Fatty acid uptake by Caco-2 human intestinal cells

Pamela J. Trotter,[1],* Shiu Ying Ho,[†] and Judith Storch[2],*,[†]

Program in Cell and Developmental Biology,* Harvard Medical School, Boston, MA 02115, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, and Department of Nutritional Sciences,[†] Cook College, Rutgers University, New Brunswick, NJ 08903

Abstract The Caco-2 human enterocytic cell line was used to study the kinetics and mechanism of intestinal long chain fatty acid uptake. Initial rates of palmitate (16:0), oleate (18:1), and octanoate (8:0) uptake were determined for adherent cells at greater than 7 days after confluence. Uptake of long chain 18:1 and 16:0 by cells grown on coverslips was saturable with an apparent K_m of 0.3 μM, but also included a notable diffusive component. Uptake of short chain 8:0, on the other hand, was linear up to 10 μM. Cells grown on permeable Transwell filters were used to study uptake at the apical versus the basolateral membrane. Uptake of long chain (18:1 and 16:0), but not short chain (8:0), fatty acid was saturable at both surfaces with a similar K_m of 0.3 μM. In addition, long chain but not short chain fatty acid uptake was competitively inhibitable. Western blot analysis demonstrated that Caco-2 cells express a protein immunoreactive with antibodies to the rat liver plasma membrane fatty acid binding protein ($FABP_{pm}$), which is thought to be involved in long chain fatty acid transport. Nevertheless, long chain fatty acid uptake was not inhibited by pretreatment of the cells with an $FABP_{pm}$ antibody, nor by pretreatment with two proteases. These data support a saturable component in the transport of long chain but not short chain fatty acids by human intestinal epithelial cells, which may involve an as yet unknown plasma membrane protein.—**Trotter, P. J., S. Y. Ho, and J. Storch.** Fatty acid uptake by Caco-2 human intestinal cells. *J. Lipid Res.* 1996. **37:** 336–346.

Supplementary key words intestinal epithelial cells • cell polarity • kinetics • Caco-2

Despite the importance of fatty acids as a metabolic energy source, as building blocks for membrane lipids, and as cellular signalling molecules, the mechanism and regulation of fatty acid uptake into cells remain unresolved. Due to the lipophilic nature of fatty acids and the membranes they must cross, passive diffusion has long been thought to be the primary uptake mechanism (1–3). Indeed, in small unilamellar vesicles composed of egg phosphatidylcholine, rapid transbilayer movement of fatty acids ($t_{1/2}$ < 1 sec) has been reported (4). Uptake of albumin-bound fatty acid from the circulation has been suggested to occur by spontaneous dissociation and passive transmembrane movement driven by a concentration gradient that is maintained by intracellular fatty acid metabolism (5, 6). Fatty acid uptake from the intestinal lumen is suggested to occur by a similar mechanism, except that the fatty acid monomer dissociates from a mixed micelle (1), and uptake is hypothesized to be limited by an "unstirred" water layer adjacent to the membrane (7) and/or the physical properties of the plasma membrane (3).

It has also been proposed that fatty acid uptake into cells occurs by a saturable membrane carrier-mediated mechanism (8, 9). It has been demonstrated that uptake of long chain fatty acids by a number of rat cell types is a saturable function of unbound fatty acid concentration and can be competitively inhibited (10–14). Fatty acid uptake is thought to be energy-independent in adipocytes and myocytes (12, 15), whereas fatty acid uptake in hepatocytes has been reported to be energy-dependent (16). Putative plasma membrane fatty acid transport proteins, designated $FABP_{pm}$ (8, 9), have been isolated from hepatocytes, adipocytes, myocytes, and enterocytes (17–20). Antibodies raised against the protein isolated from rat liver have been found to partially inhibit fatty acid uptake by rat liver, adipose and intestinal cells, and by isolated perfused intestine (14, 19, 21). In addition, a number of different genes encoding putative fatty acid transport proteins have recently been identified (22, 23). The potentially important role of

Abbreviations: AP, apical; BL, basolateral; BSA, bovine serum albumin; $FABP_{pm}$, plasma membrane fatty acid binding protein; PBS, phosphate-buffered saline; TER, transepithelial resistance; 8:0, octanoate; 16:0, palmitate; 18:1, oleate; MVM, microvillus membrane; BBMV, brush border membrane vesicle; BLMV, basolateral membrane vesicle.

[1]Present address: Lord and Taylor Laboratory for Lung Biochemistry, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

[2]To whom correspondence should be addressed at Department of Nutritional Sciences, Rutgers University, Cook College, New Brunswick, NJ 08903.

these $FABP_{pm}$ proteins in the cellular uptake of fatty acid remains uncertain.

Fatty acids derived from the diet are first absorbed by the epithelium of the small intestine via the apical (AP) membrane of the absorptive enterocytes. Data from a number of studies (24–28) are consistent with the proposal that fatty acid absorption by enterocytes may be mediated by a saturable membrane component. In 1985, Stremmel et al. (17) reported that oleate binding to rat jejunal microvillus membranes (MVM) was saturable, heat-sensitive, and inhibited by excess oleate. A 40-kD $FABP_{pm}$ was purified from MVM by its affinity to oleate, and it exhibited little affinity for lipophilic molecules other than fatty acids. $FABP_{pm}$ was detected immunochemically in the AP and basolateral (BL) membranes of rat jejunal villus cells by a polyclonal antibody to the rat liver $FABP_{pm}$ (14, 17). Fatty acids are also taken up from the circulation across the BL membrane of enterocytes (29–31), but little is known about the mechanism of BL fatty acid uptake. We have utilized the Caco-2 cell line as a model of the human intestinal epithelium to examine the kinetics and mechanism of long chain fatty acid transport at the level of the polarized absorptive cell. The results indicate saturable uptake of long chain, but not short chain, fatty acids at the AP and BL surfaces of Caco-2 cells.

