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Absorption of Monoacylglycerols by Small Intestinal Brush Border Membrane

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ABSTRACT: The absorption of monoacylglycerol by small intestinal brush border membrane is a passive process, i.e., the movement of monoacylglycerol from small unilamellar phospholipid vesicles as donor particles through the aqueous medium and the incorporation into the outer monolayer of the lipid bilayer of the brush border membrane are passive processes involving diffusion of the lipid along a concentration gradient. Small unilamellar vesicles of egg phosphatidylcholine containing 1 mol % of radiolabeled hexadecylglycerol were used as donor, and rabbit small intestinal brush border membrane vesicles or intact enterocytes isolated from pig jejunum, as acceptor. Hexadecylglycerol was employed as a lipase-resistant model compound for monoacylglycerols. Both acceptor membranes behave similarly in terms of hexadecylglycerol absorption: the kinetics of hexadecylglycerol absorption are biphasic. The initial fast phase is due to the movement of hexadecylglycerol from the donor particle through the aqueous medium to the outer lipid monolayer of the acceptor membrane, and the second slow phase probably involves the flip–flop motion of hexadecylglycerol from the outer to the inner monolayer of the acceptor membrane. The values for the pseudo-first-order rate constants of the initial fast phase for hexadecylglycerol absorption are relatively large and primarily determined by the high solubility (cmc) of hexadecylglycerol in aqueous media. The pseudo-first-order rate constants depend linearly on the protein (lipid) concentration of the acceptor membrane, indicating that the on rate of the hexadecylglycerol into the brush border membrane is rate limiting. The mechanism of the hexadecylglycerol absorption involves mainly monomer diffusion and probably collision-induced transfer.

It is now well established that triacylglycerols are hydrolyzed in the course of digestion by gastric and pancreatic lipases (Borgström, 1977; Patton, 1981; Carey et al., 1983). The two main products of hydrolysis are fatty acids and 2-acylglycerols (Hernell et al., 1990). The aqueous solubility of the products of fat hydrolysis is relatively low, ranging from nanomolar to micromolar, and therefore these products have to be emulsified and transported in some kind of dispersed form to the site of fat absorption. Lipolytic products are finally dispersed as bile salt mixed micelles and carried in this form to the site of absorption (Thomson & Dietschy, 1981). Lipid absorption takes place at the apical part of the plasma membrane of the epithelial cells or enterocytes lining the gut. Considering that 100–150 g of triacylglycerols are consumed daily by an average western adult (Hernell et al., 1990), rather large quantities of fatty acids and 2-acylglycerols have to be absorbed by the brush border membrane (BBM).[1] In the first step of lipid absorption the lipolytic products are presumably incorporated in the external half of the lipid bilayer of the BBM. Subsequently these compounds are assumed to diffuse either passively across the membrane or alternatively in a facilitated, protein-mediated process. Eventually the absorbed lipids are released into the cytosol and processed by the cell's internal machinery.

Recently we reported that the absorption of cholesterol from various donor particles by BBMVs is protein-mediated (Thurnhofer & Hauser, 1990a; Thurnhofer et al., 1991b; Lipka et al., 1991; Lipka et al., 1992). The uptake of cholesterol from both bile salt micelles as well as small unilamellar lipid vesicles is facilitated by an integral protein of the BBM which has a cholesterol-binding site exposed on the external or luminal surface of the BBM (Thurnhofer & Hauser, 1990; Thurnhofer et al., 1991a,b; Lipka et al., 1992). In the light of this finding the question arises whether other lipolytic products of fat digestion are also taken up by a protein-facilitated mechanism. In order to shed light on this question, we studied the uptake of hexadecylglycerol by small intestinal BBMVs and isolated epithelial cells or enterocytes. We used the ether lipid 1-*O*-hexadecylglycerol as a model for monoacylglycerol because ether lipids are resistant to lipases and are not hydrolysed during absorption. The use of ether analogs of glycerolipids in studies of intestinal absorption was reviewed (Paltauf, 1983).

MATERIALS AND METHODS

Materials

Egg PC and egg phosphatidic acid were purchased from Lipid Products (South Nutfield, Surrey, U.K.), cholesterol (puriss.) and D-(+)-glucose were purchased from E. Merck (Darmstadt, Germany), the sodium salt of taurocholate, heparin (grade I-A) from porcine intestinal mucosa, trypan blue solution (0.4%), hyaluronidase from bovine testes, and soybean trypsin inhibitor (both enzymes type I-S) were purchased from SIGMA (St. Louis, MO), papain from papaya latex was purchased from Boehringer (Mannheim, Germany), (phenylmethyl)sulfonyl fluoride, 2,3-dihydroxy-1,4-dithiol-

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[1] Abbreviations: BAEE, N^{α}-benzoyl-L-arginine ethyl ester; BBM, brush border membrane; BBMV, brush border membrane vesicle; BCA, bicinchoninic acid; cmc, critical micellar concentration; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid; PC, phosphatidylcholine; SUV, small unilamellar vesicles; TRIS, tris(hydroxymethyl)-aminoethane.

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butane, maleic acid, and sucrose were purchased from Fluka (Buchs, Switzerland), oleic acid was purchased from Applied Science (State College, PA), and D-[6-^{3}H]glucose was purchased from Amersham International, U.K. [9′,10′-$^3H_2$]-1-*O*-Hexadecyl-*sn*-glycerol (^{3}H-hexadecylglycerol) was prepared from 1-*O*-hexadec-9′-enyl-*sn*-glycerol by catalytic tritiation (Amersham International, U.K.). Unlabeled hexadecylglycerol was synthesized according to Baumann & Mangold (1964) and Yin et al. (1991). All lipids used in this work were pure by thin-layer chromatography standard.

Methods

Determination of the Critical Micellar Concentration (cmc). The cmc of hexadecylglycerol micelles labeled with ^{3}H-hexadecylglycerol was determined by ultrafiltration using a stirred ultrafiltration cell (from Amicon, Model 3) with Amicon YM2 filters (exclusion limit M_r = 1000). Solutions and dispersions (volume $\simeq$ 2 mL) of hexadecylglycerol in buffer A (0.01 M Hepes, 0.3 M D-mannitol, 5 mM EDTA, 0.02% NaN_3 adjusted with Tris to pH 7.3) were filtered through the Amicon ultrafiltration cell, and the radioactivities of the filtrate and of the dispersion retained on the filter were determined.

Preparation of BBMV. Details of collecting rabbit small intestines from the slaughter house and storing the intestines prior to the preparation of BBMVs were given previously (Thurnhofer et al., 1991b). The preparation of BBMVs from frozen small intestines was carried out according to Hauser et al. (1980).

Preparation of Isolated Enterocytes from Pig Jejunum. Nonfasting pigs were anaesthetized by electroshock applied to the brain and killed by bleeding at the slaughterhouse. A 7 cm long piece of upper jejunum was removed, cut open along the mesenteric border, and immediately immersed in 30 mL of ice-cold buffer B (0.01 M Hepes, 0.139 M NaCl, 2.4 mM Na_2HPO_4, 5.9 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$ adjusted to pH 7.4 with 1 M NaOH) continuously gassed with pure oxygen. In this state the tissue was transported by car from the slaughterhouse to the laboratory, which took about 15 min. Enterocytes were obtained following the procedure originally described by Stern (1966), Stern and Jensen (1966), and Weiser (1973). The enzymatic treatment was carried out according to Kimmich (1975) and Hoffman and Kuksis (1979), which proved to be optimal as to the release of intact cells from the tissue. The piece of small intestine was incubated at 35 °C for 20 min in 30 mL of buffer B containing 1 mg of hyaluronidase/mL and 0.13 mg of trypsin inhibitor/mL (660 inhibitory BAEE units), continuously gassed with pure oxygen. After the enzymatic treatment the tissue was transferred to another glass vessel containing 30 mL of buffer B at room temperature. The flask was gently handshaken for 1–3 min in order to effect the release of intact enterocytes. After removal of the tissue the cells were spun down at 400 × *g* for 2 min. In order to remove cell debris, the cells were resuspended in ~10 mL of buffer B and centrifuged at 400 × *g* for 2 min. The pelleted cells were resuspended in the appropriate volume of buffer B to yield protein concentrations ranging from 1 to 18 mg/mL.

