entrapped in them. The first is that brush border vesicles give ESR spectra after incubation with spin-labeled SUV that are distinctly different from those of the original SUV. Sufficient spin-label was incorporated into the bilayer of SUV so that the ESR spectra were characterized by spin exchange and markedly different from those obtained when the spin-label was incorporated in the brush border membrane (Figure 1A). The second observation is that the values of the maximum hyperfine splittings $2T_\parallel$ of the two

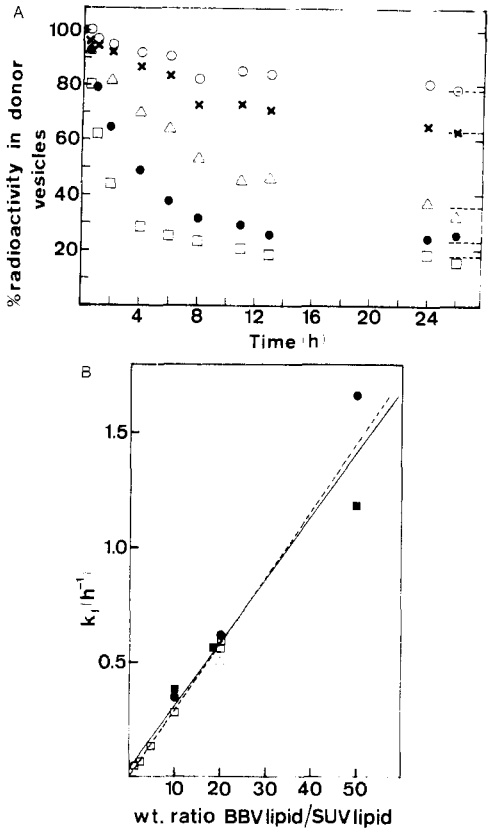

FIGURE 3: (A) Time course of the radioactivity remaining in the donor vesicles. SUV made of EPC (at 1 mg/mL) and labeled with [^{3}H]-DPPC were used as donor vesicles and incubated with brush border membrane vesicles of different concentrations: 1.25 (○), 2.5 (×), 5 (△), 10 (●), and 20 mg of brush border membrane lipid/mL (□). After timed intervals brush border vesicles were separated from the donor vesicles by centrifugation at 80000g for 10 min, and the radioactivity remaining in the supernatant SUV was determined by lipid scintillation counting. The dotted lines (right hand side) represent the equilibrium radioactivity y_∞ remaining in the SUV made of EPC (donor vesicles). (B) Pseudo-first-order rate constants k_1 (h^{-1}) as a function of the weight ratio of brush border membrane lipid to SUV phospholipid. Pseudo-first-order rate constants were derived from primary data relating fractional transfer of label to time (h). This fractional transfer of either radiolabel or spin-label from EPC vesicles (at 1 mg/mL) to brush border vesicles of different concentration was evaluated in terms of eq 1 or 3. [^{3}H]DPPC (□); [^{14}C]cholesteryl oleate (△); cholestane spin-label (●); 5-doxyl-PC (■). The dotted line is the linear least-squares fit to the experimental data obtained with [^{3}H]DPPC (□), $r^2 = 0.998$. The primary data for the [^{3}H]DPPC experiment are presented in (A). The solid line represents the linear least-squares fit to all other data points.

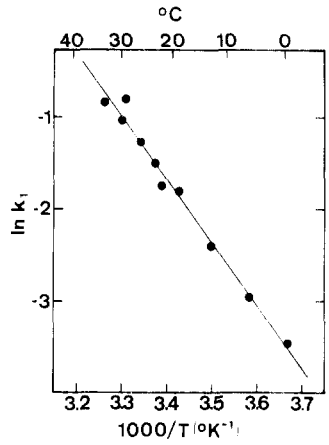

FIGURE 4: Arrhenius plot for the exchange of [^{3}H]DPPC between SUV made of EPC and brush border vesicles. SUV of EPC at a concentration of 1 mg/mL as the donor vesicles were incubated with brush border vesicles (BBV) at 10 mg of BBV protein/mL as the acceptor vesicles at the indicated temperature. At various time intervals up to 30 h brush border vesicles were separated by centrifugation at 80000g for 15 min, and the radioactivity was measured in the supernatant. First-order rate constants k_1 were determined as shown in Figure 2. The solid line is a least-squares fit to the experimental data ($y = 22 - 6.89x$, $r^2 = 0.986$). The apparent energy of activation ΔE is obtained from the slope = $\Delta E/R$ where R is the gas constant.