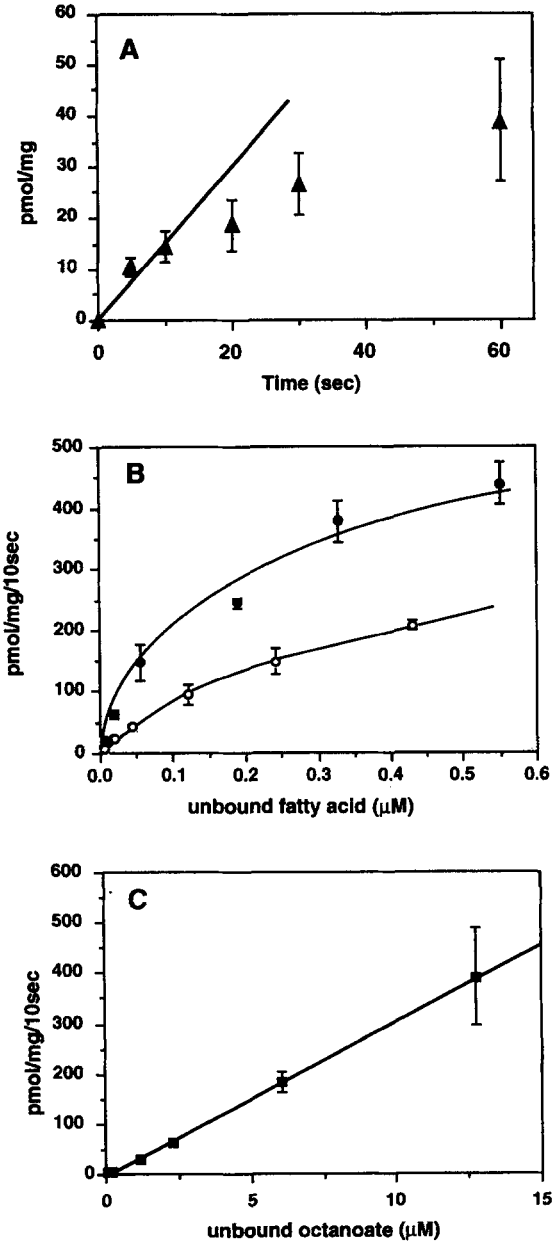

Fig. 1. Uptake of fatty acids by Caco-2 cells grown on coverslips as a function of time (A) and unbound fatty acid concentration (B and C). (A) The time course of oleic acid (complexed 0.5:1 with BSA) uptake at 23°C (▲). Data are the average ± SD (n = 4) from two experiments. The uptake of (B) long chain fatty acids oleate (○) and palmitate (●), and (C) short chain fatty acid octanoate (■) for 10 sec at 23°C. Varying amounts of fatty acid were added to a fixed concentration of BSA (100 μM). Unbound fatty acid concentrations were calculated as described under Materials and Methods. Data are the average ± SD (n = 3) from a representative one of two (B) or three (C) experiments

MATERIALS AND METHODS

Materials

Polycarbonate Transwell filter inserts (6.5 mm diameter, 0.4 μm pores) were purchased from Costar Corp. (Cambridge, MA). Tritium-labeled oleate and palmitate, and ^{14}C-labeled octanoate were from New England Nuclear (Boston, MA). Unlabeled fatty acids were obtained from Nu-Chek Prep (Elysian, MN). Triton X-100 was from National Diagnostics (Highland Park, NJ). The PVDF Immobilon-P blotting membrane was from Millipore Corp. (Bedford, MA). Bovine serum albumin (essentially fatty acid free), Dulbecco's Modified Eagle's Medium base, trypsin, and pronase E were all obtained from Sigma Chemical Co. (St. Louis, MO). The sodium taurocholate was from Calbiochem (La Jolla, CA). The rabbit polyclonal antibody to rat liver plasma membrane fatty acid binding protein ($FABP_{pm}$) was generously provided by Dr. Paul Berk (Mt. Sinai Medical Center, New York).

Cell culture

Caco-2 cells were cultured as previously described (30). Cells were plated at $10^4/cm^2$ onto 12-mm diameter glass coverslips which were placed in 24-well tissue culture plates, or were plated at $3 \times 10^4/cm^2$ onto the interior (for basolateral uptake) or exterior surface (for apical uptake) of 6.5 mm diameter polycarbonate Transwell filter inserts with 0.4 μm pores. Cells were grown to 7–12 days post-confluence, as we have shown previously that Caco-2 cells are differentiated and express fully their fatty acid metabolic activities by this time in

culture (32, 33). Transepithelial resistance (TER) measurements were made on filter-grown cells as previously described (30), and monolayers with TER less than 150–200 ohms × cm^2 were not used.

Preparation of fatty acid uptake media

The long chain fatty acids oleate (^{3}H-18:1) and palmitate (^{3}H-16:0) were complexed with bovine serum albumin (BSA) for introduction to cells, whereas the short chain fatty acid octanoate (^{14}C-8:0) was either introduced complexed to BSA or in free solution as noted in the text. Fatty acids were complexed to BSA in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM $NaHPO_4$, pH 7.5) by addition of concentrated fatty acid in ethanolic stock such that the amount of ethanol was less than 0.5% (v/v). The fatty acid-BSA solution was then incubated at room temperature for > 10 min prior to use. For the determination of ^{3}H-16:0 uptake from bile salt micelles, a concentrated ethanolic stock of ^{3}H-16:0 was added to a solution of 10 mM sodium taurocholate in PBS (ethanol < 0.5% v/v) and incubated for 1 h at 37°C before use. The specific activities of the uptake solutions were 0.2–0.5 μCi/nmol for ^{3}H-labeled fatty acid and 0.5 μCi/nmol for ^{14}C-labeled fatty acid. For competition experiments the specific activity of the ^{3}H-labeled fatty acid was 3–4 μCi/nmol. Unbound fatty acid concentrations in the fatty acid-BSA solutions were calculated from the equilibrium binding constants as reported by Spector, Fletcher, and Ashbrook (34) and Spector (35) using a step-wise calculation program generously provided by Drs. Luis Isola and Paul Berk (Mt. Sinai Medical Center, New York).

Assay of fatty acid uptake

Initial rates of fatty acid uptake were determined by a procedure based on methods reported for suspended cells (10, 12, 14), which were adapted for the adherent Caco-2 cells and optimized using oleate. Uptake was determined at 22–23°C. Cells grown on coverslips or permeable filters were washed twice with either PBS or with PBS plus 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS+). PBS+ was initially utilized to ensure the maintenance of tight junction integrity, but the pattern of uptake was similar whether PBS or PBS+ washes were used (data not shown). The radioactive fatty acid solution was then added to the cells. After a specified incubation time, the uptake medium was aspirated off, and the coverslip or filter was immediately dipped into an ice-cold 0.5% BSA solution to stop cellular uptake and remove surface-bound fatty acid (14). The coverslip or filter was then rapidly washed once more with ice-cold 0.5% BSA. To ensure that only extracellular fatty acid was removed, experiments were performed to establish the effect of BSA concentration and washing time on fatty acid removal. It was found that fatty acid removal was saturable with respect to both BSA concentration and time (data not shown), therefore the washing conditions described above were chosen. After the BSA washes, the cells were washed three times with ice-cold PBS, scraped into 0.05% Triton X-100, and sonicated for 15 sec using a Branson sonifier equipped with a microtip (Danbury, CT). Cell-associated radioactivity and protein (36) were then determined. Uptake was linear for approximately 10 sec, thus all experiments were done using 10 sec time points (see Fig. 1A). We determined that at 10 sec greater than 90% of fatty acid taken up by Caco-2 cells remained unesterified (data not shown), further ensuring that uptake measurements reflected the initial rate of fatty acid transport and not subsequent cellular metabolism. Background was determined as the apparent uptake that occurred when the radiolabeled fatty acid solution was added directly to the stop solution, and accounted for less than 10% of the total uptake at 10 sec for fatty acid:BSA ratios between 0.25 and 2.0.

Studies using the anti-$FABP_{pm}$ antibody

Western blot analysis of Caco-2 cell homogenates for a protein immunoreactive with a polyclonal rabbit anti-rat liver plasma membrane fatty acid binding protein ($FABP_{pm}$) was carried out using methods described by us previously (30). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% gels. The proteins were electrophoretically transferred to PVDF membrane and probed with antibody (at a 1:120 dilution).