Characterization of Isolated Enterocytes. The protein concentration of cell suspensions was routinely determined using the BCA method (Smith et al., 1985). Cells were counted in an improved Neubauer chamber, and the cell count was correlated to the protein concentration. Our value of about 1.5 × 10^6 enterocytes per mg of cell protein is higher than that of 1 × 10^6 villus cells/mg of protein reported by Kimmich (1975) for chicken mucosa and 0.74 × 10^6 villus cells/mg of protein and 1.21 × 10^6 crypt cells/mg of protein obtained by Hoffman and Kuksis (1979) for rat jejunum. Contaminant cells were identified mainly as lymphocytes amounting to less than 20% of the total cell count. The integrity and viability of isolated enterocytes was assessed by light microscopy using a 0.4% solution of trypan blue stain according to the supplier (Sigma). By this dye exclusion procedure the number of intact cells was determined before and after lipid uptake measurements, and in both cases estimates of 70–85% were obtained. D-Glucose uptake by enterocytes was determined in the absence and presence of a Na^+ gradient.

Proteolytic Treatment of BBMVs and Enterocytes. Papain Digestion of BBMVs. Papain was activated by diluting 0.2 mL of the papain suspension (10 mg/mL) with 40 μL of 0.5 M potassium phosphate buffer (pH 6.8) containing 50 mM 2,3-dihydroxy-1,4-dithiolbutane and 10 mM EDTA. The suspension was saturated with N_2 and incubated at room temperature for 30 min. BBMVs were suspended at 10 mg of protein/mL in 0.2 M potassium phosphate buffer, pH 6.8, containing 2 mM dihydroxy-1,4-dithiolbutane and 0.4 mM EDTA and digested with 2 mg of papain/mL (0.2 mg of papain corresponding to 6 units/mg of BBM protein) at 22 °C for 2 h. The reaction was stopped by adding (phenylmethyl)sulfonyl fluoride to a final concentration of 0.5 mM. The BBMV suspension was diluted with buffer 1:1 (by volume), BBMVs were sedimented at 100 000 × *g* for 30 min at 4 °C, and the resulting pellet of BBMVs was resuspended in buffer A to the final concentration required.

Papain Digestion of Isolated Enterocytes. Essentially the same procedure was used as described above except that isolated enterocytes suspended in buffer B were incubated with 0.2 mg of papain/mg of total protein of enterocytes at 22 °C for 1.5 h. Digested enterocytes were pelleted by centrifugation at 400 × *g* for 2 min and resuspended in buffer B. Enterocytes were washed by repeating this procedure twice except that the final suspension of the enterocytes was made up to the desired concentration.

^{3}H-Hexadecylglycerol Uptake by BBMVs and Isolated Enterocytes. Absorption isotherms of ^{3}H-hexadecylglycerol for both BBMVs and isolated enterocytes were determined at 4 and 22 °C. The fast absorption of ^{3}H-hexadecylglycerol by BBMVs taking place within less than 1 min was measured by a filtration method similar to that described before (Thurnhofer et al., 1991b). For this purpose 6 μL of BBMVs (10 mg of lipid/mL) suspended in buffer A was incubated with an equal volume of egg PC SUV (1 mg of lipid/mL) containing 1 mol % of ^{3}H-hexadecylglycerol. After timed intervals the reaction was stopped by automatic injection of 2 mL of ice-cold buffer A, and the diluted suspension was filtered through polycarbonate filters (from Nucleopore, mean pore size 0.4 μm) or cellulose nitrate filters (from Sartorius, mean pore size 0.65 μm). The radioactivities of both the filtrate and BBMVs retained on the filter were determined. Since the retention of BBMVs on the filter was incomplete, values for ^{3}H-hexadecylglycerol absorption were corrected for this effect. The absorption of ^{3}H-hexadecylglycerol after incubating BBMVs with egg PC SUV for periods longer than 1 min was determined by separating BBMVs from egg PC SUV by centrifugation at 100 000 × *g* for 10 min in a Beckman airfuge. Aliquots of the supernatant containing the radiolabeled egg PC SUV were analyzed for radioactivity.

The absorption of ^{3}H-hexadecylglycerol by isolated enterocytes was determined by incubating enterocytes (1–18

mg of cell protein/mL) suspended in buffer B with egg PC SUV containing 1 mol % of ^{3}H-hexadecylglycerol (0.063 mg of total lipid/mL). After timed intervals the cells were pelleted by centrifugation at 12 000 × *g* for 2 min, and the radioactivity remaining in the supernatant was determined.

Preparation of Egg PC SUV. SUV of phospholipids were prepared by dissolving the lipids in $CHCl_3/CH_3OH$ (2:1, by volume) and evaporating the solvent by rotary evaporation. The resulting film of mixed lipids was dried in vacuo, and the dry residue was dispersed in 2 mL of either buffer A or B by vortexing for 5 min at room temperature. The resulting lipid dispersion (2 mg/mL) was subjected to tip sonication as described by Brunner et al. (1978).

Exchange of ^{3}H-Hexadecylglycerol between Two Populations of SUV. The exchange of ^{3}H-hexadecylglycerol between two populations of SUV was measured as described for the exchange of radiolabeled PC (Thurnhofer & Hauser, 1990b). SUV of egg PC/egg phosphatidic acid/^{3}H-hexadecylglycerol (84:15:1, mole ratio) dispersed in buffer A at 0.5 mg of lipid/mL were incubated as donor particles with pure egg PC SUV at different lipid concentrations as acceptor vesicles. After timed intervals the negatively charged donor vesicles were separated from the isoelectric acceptor vesicles by absorbing the lipid dispersion on a DEAE-Sepharose CL-6B column of 0.5-cm height packed in a glass frit of 1-cm diameter. The lipid dispersions were filtered through the column under water pump vacuum. The negatively charged donor vesicles were retained on DEAE-Sepharose while the isoelectric acceptor vesicles were eluted. The amount of radiolabeled lipid transferred to the acceptor was determined.

Analytical Methods. The lipid content was determined either by radiotracer techniques or by lipid phosphorus determination (Chen et al., 1956). Protein concentrations were determined according to Lowry et al. (1951) or by the BCA method of Pierce (Smith et al., 1985). The radioactivity of radiolabeled samples was determined by counting three or four aliquots in a Beckman LS 7500 liquid scintillation counter.