spin-labeled PC incorporated in brush border membrane are distinctly different from those measured when the same spin-labels are present in SUV (Figure 1B,C). The $2T_\parallel$ values of 5-doxyl-PC present in brush border membrane are close to those reported before (Mütsch et al., 1983). This is shown in Figure 1B. In the previous work the spin-label was indeed incorporated in the brush border membrane by incubating brush border vesicles with spin-labeled SUV in the presence of PC exchange protein. In further control experiments brush border vesicles were mixed with labeled SUV, and immediately after being mixed ($t = 0$), the brush border vesicles were separated from SUV by centrifugation. No label was found to be associated with the brush border membrane under these conditions. This control supports the conclusion that there is no binding or entrapment of SUV by brush border vesicles.

From Figures 2 and 3A it is clear that the radioactivity transferred to brush border vesicles tends toward an equilibrium value. This is consistent with the equilibration of the radiolabel between two lipid pools. For true mass exchange the fractional transfer at equilibrium of label x_∞ from SUV to brush border vesicles is given by $x_\infty = a/(a + b)$; the fractional amount of radioactivity remaining in SUV is then $y_\infty = b/(a + b)$. While the donor concentration b is equal to the concentration of SUV, the acceptor concentration a is unknown. It is reasonable to assume the acceptor concentration to be equal to the total lipid pool of the brush border membrane. Information concerning this question may be derived from the equilibrium distribution of the labeled lipid as follows. We assume that the acceptor concentration or pool is given by $a = k'a'$ where a' is the protein concentration of brush border vesicles and k' is a distribution coefficient taking into account that only part of the total brush border membrane may be accessible to the lipid transferred from SUV. In the case of mass exchange of the label between the two pools eq 6 holds. With rearrangement of eq 6 and with $x + y = 1$,

$$y_\infty = x_\infty \frac{b}{a'k'} \tag{6}$$

eq 7 is obtained. The equilibrium value y_∞ is in percent. The

$$y_\infty = 100 - y_\infty \frac{a'k'}{b} \tag{7}$$

experimental y_∞ values given by the dotted lines in Figure 3A were used and plotted according to eq 7 as a function of $y_\infty a'$ (Figure 5). The above treatment assumes that there is lipid exchange and the acceptor and donor concentrations or pool sizes a and b do not change with time. The intercept at $y_\infty a' \rightarrow 0$ is close to the expected value of 100. In the limit of $y_\infty \rightarrow 0$ or at infinite brush border vesicle (acceptor) concentration, $y_\infty a' = 100b/k'$. The k' value that is derived from Figure 5 and which is a measure of the pool size is 0.3. The acceptor pool is 0.3 of the protein concentration of brush border membrane. It is not identical with the total lipid pool of brush border membrane. It is interesting to note that the acceptor pool $a'k'$ agrees well with the total amount of phospholipid and neutral lipids. This pool of phospholipids and neutral lipids has been shown to amount to 40–60% of the total membrane lipid (Hauser et al., 1980).

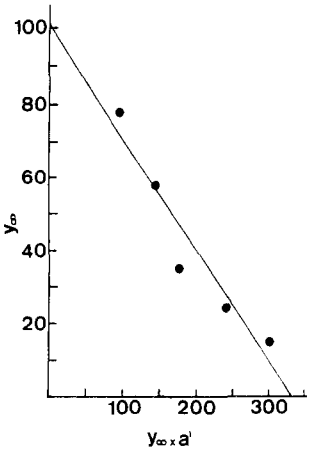

FIGURE 5: Experimentally determined equilibrium values y_∞ for the radioactivity remaining in SUV as a function of the product $y_\infty a'$. The y_∞ values given by the dotted lines in Figure 3A were plotted according to eq 7. The brush border membrane or acceptor concentration is expressed as $a'k'$ where a' is the protein concentration of brush border membrane in milligrams per milliliter and k' is a distribution coefficient. The concentration of SUV (made of EPC) as the donor is b; it is kept constant at 1 mg/mL. The solid line was obtained by a linear regression analysis of the data ($r^2 = 0.91$). The slope is k'/b from which $k' = 0.3$.