To examine the effect of the anti-$FABP_{pm}$ on cellular fatty acid uptake, adherent Caco-2 cells were washed

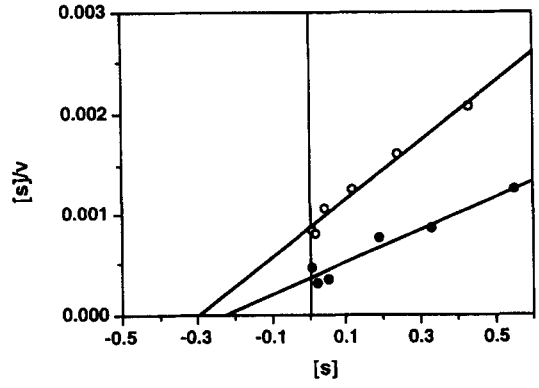

Fig. 2. Woolf plots, [s]/v versus [s], of oleate (○) and palmitate (●) uptake by Caco-2 cells. Data were from uptake experiments as described in Fig. 1. The best fit of the data points was obtained using linear regression. [s] is the substrate concentration (unbound fatty acid concentration) and v is the initial uptake velocity (pmol/mg/10 sec). The $-K_m$ is the intercept on the x axis, and the slope is $1/V_{max}$. Data are from a representative one of two experiments determined in triplicate.

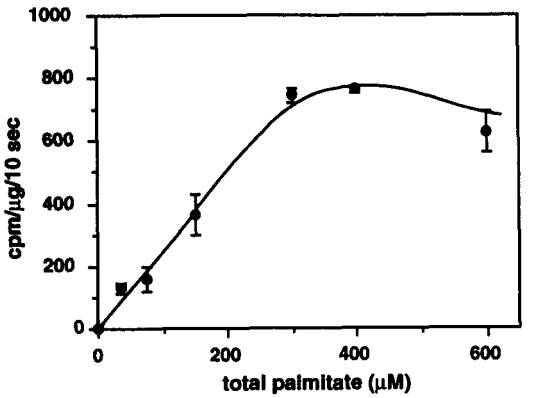

Fig. 3. Apical uptake of micelle-bound palmitate by Caco-2 cells. Uptake of 37.5–600 µM palmitate (16:0) in the presence of 10 mM Na^+-taurocholate was determined at the apical surface of filter-grown cells as described in Materials and Methods. Data are the average ± SD (n = 3) from one of two similar experiments.

twice with ice-cold Dulbecco's Modified Eagle's Medium base (without glucose, bicarbonate or phenol red) plus 4.5 g/L glucose and 10 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.5 (DMEM-HEPES). Cells (approximately 5×10^5/coverslip) were then incubated for 30 min on ice with 100 µl of the rabbit anti-rat $FABP_{pm}$ at 2.5 or 5 mg IgG/ml, normal rabbit serum or normal rabbit IgG at approximately 5 mg/ml IgG, or DMEM-HEPES alone. All antibody dilutions were in DMEM-HEPES. Similar experiments were also conducted with the antibody incubation at room temperature (~23°C). After incubation, the cells were washed twice with PBS at room temperature, and, within 5 min, uptake of ^{3}H-18:1 was determined as described above.

Protease studies

The effect of the proteases trypsin and pronase on oleate uptake in Caco-2 cells was determined based on methods described by others (10, 12) and adapted for adherent cells. Cells were incubated at 25°C with the proteases at concentrations ranging from 0.05 to 0.5 mg/ml. The cells were then washed, the TER was measured, and uptake was determined within 5 min. The concentrations and times chosen were determined based on measurements of monolayer integrity after protease treatment, as determined by TER measurements.

Statistical methods and calculations

Each experiment was performed at least two times in duplicate and data are expressed as the mean plus or minus the standard deviation (SD) or standard error (SEM) as noted in the text or figure and table legends. Kinetics of fatty acid uptake were analyzed by constructing Woolf plots as described (37). Theoretical competition curves were generated by assuming $K_m = K_i$ and using the equation (38):

$$\left[i = [I]/[I] + K_i(1 + [S]/K_m)\right]$$

where i is the fractional inhibition, [I] is the concentration of inhibitor, and [S] is the concentration of substrate.

RESULTS

Fatty acid uptake was first examined in Caco-2 cells grown on glass coverslips, in which only the apical surface of the cell is in contact with the medium. **Fig. 1B** shows the uptake of the long chain fatty acid oleate (18:1) and palmitate (16:0) and Figure 1C the uptake of the short chain fatty acid octanoate (8:0), as a function of unbound fatty acid concentration. Uptake of both long chain fatty acids showed apparent saturation, but a notable diffusion component was evident at higher concentrations. In contrast, the uptake of the shorter chain 8:0 was linear across a range of 0.05 to 12 µM unbound fatty acid. A similar linear relationship was also obtained when 8:0 was added to cells in free solution (not shown). Woolf plots, [s]/v versus [s], were constructed from the data for the long chain fatty acids to determine the apparent K_m and V_{max} of uptake (**Fig. 2**). The average apparent K_m (± SEM) for 18:1 uptake was 0.35 ± 0.07 µM (n = 6 experiments) and for 16:0 uptake was 0.27 ± 0.03 µM (n = 2 experiments). The average V_{max} of 18:1 uptake (263 ± 27 pmol/mg/10 sec) was approximately one-half that of 16:0 (570 ± 32 pmol/mg/10 sec). The K_m values obtained are well below the critical micelle concentration for these fatty acids (> 1 µM), and do not reflect the different aqueous solubilities of 18:1 and 16:0, which are reported to differ by orders of magnitude (39, 40), suggesting that uptake is not dependent on these factors. In the postprandial intestine, the products of triacylglycerol hydrolysis are incorporated primarily into bile salt micelles, and the apical surface of the absorptive cell is exposed to the micellar digestive phase. We therefore examined the uptake of 16:0 from 10 mM taurocholate micelles, and the results demonstrate that uptake is a saturable function of total 16:0 concentration, in agreement with results obtained using BSA-bound fatty acid (**Fig. 3**).

In order to compare the fatty acid uptake process at the apical (AP) and basolateral (BL) plasma membranes, Caco-2 cells were grown on permeable Transwell polycarbonate filters, which allows for separate access to the AP and BL surfaces. **Figure 4** shows uptake of 18:1 (A), 16:0 (B), and 8:0 (C) at the AP and BL surfaces of Caco-2 cells. Fatty acids were introduced as BSA complexes at both surfaces to enable direct quantitative comparison