RESULTS

Determination of the Critical Micellar Concentration (cmc). The cmc of hexadecylglycerol micelles labeled with ^{3}H-hexadecylglycerol was determined at room temperature by ultrafiltration. At hexadecylglycerol concentrations smaller than the cmc, there was a nearly perfect 1:1 correlation between the total hexadecylglycerol concentration and the hexadecylglycerol concentration in the filtrate (data not shown). The cmc was indicated by a break in this linear relationship, and the value thus obtained was (2.8 ± 0.3) × 10^{-6} M. In the same way, the monomer concentration of hexadecylglycerol was determined, which is in equilibrium with SUV of egg PC (1.0 mg of lipid/mL and 0.13 mg of lipid/mL) containing 1 mol % of ^{3}H-hexadecylglycerol. Aliquots of this phospholipid dispersion were filtered through the Amicon ultrafiltration cell, and the concentration of ^{3}H-hexadecylglycerol recovered in the filtrate was determined by radiocounting. The values obtained reproducibly for the monomer concentration of hexadecylglycerol under these conditions were (1.6 ± 0.3) × 10^{-6} M and (0.29 ± 0.19) × 10^{-6} M, respectively.

Absorption of Hexadecylglycerol by BBMVs. The absorption of ^{3}H-hexadecylglycerol by BBMVs from SUV of egg PC as donor vesicles was measured at 4 and 22 °C. As shown in Figure 1 biphasic curves were obtained at both temperatures. The initial fast phase of ^{3}H-hexadecylglycerol absorption was followed by a significantly slower phase. Linearization of the kinetic curves shown in Figure 1A was carried out according to eq 1 of the Appendix (McKay, 1938; McLean & Phillips, 1981; Mütsch et al., 1986). The resulting semilogarithmic plots shown in Figure 1B clearly reveal the biphasic nature of ^{3}H-hexadecylglycerol absorption by BBMVs. From the slopes of the straight-line relations, order-of-magnitude estimates for the pseudo-first-order rate constants k_1 can be derived. As detailed in the Appendix more precise values for these rate constants were derived from fitting the experimental curves by the sum of two exponentials. Values thus obtained are summarized in Table 1. The fast phase was characterized by pseudo-first-order rate constants k_1, which depended linearly on the acceptor concentration. Within the error of the measurement there was no difference in kinetics between 4 °C and room temperature. The second slow phase of ^{3}H-hexadecylglycerol was characterized by pseudo-first-order rate constants that were independent of the acceptor concentration. The average value for k_1 was k_1 = 1.1 ± 0.2 (h^{-1}), corresponding to a half-life of 19 ± 5 min (Table 1).

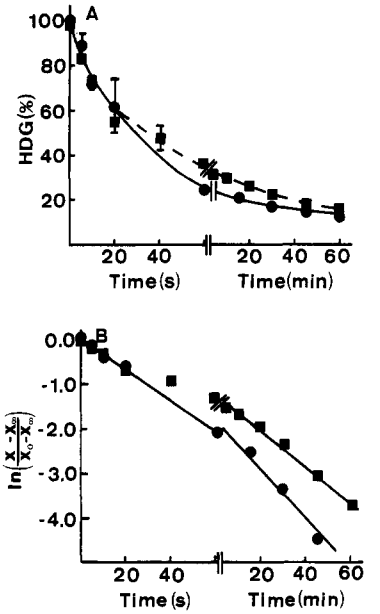

FIGURE 1: Kinetics of ^{3}H-hexadecylglycerol (HDG) absorption by BBMVs: (A) The amount of HDG present in the donor (expressed as percent) is shown as a function of time. BBMVs (10 mg of protein/mL or 5 mg of total lipid/mL) made from rabbit small intestines as described in Materials and Methods were incubated with egg PC SUV containing 1 mol % of HDG (0.5 mg of total lipid/mL) at 4 °C (■) and at 22 °C (●) for various periods of time, and HDG uptake was measured (see Materials and Methods). Both donor and acceptor vesicles were suspended in buffer A. (B) Linearization of the primary data shown in panel A was carried out according to eq 1 in the Appendix; x_0, x, and x_∞ represent the fraction of HDG present in the donor at times 0, t, and at equilibrium ($t \rightarrow \infty$), respectively. The solid lines were fitted to the experimental data by linear regression analysis.

Papain treatment of BBMVs as described under Methods had the following effect: the protein content of BBMVs was reduced to 38 ± 5% and the aminopeptidase N and sucrase activities were reduced to 25% and 18% of their original values, respectively. The cholesterol uptake by BBMVs from mixed bile salt micelles (Thurnhofer et al., 1991b) was significantly reduced. With untreated BBMVs the cholesterol uptake was practically finished after a 5-min incubation at room temperature; i.e., only 7% of the total cholesterol remained in the donor after 5 min. In contrast, with papain-treated BBMVs only 45% of the total cholesterol was taken up by BBMVs; i.e., 55% of the cholesterol remained in the donor after a 5-min incubation at room temperature. The proteins liberated from BBMVs by papain digestion catalyzed the exchange of

Table 1: ^{3}H-Hexadecylglycerol Absorption by Rabbit Small Intestinal BBMVs and Pig Jejunum Enterocytes

acceptor	temp (°C)	protein conc (mg/mL)	lipid pool a_1 (mg/mL)	lipid pool b (mg/mL)	$x_{1\infty}$[a] (%)	fast phase k_1 (h^{-1})	fast phase $t_{1/2}$ (s)[b]	lipid pool a (mg/mL)	lipid pool b (mg/mL)	x_∞[a] (%)	slow phase k'_1 (h^{-1})[c]	slow phase $t_{1/2}$[b] (min)
BBMV	22	0.8	0.125	0.5	78 ± 6	10 ± 5	52 ± 18	0.25	0.5			
	22	1.7	0.25	0.5	71 ± 2	48 ± 22	17 ± 8	0.5	0.5			
	22	10	1.5	0.5	22 ± 3	106 ± 6	18 ± 1	3	0.5	12	1.1 ± 0.2	19 ± 5
	22	12.7	1.9	0.5	20 ± 4	171 ± 42	12 ± 3	3.8	0.5			
	4	10	1.5	0.5	35 ± 10	125 ± 45	15 ± 4	3	0.5	14	1.1 ± 0.1	19 ± 3
BBMV treated with papain	22	(10)	1.5	0.5	21 ± 6	91 ± 29	20 ± 6	3	0.5			
enterocytes	22	10.8	0.42	0.5	76 ± 3	47 ± 6	24 ± 4	1.4	0.5	39	2.1 ± 0.9	10 ± 5
	22	0.7	0.027	0.063	77 ± 2	29 ± 7	26 ± 6	0.09	0.063	46	1.1 ± 0.1	18 ± 2
	22	1.8	0.070	0.063	73 ± 1	52 ± 4	25 ± 2	0.23	0.063	39	1.1 ± 0.1	19 ± 2
	22	2.9	0.113	0.063	60 ± 2	62 ± 6	24 ± 2	0.38	0.063	34	1.1 ± 0.1	18 ± 2
	22	9.2	0.36	0.063	51 ± 3	121 ± 12	18 ± 2	1.20	0.063			
	22	10.2	0.40	0.063	53 ± 3	135 ± 16	16 ± 2	1.33	0.063			
	22	10.7	0.42	0.063	46 ± 2	160 ± 12	14 ± 1	1.39	0.063			
	22	13.9	0.54	0.063	47 ± 2	177 ± 32	13 ± 2	1.81	0.063	24	1.5 ± 0.3	14 ± 3
	22	17.9	0.70	0.063	45 ± 3	198 ± 31	11 ± 2	2.32	0.063			
	4	10.7	0.42	0.063	40 ± 2	147 ± 22	15 ± 2	1.39	0.063			
enterocytes treated with heparin	22	12.1	0.47	0.063	43 ± 2	175 ± 16	13 ± 1	1.57	0.063			
enterocytes treated with papain[d]	22			0.063	45 ± 1		5 ± 0.5		0.063			