The determination of the order of the lipid exchange reaction between SUV and brush border membrane vesicles is important in considering its mechanism. In vitro, four different mechanisms have been found by which lipid from liposomes becomes incorporated into cell membranes: (I) transfer of free monomers, (II) transfer of monomers bound to an exchange protein, and (III and IV) transfer via collisional contact leading either to membrane fusion or to exchange of membrane lipid. Lipid transfer catalyzed by soluble lipid exchange protein(s) can be safely ruled out as a possible mechanism in our experiments. Lipid exchange proteins are of cytosolic origin and would have been lost during the preparation of brush border vesicles. Furthermore, if there was any significant contribution from this kind of exchange mechanism, the k_1 values or half-lifetimes are expected to depend on the nature of the lipid for lipid exchange proteins are known to be substrate specific (Demel et al., 1973; Kamp et al., 1973). This is not borne out by experiment. Table I and Figure 3B show that for all four different labels used similar rate constants were obtained. The second-order kinetics we find would be consistent with fusion. However, fusion between SUV and brush border vesicles can also be ruled out as a major contribution to lipid transfer. First, we have shown that labeled lipid is not only transferred from SUV to brush border membrane but also vice versa. Second, unlimited fusion would lead to a net transfer of label from SUV to brush border membrane. If, on the other hand, the fusion process was limited and the brush border membrane could take up only a limited amount of lipid, the quantity of lipid taken up is expected to be directly proportional to the brush border vesicle concentration. These predictions of the fusion mechanism are apparently inconsistent with the experiment. The observed equilibrium transfer of label is incompatible with either fusion mechanism. If there is some contribution from fusion, it must be within the error of our measurements. Therefore, the remaining two mechanisms which have to be considered for the lipid exchange between phospholipid SUV and brush border membrane are the following: The first one is a multistep process involving the desorption of lipid molecules from the donor vesicle into the aqueous phase, diffusion of monomers through the aqueous phase, and collision with brush border membrane vesicles as the acceptor (Hagerman & Gould, 1951). The second mechanism is lipid transfer via collisional contact between donor and acceptor membrane (Gurd, 1960). The following discussion is an attempt to differentiate between these two possible mechanisms on the basis of their kinetic predictions. As pointed out before the monomer-diffusion mechanism (Roseman & Thompson, 1980; McLean & Phillips, 1981) may follow either first- or second-order kinetics depending on the rate-limiting step in the lipid exchange. These authors studied the lipid transfer between negatively charged SUV as donor vesicles and neutral SUV as acceptor vesicles. They found that the transfer of radiolabeled cholesterol and 1-palmitoyl-2-oleoylphosphatidylcholine between these two SUV populations followed first-order kinetics independent of the acceptor vesicle concentration. Furthermore, the k_1 values (half-lifetimes) depended on the nature of the lipid, probably reflecting the solubility or cmc of the lipid in the aqueous phase. The rates of exchange of cholesterol and 1-palmitoyl-2-oleoylphosphatidylcholine at 37 °C differed by more than 1 order of magnitude with $k_1 = 0.322 \pm 0.040$ h^{-1} ($t_{1/2} = 2.3 \pm 0.3$ h) and $k_1 = 0.016 \pm 0.003$ h^{-1} ($t_{1/2} = 48 \pm 5$ h), respectively. For the rate of exchange of DPPC, values of $k_1 = 0.012 \pm 0.0015$ h^{-1} and $t_{1/2} \cong 60$ h were reported (McLean & Phillips, 1981, 1984). McLean and Phillips (1981) concluded that their results are only consistent with a diffusion mechanism in which the rate-limiting step is the desorption of the lipid molecule from the donor bilayer. Only with the desorption step being rate limiting are first-order kinetics predicted. The monomer-diffusion mechanism could follow second-order kinetics. In this case the adsorption of lipid monomers by the acceptor bilayer would be the rate-limiting step, and the rate of lipid transfer would be proportional to the concentration of acceptor vesicles. So if the mechanism of lipid transfer between phospholipid SUV and brush border vesicles reported here was a monomer-diffusion mechanism, the rate-limiting step would have to be the interaction of monomers with the acceptor membrane, i.e., the brush border membrane. This is, however, unlikely because the kinetic predictions of this monomer-diffusion mechanism are inconsistent with the experiment. Monomer diffusion would involve the desorption of lipid molecules from the SUV into the aqueous phase. The kinetics of this process can be expected to be very similar to those reported by McLean and Phillips (1981). If in our case the rate-limiting step was the adsorption of monomers by brush border membrane and not the desorption step, we would predict the rates of lipid transfer to be slower than those observed by McLean and Phillips. This is not borne out by experiment. Inspection of Table I shows that our k_1 values for DPPC exchange are significantly larger than the k_1 value for DPPC exchange ($k_1 = 0.012 \pm 0.0015$ h^{-1}, $t_{1/2} \cong 60$ h) reported by McLean and Phillips (1984). Furthermore, the relative exchange rates of different lipid molecules are expected to differ whereas we found that the rate of lipid exchange is independent of the chemical nature of the lipid. Our results appear therefore only consistent with a mechanism of lipid exchange involving collisional contacts between phospholipid SUV and brush border vesicles. The exchange of lipid occurs then by lateral diffusion within the collisional complex formed between phospholipid SUV and brush border vesicles. A simple calculation shows that the collision rate is rate limiting rather than the lateral diffusion of lipid molecules within the collision complex. Lateral diffusion of lipid molecules is a fast process with diffusion coefficients $D = 10^{-8}$ cm^2 s^{-1} (Träuble & Sackmann, 1972), and the frequency of lipid exchange by diffusion between donor and acceptor membrane will exceed the collision frequency