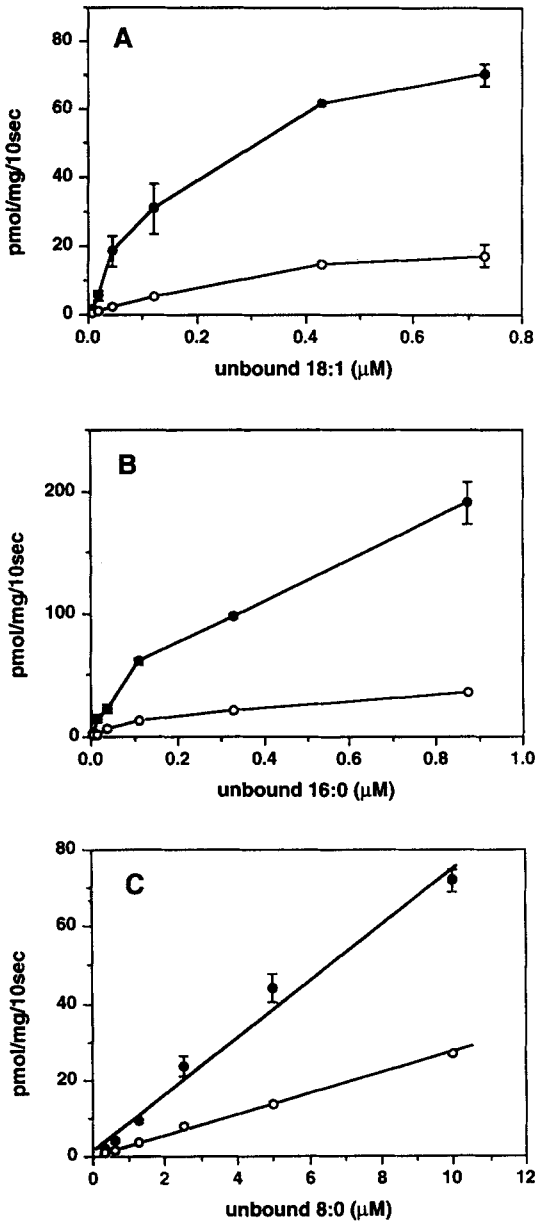

Fig. 4. Apical (AP, ●) and basolateral (BL, ○) uptake of fatty acids by filter-grown Caco-2 cells. Uptake of oleate (18:1, A), palmitate (16:0, B), or octanoate (8:0, C) introduced at the AP or BL surface of Caco-2 cells grown on Transwell filters was measured as in Fig. 1. Data are the average ± SD (n = 2) from one of two similar experiments.

relative to unbound fatty acid concentrations (see Materials and Methods). Uptake of the long chain 18:1 and 16:0 was saturable at both surfaces, whereas the uptake of the short chain 8:0 was linear at both surfaces up to 10 μM. For both the long and short chain fatty acids, uptake at the AP surface was about 3-fold higher than BL uptake. A Woolf plot constructed for 18:1 uptake at the AP and BL surfaces demonstrated that uptake at both surfaces has a similar apparent K_m between 0.3 and 0.4 μM (not shown).

To further explore the saturable nature of long chain fatty acid transport in these cells, studies were conducted to determine whether competitive inhibition of uptake occurred. The uptake of labeled fatty acid (0.02 μM unbound concentration) was measured in the presence of increasing concentrations of unlabeled fatty acid. **Figure 5** shows the experimental (solid line) and theoretical (dashed line) uptake curves for 18:1 (A) and 16:0 (B). The theoretical values, which were calculated based on competitive kinetics assuming the apparent K_m equals K_i (38), are very similar to those that were obtained experimentally. Similar competitive inhibition of uptake could be demonstrated at both the AP and the BL surfaces of the Caco-2 cells (not shown). Further, as shown in **Table 1,** neither 18:1 nor 16:0 uptake was inhibited by the addition of excess 8:0. In contrast, 8:0 was not inhibited, but rather was increased, by the addition of 18:1 or 16:0 (Table 1), perhaps due to displacement of the 8:0 from the BSA by the added long chain fatty acid.

As it has been proposed that a plasma membrane protein may be involved in fatty acid uptake into cells, it was of interest to determine whether a membrane protein is responsible for the apparent saturable and competitively inhibitable uptake of fatty acid by Caco-2 cells. The 40-kD plasma membrane protein $FABP_{pm}$ has been proposed as a long chain fatty acid transporter in a number of rat cell types (9), and Western analysis of Caco-2 cell homogenates was performed to determine whether these cells express a protein immunoreactive

Table 1. Effect of fatty acid addition on oleate, palmitate, and octanoate uptake by Caco-2 cells.

Labeled FA in Medium (ub [FA])	Added Unlabled ub [FA]	FA Uptake	Inhibition
	μM	*pmol/mg/10 sec*	%
[^{3}H]oleate (0.02 μM)	0	7.8 ± 1.7	
Plus oleate	1.3	2.3 ± 0.4	71 (82)
Plus octanoate	>6	10.9 ± 1.54	none
[^{3}H]palmitate (0.02 μM)	0	26.4 ± 1.4	
Plus palmitate	1.3	3.3 ± 0.2	88 (85)
Plus octanoate	>6	26.0 ± 0.1	none
[^{14}C]octanoate (1.2 μM)	0	9.6 ± 0.2	
Plus oleate	1.3	12.1 ± 1.3	none
Plus palmitate	1.3	16.4 ± 2.1	none

[^{3}H]oleate, [^{3}H]palmitate, or [^{14}C]octanoate were complexed to 100 μM BSA to give unbound fatty acid concentrations of 0.02, 0.02, and 1.2 μM, respectively, as noted in parentheses. Excess unlabeled fatty acids were added at the unbound concentrations listed. Uptake was monitored at 23°C for 10 sec. Data from one of at least two similar experiments are expressed as mean ± SD. The values in parentheses are the calculated theoretical levels of inhibition as depicted in Fig. 5. Abbreviations: FA, fatty acid(s); ub [FA], unbound fatty acid concentration.

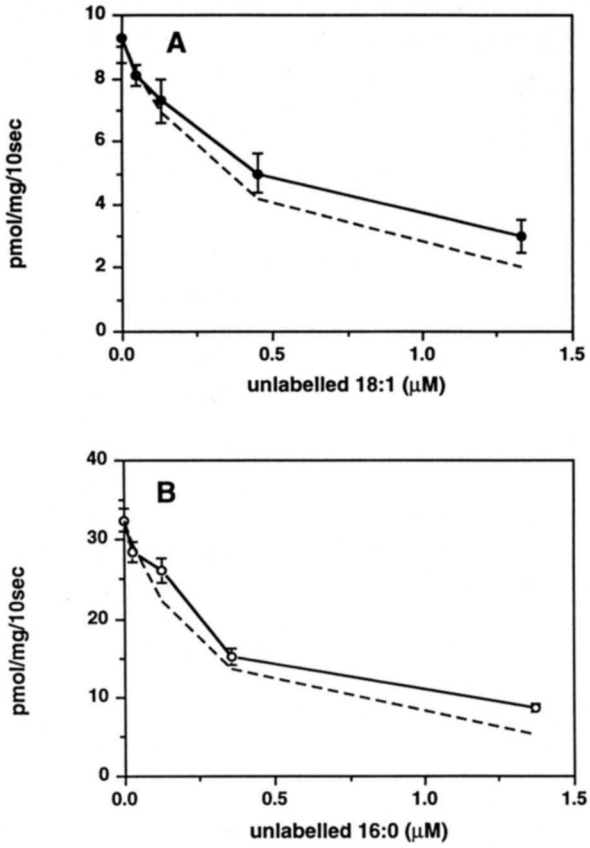

Fig. 5. Competitive inhibition of oleate (A) and palmitate (A) uptake by Caco-2 cells. To a fixed concentration of BSA (100 μM), labeled fatty acid was added to give a constant unbound concentration of 0.02 μM, and various amounts of inhibitor (unlabeled) fatty acid were added to give a range of unbound fatty acid concentrations. The solid line (—) represents the experimental values, and the dashed line (---) represents the theoretical values based on competitive kinetics and calculated as described in Materials and Methods. Data are the average ± SD (n = 2–4) from one of two experiments

to the polyclonal anti-rat $FABP_{pm}$ antibody. **Figure 6** shows that this antibody does react with a protein of the correct molecular weight in undifferentiated (day 2) and differentiated (day 10) Caco-2 cells, as well as rat jejunum, but not human red blood cell ghosts (RBC). It has been reported that this antibody blocks long chain fatty acid uptake in a number of rat cell types (9). However, oleate uptake by Caco-2 cells was not inhibited by treatment with the antibody (2.5 or 5 mg/ml IgG), as compared to treatment with normal rabbit serum or normal rabbit IgG (data not shown).