[a] Values for $x_{1\infty}$ and x_∞ were determined as described in the Appendix. [b] Half-times in columns 8 and 13 were calculated according to $t_{1/2} = \ln 2/[k_1(a_1 + b)/a_1)]$ and $\ln 2/(2k_1)$, respectively, taking care of the factors included in the two exponentials of eq 2 of the Appendix. [c] The rate constants k_1' of the second slow phase are independent of the enterocyte concentration, yielding average values of the half-time of 19 ± 5 and 16 ± 4 min for BBMVs and enterocytes, respectively. [d] $t_{1/2}$ was derived directly from the kinetic curve; a value for k_1 could not be calculated, since a_1 is unknown.

cholesterol between two populations of egg PC SUV. In the presence of the total amount of protein released by papain digestion, the extent of cholesterol exchange between donor and acceptor vesicles after a 5-min incubation at room temperature was increased by a factor of 3–4. However, as evident from Table 1, there was no difference in the kinetics of ^{3}H-hexadecylglycerol uptake by BBMVs before and after papain treatment, at least not within the error of the measurement.

Absorption of Hexadecylglycerol by Isolated Enterocytes. Isolated enterocytes behaved similarly to BBMVs as regards the absorption of hexadecylglycerol from egg PC SUV. Representative data describing the kinetics of ^{3}H-hexadecylglycerol absorption by enterocytes from egg PC SUV as the donor are shown in Figure 2. Similar to the cases of the experiments with BBMVs as acceptor, the curves were biphasic (Figure 2A–D). The absorption of ^{3}H-hexadecylglycerol approached equilibrium after about 90 min (Figure 2A). The initial fast phase was followed by a significantly slower phase of lipid absorption. The solid lines in Figure 2 represent the sum of two exponentials computer fitted to the experimental data according to eq 2 of the Appendix, and pseudo-first-order rate constants derived from the computer fit are summarized in Table 1. Within the experimental error the values of the pseudo-first-order rate constants obtained for enterocytes agreed well with those measured for BBMV at comparable effective lipid concentrations of donor and acceptor membranes. As evident from Figure 2B and Table 1, the pseudo-first-order rate constants for the initial fast phase of hexadecylglycerol absorption depended on the amount of enterocytes. In contrast, the pseudo-first-order rate constants characterizing the second slow phase of ^{3}H-hexadecylglycerol absorption were independent of the concentration of enterocytes. The average value for k'_1 characterizing the slow phase was $k'_1 = 1.38 \pm 0.44\ h^{-1}$, corresponding to a half-time of 16 ± 4 min. As shown in Figure 2C, there was no temperature dependence between 4 and 22 °C within the error of the measurement.

Figure 3 shows that the pseudo-first-order rate constants k_1 for the initial fast phase of ^{3}H-hexadecylglycerol absorption by enterocytes depend linearly on the protein concentration and, related to it, on the lipid pool of the enterocytes (closed circles, Figure 3). One experiment describing the ^{3}H-hexadecylglycerol absorption at 4 °C is included, emphasizing the lack of temperature dependence of ^{3}H-hexadecylglycerol absorption (open circles, Figure 3). Also included in Figure 3 is the pseudo-first-order rate constant obtained with enterocytes treated with heparin, indicating that removal of triacylglycerol lipase from BBM (Bosner et al., 1989) did not impair ^{3}H-hexadecylglycerol absorption (triangle, Figure 3).

Papain digestion of enterocytes had a destabilizing effect: only about 20% of the enterocytes survived this treatment, but more than 95% of the cells that survived were intact, as judged by trypan blue exclusion. As a control enterocytes suspended in buffer B were incubated at room temperature for 1.5 h. Proteins released during incubation were removed by washing the enterocytes three times. Papain-treated enterocytes and enterocytes of the control were resuspended to 5.7 and 5.4 mg of protein/mL, respectively. The aminopeptidase N activity of the papain-treated enterocytes was one tenth of that of the control. The uptake of ^{3}H-hexadecylglycerol was, however, more efficient than that in the control (cf. Figure 2D). The initial, fast phase of ^{3}H-hexadecylglycerol uptake by papain-treated enterocytes was characterized by a half-time of 5 ± 0.5 s (Table 1). The control yielded a value for the half-time of 10 ± 3 s, indicating that the ^{3}H-hexadecylglycerol uptake in enterocytes incubated at room temperature for 1.5 h is accelerated compared to that for freshly prepared, untreated enterocytes.

As expected for true mass exchange, the equilibrium values (x_∞) depended on the protein concentration of enterocytes (Figure 4). An unexpected feature of the relationship shown in this figure was that the x_∞ values did not asymptotically approach zero with infinite protein concentration but reached a finite value of 22%. This finding was accounted for by introducing a correction factor in the equation used to fit the experimental data in Figure 4 (see the Appendix). The effective lipid concentration (a) of isolated intact enterocytes, which was tentatively related to the total protein concentration of the cells, comprised the lipid bilayers of the BBM and the basolateral plasma membrane and probably also a lipid pool of the cell interior.

^{3}H-Hexadecylglycerol Exchange between Two Populations of Phospholipid SUV. ^{3}H-Hexadecylglycerol exchange be-

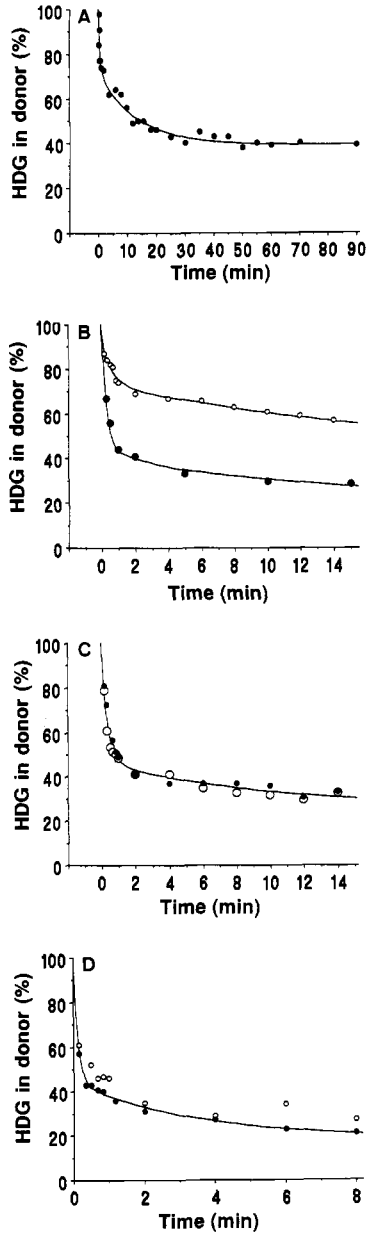

FIGURE 2: Kinetics of ^{3}H-hexadecylglycerol (HDG) uptake by enterocytes isolated from pig jejunum. Suspensions of isolated enterocytes in buffer B were incubated with egg PC SUV containing 1 mol % of HDG at room temperature unless otherwise stated. Enterocytes were spun down at 12 000 × *g* for 2 min, and the radioactivity in the supernatant was determined: (A) Donor concentration 0.5 mg of lipid/mL; acceptor concentration 10.8 mg of protein/mL. (B) The donor concentration was kept constant at 0.063 mg of lipid/mL, and two acceptor concentrations were used at 1.8 mg of protein/mL (○) and 17.9 mg of protein/mL (●). (C) Temperature dependence of ^{3}H-hexadecylglycerol uptake by enterocytes measured at 4 °C (●) and 22 °C (○). The donor concentration was 0.063 mg of lipid/mL, and the acceptor concentration was 10.7 mg of protein/mL. (D) The ^{3}H-hexadecylglycerol uptake by papain-treated enterocytes (●) is compared to that of untreated enterocytes (○). The donor concentration was 0.063 mg of lipid/mL, and the acceptor concentration was 5.5 mg of protein/mL. The solid lines were obtained by curve fitting using eq 2 described in the Appendix.