between SUV and brush border vesicles of a mean diameter of 200 nm by more than 3 orders of magnitude (Sheludko, 1966; McLean & Phillips, 1981). If the collision frequency between vesicles determines the rate of lipid exchange, then the relative rates of exchange of different lipid molecules are expected to be similar. This is indeed the case in our lipid exchange experiments (see Table I and Figure 3B). When the mechanism of lipid exchange is discussed, another observation is relevant. SUV made of EPC and containing radiolabeled inulin[^{14}C]carboxylic acid (M_r 5200) entrapped in their aqueous cavity were incubated with an approximately 10-fold excess of brush border membrane lipid at room temperature for 6 h. After incubation the mixed vesicle dispersion was applied to a Sepharose 4B column. Brush border vesicles eluted in the void volume of the column well separated from the peak of EPC–SUV. Less than 5% of the total radioactivity eluted together with the brush border vesicles, 26% was associated with the SUV peak, and the remainder of the radioactivity was eluted in the total column volume. The radioactivity associated with brush border vesicles probably originates from fusion of SUV with brush border vesicles. Consistent with our discussion, fusion is apparently a minor process. The radioactivity eluted in the total volume represents inulin released from the internal vesicle cavity during incubation. In the absence of brush border vesicles the release of trapped inulin was negligible. The release of inulin is consistent with the formation of collisional complexes; it suggests that the vesicles partially leak out their content upon interaction with brush border vesicles. This result would be, however, difficult to reconcile with a monomer-diffusion mechanism. Desorption of single lipid molecules from SUV can hardly effect the integrity and permeability of the lipid bilayer. Furthermore, the activation energy of $\Delta E = 57 \pm 2$ kJ mol^{-1} for DPPC exchange is similar to that reported for lateral diffusion (Kuo & Wade, 1979), indicating that in both cases similar energy barriers are involved. Evidently under the experimental conditions used here the mechanism involving collisional contact and exchange diffusion within the collisional complex is predominant. However, when the acceptor and/or donor vesicle concentration is reduced sufficiently, it should be possible to significantly slow down the lipid exchange via collisional contacts. Under these conditions lipid exchange by monomer diffusion should become the predominant mechanism.

Physiological Significance. As discussed by McLean and Phillips (1981), the diffusion of lipid monomers seems to be a general lipid exchange mechanism. Cholesterol has been reported to exchange between SUV or lipoproteins via this mechanism. For less soluble molecules such as diacyl phospholipids nature uses exchange proteins to increase the rate of exchange of these lipids. For instance, the exchange of phosphatidylcholine is catalyzed by a specific exchange protein which also operates according to the monomer-diffusion mechanism; in this case, the phosphatidylcholine bound to the exchange protein diffuses through the aqueous phase. The exchange rate of cholesterol in the absence of protein is comparable to that of phosphatidylcholine in the presence of exchange protein, suggesting that exchange proteins are required for less soluble lipids but not for the relatively more soluble cholesterol molecule; it would seem that the water solubility or cmc of cholesterol is sufficiently high to give rise to physiologically adequate exchange rates. In contrast, we find that the lipid exchange between SUV made of EPC and brush border vesicles takes place by collisional contacts. This mechanism gives rise to relatively high exchange rates of lipid molecules differing widely in chemical nature. The question whether this mechanism of lipid exchange plays a physiological role in the lipid uptake by the enterocyte cannot be answered from the experiments presented here. Another related question is whether or not the lipid transfer is catalyzed by protein bound to brush border membrane. Undoubtedly more experimental work is required to answer these questions. A comparative study using other membranes such as the erythrocyte or inner mitochondrial membrane that are not involved in lipid uptake could be enlightening.

Registry No. DOPC, 4235-95-4; DPPC, 2644-64-6; 5-doxyl-PC, 66642-40-8; 8-doxyl-PC, 76743-18-5; 3-doxylcholestane, 18353-76-9; cholesteryl oleate, 303-43-5.

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