The role of a plasma membrane protein in long chain fatty acid uptake by Caco-2 was further investigated by pretreatment of the cells with the proteases trypsin and pronase (**Table 2**). Cells were treated under conditions in which the enzyme did not significantly impair the transepithelial resistance of the monolayers. Uptake of 18:1 at both surfaces was resistant to protease treatment. Uptake at the BL surface was increased by low concentrations (0.05 mg/ml) of both proteases, most likely indicating an increase in transport surface area and cell permeabilization. The AP surface was resistant up to much higher concentrations of protease (0.5 mg/ml). The BL membrane is probably more sensitive to proteases than the AP membrane because of differences in composition of these two membranes in intestinal epithelial cells (41).

DISCUSSION

It has long been known that in the intestine short chain fatty acids traverse the epithelium to the portal circulation intact and unesterified, whereas long chain fatty acids are taken into enterocytes and esterified before secretion into the lymph as components of lipoproteins (42). This difference has been attributed primarily to the greater degree of esterification activity for the long chain fatty acids within the enterocytes (42), but it could also reflect a specific absorption mechanism for long chain fatty acids into the cells.

The kinetic data obtained for fatty acid uptake by Caco-2 cells indicate a saturable component in the uptake of long chain but not short chain fatty acids at both the AP and BL membranes (Figs. 1, 3, and 4). The demonstration of competitive inhibition for long chain fatty acid uptake further supports the saturable nature of long chain fatty acid uptake (Fig. 5, Table 1), while the inability of the short chain 8:0 to inhibit long chain

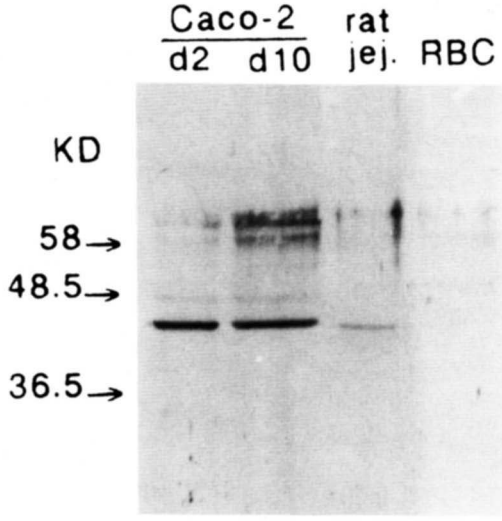

Fig. 6. Immunoblot of FABPpm in Caco-2 cells. Proteins were separated by SDS-PAGE, electrophoretically blotted to PVDF membrane, and reacted with anti-FABPpm antibody as described in Materials and Methods. Lane 1, 100 μg homogenate protein from undifferentiated (day 2) Caco-2 cells; lane 2, 100 μg protein from differentiated (day 10) Caco-2 cells; lane 3, 50 μg rat jejunal homogenate; lane 4, 100 μg human red blood cell ghosts.

Table 2. Effect of proteases on oleate uptake by filter-grown Caco-2 cells

Surface	Protease	Time	Uptake
		min	*pmol/mg/10 sec*
AP	Control		26 ± 3
	Trypsin (0.5 mg/ml)	10	30 ± 10
		20	26 ± 6
	Pronase (0.5 mg/ml)	10	19 ± 1
		20	20 ± 2
BL	Control		6.8 ± 1.0
	Trypsin (0.05 mg/ml)	10	7.6 ± 2.2
		20	21 ± 2
	Pronase (0.05 mg/ml)	10	35 ± 16
		20	88 ± 31

Filter-grown cells were treated with protease for the given time periods under conditions in which the transepithelial resistance of the monolayers were not affected, as described in Materials and Methods. Oleate uptake was subsequently determined within 5 min at an oleate:BSA ratio of 1:1. Data are the mean ± SD (n = 3) from a representative one of 3 similar experiments.

fatty acid uptake, as well as the inability of 8:0 uptake to be inhibited by long chain fatty acids (Table 1), also indicate that short chain and long chain fatty acids are taken up by different mechanisms. Although there is a diffusive component in the uptake of long chain 18:1 and 16:0 by Caco-2 cells, as shown by the incomplete saturation of uptake at high fatty acid concentrations, the presence of a saturable component is suggestive of a carrier-mediated transport process (12, 13). The linear relationship between 8:0 concentration and cellular uptake implies that uptake of this short chain fatty acid likely occurs entirely by a passive diffusion process (1, 5).

Calculation of the kinetic parameters of long chain fatty acid uptake by Caco-2 cells gives an apparent K_m for uptake of approximately 0.3 μM unbound fatty acid. The apparent K_m values for long chain fatty acid uptake by Caco-2 cells are similar to those reported for other intestinal cell preparations, which range from 0.1 μM (14) up to 0.4 μM (28). Stremmel (14) reported that uptake of fatty acid from taurocholate micelle solutions by isolated perfused rat intestinal segments consisted of a saturable component predominant at low unbound fatty acid concentrations (< 0.1 μM), as well as a diffusive component prevalent at higher unbound fatty acid concentrations. Goré and Hoinard (28) reported that uptake of linolenic acid from taurocholate micelles by isolated hamster intestinal cells was saturable with a K_m of 0.38 μM. Ranheim et al. (43) compared uptake and metabolism of albumin-bound versus micelle-bound fatty acids by Caco-2 cells, and demonstrated that micellar fatty acids were absorbed and metabolized more rapidly, although transport K_ms were not determined. The physiological range of albumin:fatty acid ratios (0.15–2) corresponds to unbound fatty acid concentrations in serum of below 0.4 μM (9). In situ concentrations of unbound fatty acid at the lumenal surface are less clear. Utilizing a polyethylene partitioning method, Sallee (44, 45) estimated that the concentration of aqueous monomeric long chain fatty acid in complex mixed micellar solutions ranges between 0.01 and 10 μM. It is possible that in the postpradial state after a lipid-rich meal, fatty acid levels exceed the apparent saturable uptake mechanism. In the fasted state, where dietary lipid levels are low and hydrolysis is restricted to sloughed cells and biliary secretions, the saturable mechanism may predominate. Thus, the saturable component of uptake determined in the present studies would appear to operate at relevant fatty acid concentrations at the AP and BL surfaces of the absorptive epithelium.