tween two populations of SUV was measured keeping the donor concentration constant at 0.5 mg of lipid/mL and varying the acceptor concentrations at 0.05, 0.25, 0.5, and 5 mg of lipid/mL. The equilibrium distribution of hexadecylglycerol between the lipid pools of the donor and acceptor was reached within less than 60 s (data not shown). The

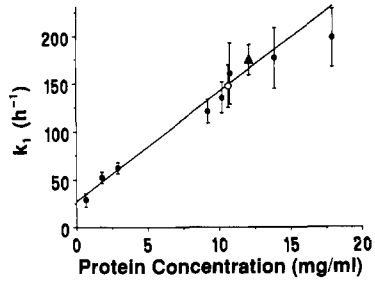

FIGURE 3: Pseudo-first-order rate constants k_1 of the initial fast phase of HDG uptake as a function of the protein concentration of isolated enterocytes as acceptors. Pseudo-first-order rate constants k_1 (h^{-1}) for the ^{3}H-hexadecylglycerol absorption by enterocytes isolated from pig jejunum were derived from kinetic data, as for example shown in Figure 2. The concentration of the donor (egg PC SUV containing 1 mol % of ^{3}H-hexadecylglycerol) was kept constant at 0.063 mg of total lipid/mL. Other experimental conditions were as described in the legends to Figure 2: closed symbols, k_1 values obtained for the initial, fast phase of ^{3}H-hexadecylglycerol absorption at 22 °C; open symbols, k_1 values obtained at 4 °C; triangle, k_1 value obtained after heparin treatment of a cell suspension of isolated enterocytes at ~2 mg of protein/mL with 5 mg of heparin per milliliter of cell suspension. The error bars represent the standard deviations of the measurements.

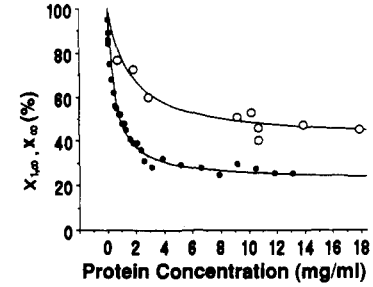

FIGURE 4: Equilibrium distributions $x_{1,\infty}$ (○) and x_∞ (●) of ^{3}H-hexadecylglycerol in the donor vesicles. Equilibrium values x_∞ were determined experimentally as follows: egg PC SUV containing 1 mol % of ^{3}H-hexadecylglycerol (0.063 mg of total lipid/mL) as the donor were incubated with increasing quantities of enterocytes isolated from pig jejunum (expressed as mg of total cell protein/mL) at 22 °C for 90 min. After incubation enterocytes were sedimented by centrifugation at 12 000 × *g* for 2 min and the radioactivity remaining in the donor vesicles was determined by radiocounting aliquots of the supernatant. The solid line represents a least-squares fit to the experimental data (closed symbols) according to $x_\infty = 100(b + ca)/(a + b)$, where a and b are the effective lipid pools of the acceptor and donor membranes, respectively, and c is $100x_\infty$ at a large excess of enterocytes ($a \gg b$). The least-squares fit yielded $c = 0.22$ and $a = 0.13$ [total cell protein (mg/mL)]. The open symbols are $x_{1,\infty}$ values derived from the computer simulation of kinetic curves using eq 2. The solid line was fitted to these $x_{1,\infty}$ values according to eq 4: $x_{1,\infty} = 100(b + c_1a_1)/(a_1 + b)$, where a_1 is the effective lipid pool of the acceptor available in the initial fast phase of ^{3}H-hexadecylglycerol uptake and c_1 approaches $x_{1,\infty}$ at a large excess of acceptor ($a \gg b$). The least-squares fit accomplished by varying a_1 and c_1 yielded $c_1 = 0.4$ and $a_1 = 0.039$ [total cell protein (mg/mL)].

evaluation of the kinetic data was hampered by the limited time resolution and precision of the exchange measurement. The kinetic curves were tentatively fitted by single exponentials, yielding k_1 values which were independent of the acceptor concentration within the error of the determination. The average k_1 value thus obtained was $k_1 = 250 \pm 150$ (h^{-1}).

DISCUSSION

[^{3}H]-1-*O*-Hexadecyl-*sn*-glycerol is used here as a model for 2-acyl-*sn*-glycerols, which are known to be one of the main products of triacylglycerol hydrolysis (Thomson & Dietschy, 1981; Carey et al., 1983; Tso, 1985; Shiau, 1987). It is reasonable to assume that the absorption properties of

alkylglycerols are similar to those of acylglycerols of comparable hydrocarbon chain length. The ether analogs such as hexadecylglycerol have the advantage over acylglycerols of being resistant to lipases. This property is particularly useful for the study of lipid absorption using in vitro acceptor systems such as BBMVs and isolated intact enterocytes. The term absorption is defined here as the transfer of lipid molecules from the donor to the acceptor membrane and incorporation of the lipid into the outer half of the lipid bilayer of the acceptor membrane. Upon incubation of BBMVs with small unilamellar lipid vesicles as the donor, donor lipids are transferred to BBMVs and incorporated into the lipid bilayer of the BBM (Mütsch et al., 1986). It was shown (Lipka et al., 1991) that simultaneously intrinsic lipids of BBMVs are transferred from BBMVs to donor vesicles. Evidence that ^{3}H-hexadecylglycerol indeed ends up in the lipid bilayer of BBMVs is provided by ESR spin labeling (data not shown).

BBMVs and isolated enterocytes were used here as in vitro acceptor membranes. The preparation of isolated enterocytes from fresh pig jejunum is simple and quick, yielding isolated intact cells to about 80%. Furthermore, by the criterion of the dye exclusion test and glucose transport, enterocytes dispersed in buffer B remained intact and viable during the course of lipid uptake. Almost equal D-glucose transport into intact enterocytes was measured in the presence and absence of a Na^+ gradient. The differentiation between active D-glucose transport across the BBM in the presence of a Na^+ gradient (Semenza et al., 1984) and facilitated D-glucose transport across the basolateral membrane (Hediger et al., 1987) is probably hampered because transport functions associated with the BBM or apical part of the plasma membrane are diluted or may even be swamped by transport across the basolateral membrane. However, we noted a significant reduction in D-glucose uptake at 4 °C compared to 37 °C and upon depolarization of the plasma membrane of enterocytes. Furthermore, we were able to unambiguously demonstrate protein-mediated absorption of cholesterol by isolated enterocytes (Schulthess & Hauser, unpublished observation). This finding is indeed reassuring, considering that most of our previous work was carried out with BBMVs. As pointed out repeatedly by us and other groups in the past, BBMVs have serious disadvantages and shortcomings, and results obtained with this model system have to be treated cautiously. We conclude that enterocytes handled as described here are sufficiently stable and lend themselves well to lipid absorption studies.

The mechanism of lipid absorption in the small intestines is still elusive. We do not know how lipid molecules dispersed in the luminal content gain entrance into the enterocyte lining the intestinal lumen (Thomson & Dietschy, 1981; Carey et al., 1983; Tso, 1985; Shiau, 1987). Work from several laboratories has shown that under normal conditions intestinal absorption occurs mainly from mixed bile salt micelles containing in addition to bile salt the main products of fat digestion: monoacylglycerols, fatty acids, cholesterol, and lysophospholipids (Thomson & Dietschy, 1981; Carey et al., 1983; Carey & Hernell, 1992). However, in the complete absence of bile salts, lipid absorption takes place all along the small intestine with an efficiency of up to 75% (Porter et al., 1971; Poley, 1976; Carey & Hernell, 1992). This finding indicates that lipid absorption also occurs efficiently from lipid particles other than mixed bile salt micelles. SUV have been proposed as the most likely candidates. There is also general agreement that the endocytotic uptake of total mixed micelles and/or SUV by enterocytes can be ruled out on the basis that the various constituent molecules of the donor particles are absorbed by independent rates (Borgström, 1960; Spener et al., 1968; Wilson et al., 1971; Wilson & Dietschy, 1972; Thomson & Dietschy, 1981; Carey et al., 1983; Tso, 1985; Shiau, 1987; Carey & Hernell, 1992). Considering the high intraluminal concentration of lipids, it is conceivable that lipids move passively down their concentration gradients from the lumen through the BBM to the cytosol of the enterocyte. Indeed, the notion that lipid absorption is mediated by lipid monomers present in the aqueous phase and in equilibrium with the mixed micelles is favored by a number of research workers in the field (Thomson & Dietschy, 1981).

The main question the present work addressed is whether or not the absorption of monoacylglycerol by BBM is a simple passive process or a protein-mediated process similar to the absorption of cholesterol. From the evidence presented we conclude that hexadecylglycerol absorption as defined here is a passive process. This conclusion is mainly based on the observation that hexadecylglycerol absorption is a fast process which is temperature-independent between 4 °C and room temperature. This is true for BBMVs as well as isolated enterocytes. The above conclusion is supported by the following observations: (I) the exchange of ^{3}H-hexadecylglycerol between two populations of SUV was measured at room temperature in the absence of protein, as described in Methods. This undoubtedly is a passive process, and it exhibited even faster kinetics than the absorption of hexadecylglycerol by BBMVs (Figure 1). (II) In one series of experiments absorption by BBM of ^{3}H-hexadecylglycerol was measured as described in Figure 1 except that the pH of the dispersion medium was lowered to pH = 1.5–2. The experimental data points were close to the absorption curve of hexadecylglycerol at 4 °C shown in Figure 1A. This result is good evidence that proteins are not involved in hexadecylglycerol absorption. Any protein on the luminal surface of BBM exposed to a low pH (<3) is expected to unfold and denature. Protein denaturation would manifest itself in a significant reduction of hexadecylglycerol absorption. This is clearly not the case. (III) Papain treatment of BBMVs has no significant effect on hexadecylglycerol absorption (Table 1). Similarly, papain treatment of enterocytes has little effect: the absorption of hexadecylglycerol appears to be even faster compared to that of untreated enterocytes (cf. Figure 2D). (IV) The good quantitative agreement between the hexadecylglycerol absorption by BBMVs and enterocytes is yet another indication of passive absorption. In terms of passive absorption, the BBM is expected to behave very similarly to any other part of the plasma membrane. If, however, hexadecylglycerol absorption is protein-mediated, pure BBMs of enterocytes are diluted with the basolateral membrane. (V) Exposure of enterocytes (~2 mg of protein/mL) to heparin (5 mg/mL cell suspension) for 15 min had no effect on the kinetics of hexadecylglycerol absorption. Heparin was reported to liberate BBM-associated enzymes such as triacylglycerol lipase (Bosner et al., 1989). Since these proteins have been implicated in lipid digestion and absorption, the lack of an effect of heparin eliminates these proteins as direct contributors to monoacylglycerol absorption. All the experimental evidence taken together points therefore to hexadecylglycerol absorption being a passive process.

The pseudo-first-order rate constants of the initial fast phase of hexadecylglycerol absorption (Table 1) are characteristic of the transfer of the lipid molecule from the donor to the acceptor membrane. As shown in Figure 3 these rate constants depend linearly on the total protein concentration of entero-

cytes. This is also true for the pseudo-first-order rate constant measured for the initial, fast phase of ^{3}H-hexadecylglycerol uptake by BBMVs (Table 1). This result indicates that hexadecylglycerol absorption from egg PC SUV as the donor is not a true first-order reaction but probably a second-order one. The mechanism of hexadecylglycerol absorption would be consistent with lipid transfer via collisional contact, leading to either membrane fusion or lipid exchange between the donor and acceptor membranes. A second-order reaction is also compatible with a monomer diffusion mechanism. In this case lipid transfer involves the desorption of the hexadecylglycerol molecule from the outer monolayer of the egg PC SUV, Brownian diffusion of the lipid molecule through the aqueous phase, and incorporation into the outer monolayer of the acceptor membrane. A second-order reaction implies that the incorporation of hexadecylglycerol into the acceptor membrane is the rate-limiting step. It should be noted that with isolated enterocytes as the acceptor, hexadecylglycerol is incorporated into the total plasma membrane comprising the BBM as well as the basolateral plasma membrane. The cmc and monomeric concentration of hexadecylglycerol in equilibrium with egg PC SUV (1 mg of lipid/mL) containing 1 mol % of hexadecylglycerol are on the order of 10^{-6} M, which compared to the cmc of cholesterol (Haberland & Reynolds, 1973; Bruckdorfer & Sherry, 1984) and phospholipids (Smith and Tanford, 1972) is 2–4 orders of magnitude higher. The monomeric concentration of hexadecylglycerol is therefore regarded as significant, and the monomer diffusion mechanism can be expected to contribute to the hexadecylglycerol absorption accordingly. We believe that this mechanism and the transfer through collisional contact are the main contributions to hexadecylglycerol absorption, at least under the conditions used in this study. Membrane fusion can be safely ruled out on the basis of the observation that with both acceptor membranes used here there is true mass transfer: at equilibrium hexadecylglycerol is evenly distributed between donor and acceptor membrane. That fusion of egg PC SUV with BBMVs cannot contribute significantly is also indicated by the observation that hexadecylglycerol absorption is not associated with a significant change in the protein/PC wt ratio. We have shown before that both cholesterol and PC uptake by BBMVs from egg PC SUV as the donor are protein-mediated and the mechanism involves collision-induced lipid transfer (Thurnhofer & Hauser, 1990a,b). Although hexadecylglycerol absorption is a passive process, the pseudo-first-order rate constants for this reaction are still larger than the pseudo-first-order rate constants measured for cholesterol and PC absorption by BBMVs from SUV as the donor (Thurnhofer & Hauser, 1990a,b). Such a comparison of pseudo-first-order rate constants lends support to the notion that the absorption of hexadecylglycerol is primarily determined by the relatively high monomer concentration of this lipid in the aqueous phase and that monomer diffusion is the dominant mechanism of hexadecylglycerol absorption.