The uptake experiments consistently showed a 2-fold lower V_{max} for oleate (18:1) as compared to palmitate (16:0) uptake (Fig. 1). In order to determine whether this difference was due to a greater diffusive component for 16:0, the theoretical V_{max} of the carrier alone was estimated graphically utilizing curves of initial uptake velocity versus substrate concentration and assuming that the putative carrier would be saturated at > 0.5 μM of fatty acid uptake, as described by Neame and Richards (37). A similar 2-fold difference between 18:1 and 16:0 V_{max} was obtained when the values were determined in this manner. If uptake occurs via passive diffusion, the greater uptake of 16:0 may be due to a greater membrane solubility than 18:1. In fact, equilibrium binding experiments utilizing fatty acid solubilized in bile salt micelles indicated that rabbit brush border membranes bind more 16:0 than 18:1 (46). On the other hand, if a carrier is involved in the saturable component of long chain fatty acid uptake, the difference may be due to a greater efficiency of the carrier in transporting 16:0, despite a similar affinity for 16:0 and 18:1. Finally, if the putative carrier is embedded deep in the membrane, it may be exposed only to the long chain fatty acids that have already partitioned into the membrane, and, as mentioned, the effective concentration of 16:0 within the membrane may be greater than that of 18:1 (46).

Previous studies using Caco-2 cells reported that fatty acid is more efficiently taken up from micelles than from BSA solutions (31, 43). In those studies, net uptake and metabolism were determined after long incubation times (time scale of hours). Total fatty acid levels were equivalent in the BSA and micelle solutions, however, the levels of unbound fatty acid were likely quite different. It is generally thought that in the absence of other

variables, unbound fatty acid concentrations determine the level of uptake into cells (13). Thus, the apparently greater uptake from micellar solutions may reflect greater unbound fatty acid concentrations relative to those in the fatty acid-albumin complexes, rather than an inherent difference in albumin compared to micelles per se. In order for there to be a mechanistic difference in the effectiveness of albumin compared with micelles as a vehicle for fatty acid uptake, the albumin or micelle would have to directly interact with the surface of the cell. While the presence of albumin receptors on the surface of cells has been proposed (47), a direct interaction of micelles with the enterocyte apical membrane has not been described. In the present studies, we have examined the initial rates of fatty acid uptake rather than net accumulation and metabolism, and observed saturable uptake at the apical surface from both micellar and albumin solutions.

Very few studies have investigated fatty acid uptake at the two surfaces of polarized cells. Shasby, Stoll, and Spector (48) reported differences in the net uptake and metabolism of various long chain fatty acids at the AP and BL surfaces of filter-grown bovine aortic endothelial cells, but the kinetics and mechanism of uptake at the two surfaces were not investigated. Trimble (49, 50) studied the uptake of long chain fatty acids by rat renal cortical brush border (BBMV) and basolateral membrane vesicles (BLMV). Results from these experiments indicate that fatty acid uptake by BLMV, but not BBMV may occur via an ion exchange mechanism, which is blocked by the anion transport inhibitor 4,4′-diisothiocyano-stilbene-2,2¢-disulfonate (DIDS). The current experiments with filter-grown Caco-2 cells show that uptake of long chain fatty acid is apparently saturable with a similar K_m at both the AP and BL membranes, indicating that the saturable component of uptake at the two surfaces may be similar. Short chain fatty acid uptake, on the other hand, was linear and likely to be diffusional at both surfaces (Fig. 4). Immunocytochemical localization of $FABP_{pm}$ in sections of rat intestinal epithelium has demonstrated the presence of this protein at both the AP and BL membranes. Reactivity was reportedly greater in the AP membrane (14). Thus, if a plasma membrane protein carrier is involved, it would be expected to be localized at both surfaces of Caco-2 cells.

Uptake of fatty acid was greater at the AP as compared to the BL membrane (Fig. 4). As the difference is observed for both long as well as short chain fatty acids, this suggests that the diffusive parameters of uptake at the two surfaces differ. Although previous measurements of AP versus BL surface area of Caco-2 cells indicated that the BL membrane surface area is 3-fold greater than the AP surface area (30), the basolateral membrane is in contact with neighboring cells and the substratum, and may therefore not be readily accessible for fatty acid uptake. Also, there are large compositional differences as well as differences in membrane lipid order between the AP and BL membranes of intestinal epithelial cells, including Caco-2 (41, 51, 52) that may affect the diffusive component of uptake. The differential effect of protease treatment on uptake at the AP and BL surfaces (Table 2) underscores the presence of significant differences in the characteristics of these two surfaces.

Attempts were made to demonstrate more directly the involvement of a plasma membrane protein in fatty acid uptake by Caco-2 cells. Western analysis of Caco-2 cell homogenates (Fig. 6) demonstrated that these cells express significant levels of a protein immunoreactive with a polyclonal antibody to the rat liver $FABP_{pm}$. Although this antibody blocks uptake of fatty acid by a number of rat cell types (9), no effect on oleate uptake by Caco-2 cells was observed (data not shown). It is possible that the protein detected by Western analysis is not involved in fatty acid uptake (see below). Alternatively, as the antibody was made against the rat protein, it may not recognize an epitope involved in uptake by human cells, or may not have sufficient affinity for the human protein to block uptake. Attempts to localize the protein in Caco-2 cells by immunocytochemical methods failed to show consistent plasma membrane staining (data not shown), suggesting that the antibody may bind to the denatured protein in the Western blot, but may be unable to bind the native protein on the cell surface. This could explain the inability of this antibody to block fatty acid uptake by Caco-2 cells. Schurer et al. (53) also found that saturable uptake of fatty acids into cultured human keratinocytes was not inhibited by an antibody to the rat liver $FABP_{pm}$. As mentioned earlier, a number of distinct proteins have been proposed as membrane fatty acid transporters, and it will be of interest to determine the presence of these proteins in Caco-2 cells and the effects of specific antibodies on fatty acid uptake.

Trypsin and pronase did not significantly inhibit oleate uptake by Caco-2 cells at either the AP or BL surfaces (Table 2). Transepithelial resistance was disrupted at relatively low protease concentrations, perhaps too low to effect proteolysis of a putative fatty acid transporter. It is conceivable that an apical plasma membrane transport protein in intestinal epithelial cells might be particularly resistant to protease treatment, as intestinal cells are frequently exposed to significant levels of digestive enzymes. The protection of epithelial plasma membrane proteins from digestive enzymes is generally thought to be a function of the glycocalyx, a layer of carbohydrate at the cell surface made up of the oligosaccharide chains of plasma membrane glycopro-

teins and glycolipids (54, 55). The glycocalyx is prominent at the AP surface of the epithelium and is largely removed when plasma membrane vesicles are prepared from intestinal epithelial cells (54), which may account for the sensitivity of fatty acid uptake to trypsin in rat jejunal microvillus membrane vesicles (17). It has been reported that the glycolipid glucosylceramide is enriched in the AP membrane of Caco-2 cells (52) indicating that cultured Caco-2 cells may possess a glycocalyx capable of protecting its plasma membrane proteins from proteases.

The lack of effect of proteases on long chain fatty acid uptake may also indicate that a plasma membrane protein is not involved in the saturable component of long chain fatty acid uptake by Caco-2 cells. A number of alternate mechanisms might explain the current data. The saturable component could reflect a limiting solubility of fatty acid in an unstirred water layer at the cell surface (27). Such saturation of palmitate uptake would be expected to occur at much lower concentration than oleate, as its aqueous solubility is orders of magnitude lower than oleate (40). However, as the apparent K_m of uptake determined here for palmitate and oleate was found to be similar, this mechanism is not probable. In addition, apparent saturable uptake may reflect binding of fatty acid to membrane lipids rather than a protein, and the rate thus reflects the diffusion across the bilayer. Although this mechanism has been suggested by others (2, 3, 5), it does not seem likely as the cells were washed with excess fatty acid-free BSA after uptake to remove extracellular and exofacial fatty acid, and as competitive kinetics are observed at very low fatty acid concentrations. In addition, the fact that protease treatment at the BL surface of Caco-2 increased fatty acid uptake suggests that the plasma membrane represents a barrier to transmembrane transport. It would not be expected that passive diffusion-mediated uptake through the lipid bilayer would be influenced by membrane protein disruption.

The saturable component of long chain fatty acid uptake may represent titration of cytosolic fatty acid binding proteins ($FABP_c$). Caco-2 cells express the liver form of $FABP_c$ (L-$FABP_c$, (30)), and this protein is proposed to be involved in intracellular fatty acid transport in the intestine (56, 57). The observed K_m of long chain fatty acid uptake by Caco-2 cells is similar to the reported affinities (K_d) for long chain fatty acid binding to L-$FABP_c$ (57). The amount of L-$FABP_c$ in Caco-2 cells was estimated to be about 1/10 of that in rat jejunum, or < 40 pmol L-$FABP_c$ in 1 mg homogenate protein (30). The V_{max} of fatty acid uptake was determined to be an order of magnitude greater than this (> 200 pmol/mg per 10 sec), suggesting that uptake is not directly limited by the concentration of $FABP_c$.

The current investigation examined long chain fatty acid transport in the Caco-2 cell model of polarized intestinal epithelial cells. The kinetic data obtained are consistent with a similar saturable uptake mechanism at both plasma membrane surfaces. This, in turn, is consistent with the reported subcellular localization of a putative plasma membrane fatty acid transporter in rat jejunum (14). Further, it was found that Caco-2 cells express a protein immunoreactive with the anti-rat $FABP_{pm}$ antiserum, although preincubation with this antibody did not inhibit long chain fatty acid uptake by Caco-2 cells. Similarly, protease pretreatment did not inhibit long chain fatty acid uptake. Elucidation of the mechanism of long chain fatty acid uptake by Caco-2 intestinal cells will require localization of the saturable component and isolation and reconstitution of the protein, if any, that is involved.

We thank Dr. Paul Berk (Mt. Sinai Medical Center, NY) for kindly providing the anti-$FABP_{pm}$ antiserum and the computer program for calculating unbound fatty acid concentrations. This work was supported by National Institutes of Health Grants DK38389 (J.S.) and DK34854 (Harvard Digestive Diseases Center) and a National Science Foundation Graduate Fellowship (P.J.T.). J.S. is an Established Investigator of the American Heart Association.

Manuscript received 3 August 1995 and in revised form 9 November 1995.

REFERENCES

1. Thomson, A. B. R., and J. M. Dietschy. 1981. Intestinal lipid absorption: major extracellular and intracellular events. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 1147–1220.
2. Thomson, A. B. R., M. Keelan, M. L. Garg, and M. T. Clandinin. 1989. Intestinal aspects of lipid absorption: in review. *Can. J. Physiol. Pharmacol.* **67:** 179–191.
3. Meddings, J. B. 1988. Lipid permeability of rat jejunum and ileum: correlation with physical properties of the microvillus membrane. *Biochim. Biophys. Acta.* **943:** 305–314.
4. Kamp, F., and J. A. Hamilton. 1993. Movement of fatty acids, fatty acid analogues, and bile acids across phospholipid bilayers. *Biochemistry.* **32:** 11074–11086.
5. Noy, N., T. M. Donnelly, and D. Zakim. 1986. Physical-chemical model for entry of water-insoluble compounds into cells. Studies of fatty acid uptake by the liver. *Biochemistry.* **25:** 2013–2021.
6. Cooper, R. B., N. Noy, and D. Zakim. 1989. Mechanism for binding of fatty acids to hepatocyte plasma membranes. *J. Lipid Res.* **30:** 1719–1726.
7. Westergaard, H., and J. M. Dietschy. 1974. Delineation of dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in rabbit intestine. *J. Clin. Invest.* **54:** 718–732.
8. Stremmel, W. 1989. Mechanism of hepatic fatty acid uptake. *J. Hepatol.* **9:** 374–382.