In contrast to the pseudo-first-order rate constants of the initial fast phase, the pseudo-first-order rate constants k'_1 of the second slow phase of hexadecylglycerol absorption by both BBMVs and isolated enterocytes are independent of the acceptor concentration within the error of the measurement. We propose that the second slow phase of the absorption of hexadecylglycerol by isolated enterocytes is due to the flip–flop motion of hexadecylglycerol from the outer to the inner layer of the acceptor membrane and probably to further movement of this molecule into the cell interior. This interpretation is supported by the observation that the kinetics of hexadecylglycerol exchange between two populations of SUV are significantly faster than the kinetics of the second slow phase of hexadecylglycerol absorption by both BBMV and isolated enterocytes. It should be noted that the values for the half-time of the flip–flop (Table 1) were obtained under conditions where the acceptor membrane (BBM or plasma membrane of the enterocytes) is coupled to the donor membrane (egg PC SUV) and that these values may not be representative of the intrinsic flip–flop motion of the lipid molecule in BBMs.

The kinetics of the exchange reaction (including the flip–flop motion) of ^{3}H-hexadecylglycerol between two populations of SUV are so fast that the equilibrium distribution is reached in less than 1 min. With BBMVs or enterocytes as the acceptor, both the initial fast uptake of ^{3}H-hexadecylglycerol and the subsequent flip–flop motion of this lipid are significantly slower (Table 1). This finding suggests that the on rate of hexadecylglycerol movement into the BBM is the rate-limiting step of hexadecylglycerol absorption. This interpretation is also consistent with the observation that the pseudo-first-order rate constants depend on the acceptor concentration. The structural principle underlying the mechanism of hexadecylglycerol absorption by BBMs is probably the tightly packed lipid bilayer of this membrane. As was shown by several research groups, the lipids of the BBMs are significantly more tightly packed than phospholipid bilayers and most other plasma membranes (Schachter & Shinitzky, 1977; Brasitus et al., 1980; Hauser et al., 1982).

Physiological Implication. We have studied here the absorption of hexadecylglycerol as a model compound for long-chain 2-acylglycerols, which represent one of the major classes of lipids absorbed by small intestinal BBMs. Using egg PC SUV as the donor and BBMVs or isolated intact enterocytes as the acceptor, we present evidence that the absorption of hexadecylglycerol is a passive process. This is different from cholesterol absorption, which under comparable conditions is protein-mediated. In spite of its passive nature the absorption of monoacylglycerol by BBMs is an efficient process characterized by half-times on the order of seconds. This fact is explained in terms of the relatively high solubility of long-chain monoacylglycerols, which is on the order of 10^{-6} M and about 2 orders of magnitude higher than that of cholesterol. The solubility of lipids in water is apparently a crucial parameter regarding the transport and equilibration of lipids between membranes. This work shows that monomeric lipid concentrations in excess of 10^{-6} M ensure the efficient lipid transport between and absorption by membranes without the help of catalysts, i.e., in the absence of lipid carriers and/or receptors. If the lipid solubility drops significantly below 10^{-6} M, the passive monomer diffusion mechanism becomes ineffective. Nature then apparently compensates by providing appropriate carriers and membrane receptors.

The data presented here, however, still do not rule out the possibility that monoacylglycerol absorption in the small intestine is a protein-mediated process. In our model system we used egg PC SUV as the donor while in vivo digested fat molecules are mainly absorbed from mixed bile salt micelles. Our work shows that there is no receptor-like molecule in the BBMs recognizing monoacylglycerol and facilitating the uptake of this molecule. It is, however, feasible that bile salt micelles that act as a vehicle transporting monoacylglycerol and other digested lipids to the site of absorption interact with proteins and/or glycoproteins exposed at the BBM surface. Transient surface absorption or weak binding of bile salt micelles by BBMs cannot be ruled out. Such binding

mechanisms would have the effect of increasing the local concentration of lipids to be absorbed, and such an effect would be classified as protein-mediated. Experiments to shed light on this important question are presently under way in our laboratory.

APPENDIX

The uptake of ^{3}H-hexadecylglycerol by BBMVs as the acceptor membrane from egg PC SUV as the donor membrane is characterized by true mass exchange: at equilibrium ^{3}H-hexadecylglycerol is evenly distributed between the lipid pools of donor and acceptor membranes. For true mass exchange of lipid between donor and acceptor membranes, it was shown (Mütsch et al., 1986) that the fraction of the exchangeable lipid (^{3}H-hexadecylglycerol) remaining in the donor vesicles after incubation with the acceptor membrane for a period of time t is given by

$$\ln\left(\frac{x - x_\infty}{x_0 - x_\infty}\right) = -k_1\left(\frac{a+b}{a}\right)t \qquad (1)$$

where x_0, x, and x_∞ are the fractions or percentages of exchangeable lipid in the donor at times 0, t, and at equilibrium, respectively, and a and b are the effective lipid pools of acceptor and donor, respectively. Equation 1 is based on the assumption that the transfer of exchangeable lipid between donor and acceptor membrane is a simple first-order reaction and that there is simultaneous back transfer from the acceptor to the donor membrane, i.e. that there is true mass exchange between donor and acceptor.

From an inspection of Figures 1 and 2 it is clear that the exchange of ^{3}H-hexadecylglycerol between donor and acceptor is not characterized by a single exponential decay of the radioactivity in the donor: with both BBMVs and isolated enterocytes as the acceptor the kinetics of ^{3}H-hexadecylglycerol exchange are biphasic. Their biphasic character is revealed by linearization of the curves shown in Figures 1A and 2 according to eq 1. As shown in Figure 1B each curve of Figure 1A gives two straight-line portions that differ significantly in slope. Pseudo-first-order rate constants k_1 derived from the slopes of the straight lines are rough (order-of-magnitude) estimates. The kinetic data such as those of Figures 1A and 2 are, however, adequately fitted by the sum of two exponentials, and more precise k_1 values are derived from such a fit:

$$x = x_\infty + (x_0 - x_{1,\infty})\exp\left[-k_1\left(\frac{a_1+b}{a_1}\right)t\right] + (x_{1,\infty} - x_\infty)\exp[-2k'_1 t] \qquad (2)$$

where k_1 and k'_1 are the pseudo-first-order rate constants of the initial fast phase and the second slow phase, respectively, and $x_{1,\infty}$ and a_1 are discussed under eq 4. Pseudo-first-order rate constants k_1 and k'_1 derived from curve fitting using eq 2 are summarized in Table 1. In addition to the rate constants k_1 and k'_1, the parameter $x_{1,\infty}$ was used as a variable in the curve-fitting program. The factor $[(a_1 + b)/a_1]$ in the first exponential of eq 2 is related to the lipid pools available during the initial fast phase. The factor of 2 in the second exponential of eq 2 accounts for the fact that in BBMs the ratio of lipid molecules in the inner and outer monolayer of the bilayer is ~ 1.

The percentage of ^{3}H-hexadecylglycerol present in the donor membrane at equilibrium is $x_\infty = 100b/(a + b)$, where a and b are the effective lipid pools of acceptor and donor membranes, respectively. While the effective lipid pool (b) of the donor is identical to the total lipid concentration, the effective acceptor pool has to be determined experimentally. For BBMVs this pool was shown to be related to the total lipid concentration of BBMVs by a (mg of lipid/mL) = 0.6 [total lipid (mg/mL)] (Mütsch et al., 1986) or a (mg of lipid/mL) = 0.3[protein (mg/mL)], since the lipid/protein wt ratio of BBMVs was shown to be 0.5 (Hauser et al., 1980).

The effective lipid pool (a) for isolated enterocytes was derived from eq 3. The equilibrium distribution x_∞ of ^{3}H-hexadecylglycerol was approached after incubation of donor and acceptor membranes for about 90 min, and experimental x_∞ values are shown as a function of cell mass in Figure 4 (cf. closed symbols). In the presence of excess enterocytes the curve approached $x_\infty = 100c$ rather than zero. This effect was attributed to cell debris released from enterocytes, which are linearly related to the mass of enterocytes. These cell debris apparently absorb ^{3}H-hexadecylglycerol but are not sedimented at the centrifugal field of 12 000g for two min routinely used to separate enterocytes from donor vesicles. The total lipid concentration (b) was kept constant at b = 0.063 mg of lipid/mL, and a values were calculated from eq 3:

$$x_\infty = 100(b + ca)/(a + b) \qquad (3)$$

The solid line fitted to the closed symbols in Figure 4 was obtained by a least-squares procedure using eq 3 and varying a and c. The values thus obtained were c = 0.22 and for the effective lipid pool of isolated enterocytes a (mg of lipid/mL) = 0.13[total cell protein (mg/mL)]. The effective lipid pool (a) of isolated intact enterocytes comprises not only the lipid bilayer of the total plasma membrane but also a lipid pool of the cell interior.

Similarly the effective lipid pool (a_1) of enterocytes available during the initial, fast phase of the reaction was derived from eq 4

$$x_{1,\infty} = 100(b + c_1 a_1)/(a_1 + b) \qquad (4)$$

where $(c_1 a_1)$ is the amount of cell debris operative in the initial fast phase. The open symbols in Figure 4 represent $x_{1,\infty}$ values derived from curve fitting of the kinetic data, as for instance shown in Figure 2 using eq 2. The solid line fitted to the open symbols in Figure 4 represents a least-squares fit using eq 4 and varying a_1 and c_1. The values thus obtained were c_1 = 0.4 and for the effective lipid pool a_1 (mg of lipid/mL) = 0.039[total cell protein (mg/mL)] = 0.3a. The ratio of cell debris active during the initial fast phase and the second slow phase is $(c_1 a_1)/(ca)$ = 0.55.

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REFERENCES

Baumann, W. J., & Mangold, H. K. (1964) *J. Org. Chem. 29*, 3055–3057.

Borgström, B. (1960) *J. Clin. Invest. 39*, 809–815.

Borgström, B. (1977) *Int. Rev. Physiol. 12*, 305–323.

Bosner, M. S., Gulick, T., Riley, D. J. S., Spilburg, C. A., & Lange, L. G. (1989) *J. Biol. Chem. 264*, 20261–20264.

Brasitus, T. A., Tall, A. R., & Schachter, D. (1980) *Biochemistry 19*, 1256–1261.

Bruckdorfer, K. R., & Sherry, M. K. (1984) Biochim. *Biophys. Acta 769*, 187–196.
Brunner, J., Hauser, H., & Semenza, G. (1978) *J. Biol. Chem. 253*, 7538–7546.
Carey, M. C., & Hernell, O. (1992) *Seminars in Gastrointestinal Disease 3*, 189–208.
Carey, M. C., Small, D. M., & Bliss, C. M. (1993) *Annu. Rev. Physiol. 45*, 651–677.
Chen, P. S., Toribara, T. Y., & Warner, H. (1956) *Anal. Chem. 28*, 1756–1758.
Haberland, M. E., & Reynolds, J. A. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2313–2316.
Hauser, H., Howell, K., Dawson, R. M. C., & Bowyer, D. E. (1980) *Biochim. Biophys. Acta 602*, 567–577.
Hauser, H., Gains, N., Semenza, G., & Spiess, M. (1982) *Biochemistry 21*, 5621–5628.
Hediger, M. A., Coady, M. J., Ikeda, T. S., & Wright, E. M. (1987) *Nature (London) 330*, 379–381.
Hernell, O., Staggers, J. E., & Carey, M. C. (1990) *Biochemistry 29*, 2041–2056.
Hoffman, A. G. D., & Kuksis, A. (1979) *Can. J. Physiol. Pharmacol. 57*, 832–842.
Kimmich, G. A. (1975) in *Methods in Membrane Biology* (Korn, E. D., Ed.) Vol. 5, pp 58–78, Plenum Press, New York and London.
Lipka, G., Op den Kamp, J. A. F., & Hauser, H. (1991) *Biochemistry 30*, 11828–11836.
Lipka, G., Imfeld, D., Schulthess, G., Thurnhofer, H., & Hauser, H. (1992) in *Structural and Dynamic Properties of Lipids and Membranes* (Quinn, P. J., & Cherry, R. J., Eds.) pp 7–18, Portland Press, London.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
McKay, H. A. C. (1938) *Nature (London) 142*, 997–998.
McLean, L. R., & Phillips, M. C. (1981) *Biochemistry 20*, 2893–2900.
Mütsch, B., Gains, N., & Hauser, H. (1986) *Biochemistry 25*, 2134–2140.
Paltauf, F. (1983) in *Ether Lipids. Biochemical and Biomedical Aspects* (Mangold, H. K., & Paltauf, F., Eds.) pp 211–227, Academic Press, New York.
Patton, J. S. (1981) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., Ed.) 2nd ed., pp 1123–1146, Raven Press, New York.
Poley, J. R. (1976) in *Lipid Absorption: Biochemical and Clinical Aspects* (Rommel, K., Goebell, H., & Boehmer, R., Eds.) pp 151–199, MTP Press, Lancester, PA.
Porter, H. P., Sanders, D. R., Tytgat, G., Brunser, O., & Rubin, E. (1971) *Gastroenterology 60*, 1008–1019.
Poznansky, M. J., & Czekanski, S. (1979) *Biochem. J. 177*, 989–991.
Schachter, D., & Shinitzky, M. (1977) *J. Clin. Invest. 59*, 536–548.
Semenza, G., Kessler, M., Hosang, M., Weber, J., & Schmidt, U. (1984) *Biochim. Biophys. Acta 779*, 343–379.
Shiau, Y.-F. (1987) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., Ed.) 2nd ed., pp 1527–1556, Raven Press, New York.
Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., & Klenk, D. C. (1985) *Anal. Biochem. 150*, 76–85.
Smith, R., & Tanford, C. (1972) *J. Mol. Biol. 67*, 75–83.
Spener, F., Paltauf, F., & Holasek, A. (1968) *Biochim. Biophys. Acta 152*, 368–371.
Stern, B. K. (1966) *Gastroenterology 51*, 855–867.
Stern, B. K., & Jensen, W. E. (1966) *Nature (London) 209*, 789–790.
Thomson, A. B. R., & Dietschy, J. M. (1981) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., Ed.) 2nd ed., pp 1147–1220, Raven Press, New York.
Thurnhofer, H., & Hauser, H. (1990a) *Biochemistry 29*, 2142–2148.
Thurnhofer, H., & Hauser, H. (1990b) *Biochim. Biophys. Acta 1024*, 249–262.
Thurnhofer, H., Lipka, G., & Hauser, H. (1991a) *Eur. J. Biochem. 201*, 273–282.
Thurnhofer, H., Schnabel, J., Betz, M., Lipka, G., Pidgeon, C., & Hauser, H. (1991b) *Biochim. Biophys. Acta 1064*, 275–286.
Tso, P. (1985) *Adv. Lipid Res. 21*, 143–186.
Weiser, M. M. (1973) *J. Biol. Chem. 248*, 2536–2541.
Wilson, F. A., & Dietschy, J. M. (1972) *J. Clin. Invest. 51*, 3015–3025.
Wilson, F. A., Sallee, V. L., & Dietschy, J. M. (1971) *Science 174*, 1031–1033.
Yin, C.-C., Schurtenberger, P., Wehrli, E., Paltauf, F., & Hauser, H. (1991) *Biochim. Biophys. Acta 1070*, 33–42.