9. Potter, B. J., D. Sorrentino, and P. D. Berk. 1989. Mechanisms of cellular uptake of free fatty acids. *Annu. Rev. Nutr.* **9:** 253–270.
10. Stremmel, W., and P. D. Berk. 1986. Hepatocellular influx of [^{14}C]oleate reflects membrane transport rather than intracellular metabolism or binding. *Proc. Natl. Acad. Sci. USA.* **83:** 3086–3090.
11. Abumrad, N. A., R. C. Perkins, J. H. Park, and C. R. Park. 1981. Mechanism of long chain fatty acid permeation in the isolated adipocyte. *J. Biol. Chem.* **256:** 9183–9191.
12. Abumrad, N. A., J. H. Park, and C. R. Park. 1984. Permeation of long chain fatty acid into adipocytes. *J. Biol. Chem.* **259:** 8945–8953.
13. Sorrentino, D., R. B. Robinson, C. L. Kiang, and P. D. Berk. 1989. At physiologic albumin/oleate concentrations oleate uptake by isolated hepatocytes, cardiac myocytes and adipocytes is a saturable function of the unbound oleate concentration. *J. Clin. Invest.* **84:** 1325–1333.
14. Stremmel, W. 1988. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J. Clin. Invest.* **82:** 2001–2010.
15. DeGrella, R. F., and R. J. Light. 1980. Uptake and metabolism of fatty acids by dispersed adult rat heart myocytes. II. Inhibition by albumin and fatty acid homologues and the effect of temperature and metabolic reagents. *J. Biol. Chem.* **255:** 9739–9745.
16. Stremmel, W., G. Strohmeyer, and P. D. Berk. 1986. Hepatocellular uptake of oleate is energy dependent, sodium linked and inhibited by an antibody to a hepatocyte plasma membrane fatty acid binding protein. *Proc. Natl. Acad. Sci. USA.* **83:** 3584–3588.
17. Stremmel, W., G. Lotz, G. Strohmeyer, and P. D. Berk. 1985. Identification, isolation, and partial characterization of a fatty acid binding protein from rat jejunal microvillus membranes. *J. Clin. Invest.* **75:** 1068–1076.
18. Stremmel, W., G. Strohmeyer, F. Borchard, S. Kochwa, and P. D. Berk. 1985. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. USA.* **82:** 4–8.
19. Schweiterman, W., D. Sorrentino, B. J. Potter, J. Rand, C. L. Kiang, D. Stump, and P. D. Berk. 1988. Uptake of oleate by isolated rat adipocytes is mediated by a 40-kDa plasma membrane fatty acid binding protein closely related to that in liver and gut. *Proc. Natl. Acad. Sci. USA.* **85:** 359–363.
20. Fujii, S., H. Kawaguchi, and H. Yasuda. 1987. Purification of high affinity fatty acid receptors in rat myocardial sarcolemmal membranes. *Lipids.* **22:** 544–546.
21. Stremmel, W., and L. Theilmann. 1986. Selective inhibition of long chain fatty acid uptake in short-term cultured rat hepatocytes by an antibody to rat liver plasma membrane fatty acid-binding protein. *Biochim. Biophys. Acta.* **877:** 191–197.
22. Abumrad, N. A., M. R. El-Maghrabi, E-Z. Amri, E. Lopez, and P. A. Grimaldi. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. *J. Biol. Chem.* **268:** 17665–17668.
23. Schaffer, J. E., and H. F. Lodish. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell.* **79:** 427–436.
24. Chow, S. L., and D. Hollander. 1979. A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro. *J. Lipid Res.* **20:** 349–356.
25. Ockner, R. K., J. P. Pittman, and J. L. Yager. 1972. Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids. *Gastroenterology.* **62:** 981–992.
26. Bennett Clark, S. 1971. The uptake of oleic acid by rat small intestine: a comparison of methodologies. *J. Lipid Res.* **12:** 43–55.
27. Hollander, D., S. L. Chow, and V. D. Dedufalza. 1984. Intestinal absorption of free oleic acid in the unanesthetized rat: evidence for a saturable component? *Can. J. Physiol. Pharmacol.* **62:** 1136–1140.
28. Goré, J., and C. Hoinard. 1993. Linolenic acid transport in hamster intestinal cells is carrier-mediated. *J. Nutr.* **123:** 66–73.
29. Gangl, A., and R. K. Ockner. 1975. Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. *J. Clin. Invest.* **55:** 803–813.
30. Trotter, P. J., and J. Storch. 1991. Fatty acid uptake and metabolism in a human intestinal cell line (Caco-2): comparison of apical and basolateral incubation. *J. Lipid Res.* **32:** 293–304.
31. Levin, M. S., V. D. Talkad, J. I. Gordon, and W. F. Stenson. 1992. Trafficking of exogenous fatty acids within Caco-2 cells. *J. Lipid Res.* **33:** 9–19.
32. Trotter, P. J., and J. Storch. 1993. Nutritional control of fatty acid esterification in differentiating Caco-2 intestinal cells is mediated by cellular diacylglycerol concentrations. *J. Nutr.* **123:** 728–736.
33. Trotter, P. J., and J. Storch. 1993. Fatty acid esterification during differentiation of the human intestinal cell line Caco-2. *J. Biol. Chem.* **268:** 10017–10023.
34. Spector, A. A., J. E. Fletcher, and J. D. Ashbrook. 1971. Analysis of long-chain free fatty acid binding to bovine serum albumin by determination of stepwise equilibrium constants. *Biochemistry.* **10:** 3229–3232.
35. Spector, A. A. 1975. Fatty acid binding to plasma albumin. *J. Lipid Res.* **16:** 165–179.
36. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
37. Neame, K. D., and T. G. Richards. 1972. Elementary Kinetics of Membrane Carrier Transport. Blackwell Scientific Publications, Oxford, England.
38. Segal, I. H. 1975. Simple inhibition systems. *In* Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems. John Wiley & Sons, New York. 100–125.
39. Small, D. M. 1986. The Physical Chemistry of Lipids: From Alkanes to Phospholipids. Plenum Press, New York. 276.
40. Hofmann, A. F., and H. S. Mekhjian. 1973. Bile acids and the intestinal absorption of fat and electrolytes in health and disease. *In* The Bile Acids, Vol. 2. P. P. Nair, and D. Kritchesvky, editors. Plenum Press, New York. 103–152.
41. Brasitus, T. A., and D. Schachter. 1980. Lipid dynamics and lipid-protein interactions in rat enterocyte basolateral and microvillus membranes. *Biochemistry.* **19:** 2763–2769.
42. Jackson, M. J. 1974. Transport of short chain fatty acids. *In* Biomembranes. Vol. 4B. D. H. Smyth, editor. Plenum Press, London. 673–709.
43. Ranheim, T., A. Gedde-Dahl, A. C. Rustan, and C. A. Drevon. 1994. Fatty acid uptake and metabolism in CaCo-2 cells: eicosapentaenoic acid (20:5(n–3)) and oleic acid (18:1(n–9)) presented in association with micelles or albumin. *Biochim. Biophys. Acta.* **1212:** 295–304.

44. Sallee, V. L. 1974. Apparent monomer activity of saturated fatty acids in micellar bile salt solutions measured by a polyethylene partitioning system. *J. Lipid Res.* **15:** 56–64.
45. Sallee, V. L. 1978. Determinants of fatty acid and alcohol monomer activities in mixed micellar solutions. *J. Lipid Res.* **19:** 207–214.
46. Proulx, P., H. Aubury, I. Brglez, and D. G. Williamson. 1984. Studies on the uptake of fatty acids by brush border membranes of rabbit intestine. *Can. J. Biochem. Cell Biol.* **63:** 249–256.
47. Reed, R. G., and C. M. Burrington. 1989. The albumin receptor effect may be due to a surface-induced conformational change in albumin. *J. Biol. Chem.* **264:** 9867–9872.
48. Shasby, D. M., L. L. Stoll, and A. A. Spector. 1987. Polarity of arachidonic acid metabolism by bovine aortic endothelial cell monolayers. *Am. J. Physiol.* **253:** H1177–H1183.
49. Trimble, M. E. 1989. Mediated transport of long-chain fatty acids by rat renal basolateral membranes. *Am. J. Physiol.* **257:** F539–F546.
50. Trimble, M. E. 1990. Renal palmitate transport: possible sites for interaction with a plasma membrane fatty acid-binding protein. *Mol. Cell. Biochem.* **98:** 201–207.
51. Hansson, G. C. 1983. The subcellular localization of the glycosphingolipids in the epithelial cells of rat small intestine. *Biochim. Biophys. Acta.* **733:** 295–299.
52. Van't Hof, W., and G. Van Meer. 1990. Generation of lipid polarity in intestinal epithelial (Caco-2) cells: sphingolipid synthesis in the Golgi complex and sorting before vesicular traffic to the plasma membrane. *J. Cell Biol.* **111:** 977–986.
53. Schurer, N. Y., W. Stremmel, J. U. Grundmann, V. Schliep, H. Kleinert, N. M. Bass, and M. L. Williams. 1994. Evidence for a novel keratinocyte fatty acid uptake mechanism with preference for linoleic acid: comparison of oleic and linoleic acid uptake by cultured human keratinocytes, fibroblasts and a human hepatoma cell line. *Biochim. Biophys. Acta.* **1211:** 51–60.
54. Ito, S. 1969. Structure and function of the glycocalyx. *Fed. Proc.* **28:** 12–25.
55. Egberts, H. J. A., J. F. J. G. Koninkx, J. E. van Dijk, and J. M. V. M. Mouwen. 1984. Biological and pathobiological aspects of the glycocalyx of the small intestinal epithelium. A review. *Vet. Q.* **6:** 186–199.
56. Ockner, R. K., and J. A. Manning. 1974. Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J. Clin. Invest.* **54:** 326–338.
57. Bass, N. M. 1985. Function and regulation of hepatic and intestinal fatty acid binding proteins. *Chem. Phys. Lipids.* **38:** 95–114.