Comparison of Cholesterol and Sitosterol Uptake in Different Brush Border Membrane Models†

Sabina Compassi,‡ Moritz Werder,‡ Franz E. Weber,‡ Dario Boffelli,‡ Helmut Hauser,*,‡ and Georg Schulthess§

Laboratorium für Biochemie, Eidgenössische Technische Hochschule Zürich, ETH Zentrum, Universitätstrasse 16, CH-8092 Zürich, Switzerland, and Department für Innere Medizin, Medizinische Poliklinik, Universitätsspital Zürich, CH-8091 Zürich, Switzerland

Received August 16, 1996; Revised Manuscript Received March 18, 1997⊗

ABSTRACT: (I) There is little discrimination between cholesterol and the plant sterol sitosterol in the uptake at the brush border membrane (BBM). (II) This difference cannot account for the marked discrimination between cholesterol and sitosterol observed in the absorption of these two sterols by the small-intestinal epithelium. (III) This discrimination occurs during intracellular processing involving the esterification and incorporation into lipoprotein particles of the two sterols. This conclusion is based on a comparative study of sterol uptake by brush border membrane vesicles (BBMV) and sterol absorption by Caco-2 cells. (IV) The uptake of sitosterol by the BBM is energy-independent and facilitated in a manner analogous to cholesterol uptake [Thurnhofer, H., & Hauser, H. (1990a) *Biochemistry 29*, 2142−2148]. (V) The rate of cholesterol and sitosterol uptake by BBMV from both mixed bile salt micelles and small unilamellar vesicles (SUV) as the donor is directly proportional to the sterol content of the donor. (VI) The pseudo-first-order rate constants k_1 for sterol uptake from SUV are independent of the sterol content up to 10−20 mol %. Above that, competition between the two sterols leads to a reduction of the k_1 values.

We reported that the uptake of free and esterified cholesterol in the small-intestinal brush border membrane (BBM)[1] is energy-independent and protein-mediated (Thurnhofer & Hauser, 1990a; Compassi et al., 1995; Schulthess et al., 1996). This was demonstrated when both mixed bile salt micelles and phospholipid small unilamellar vesicles (SUV) were donor particles. Evidence for protein-mediated sterol uptake at the BBM was first provided using brush border membrane vesicles (BBMV) as the acceptor (Thurnhofer & Hauser, 1990a; Compassi et al., 1995). The conclusions derived from this model were then verified in other BBM models more closely related to the in vivo situation such as intact enterocytes and Caco-2 cells (Schulthess et al., 1996).

Plant sterols (phytosterols) are ingredients of increasing importance in our diet. They comprise compounds of diversified chemical structure, but one of the most abundant and widely spread plant sterols is β-sitosterol which contains a β-ethyl group at carbon atom 24 of the cholesterol side chain. We have been using β-sitosterol as a representative of the class of plant sterols. Another reason for our interest in β-sitosterol is an inherited disease of lipid metabolism, sitosterolemia, which is characterized by enhanced sterol absorption in the small intestine and increased plasma levels of cholesterol and plant sterols. The small-intestinal mucosa has the capacity to discriminate between cholesterol and plant sterols in that cholesterol is preferentially absorbed and transferred from the lumen of the small intestine to the lymph. It was reported that a major fraction (50−60%; Crouse & Grundy, 1971) of intragastrically administered cholesterol but less than 2% of plant sterols are absorbed in a rat model (Ikeda et al., 1988a). Consistent with this report, the fecal recovery of administered plant sterols in humans was found to exceed 98% (Becker et al., 1993). It is still unknown whether the discrimination between cholesterol and plant sterols takes place at the level of the BBM or the intracellular processing of the sterols. The latter involves the esterification and packaging of sterols into chylomicrons. Both the sterol uptake at the level of the BBM (Child & Kuksis, 1983) and the esterification of sterols by cytosolic cholesterol esterase and/or microsomal acyl-CoA:cholesterol acyltransferase (ACAT) (Ikeda et al., 1988a) were reported to be less efficient for plant sterols than for cholesterol.

Evidence that plant sterols decrease small-intestinal cholesterol absorption has accrued (Subbiah, 1973; Pollak & Kritchevsky, 1980; Vahouny & Kritchevsky, 1981; Becker et al., 1993). Plant sterols were reported to compete with cholesterol for micellar solubilization and might in this way limit small-intestinal cholesterol uptake at the BBM (Ikeda et al., 1983, 1988a,b). Alternatively, plant sterols might compete with cholesterol for the protein(s) facilitating sterol uptake in the small-intestinal BBM.

The work presented here is addressed to the question of (I) whether the protein(s) responsible for the energy-independent, facilitated uptake of cholesterol in the small-intestinal BBM discriminates between cholesterol and the plant sterol sitosterol and, related to it, if there is competition between sterols at the BBM. By using different models of

† This work was supported by the Swiss National Science Foundation (Grant 32-46810.96/1).

* To whom correspondence should be addressed.

‡ Eidgenössische Technische Hochschule Zürich.

§ Universitätsspital Zürich.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

[1] Abbreviations: BBM, brush border membrane(s); BBMV, brush border membrane vesicle(s); DMEM, Dulbecco's modified Eagle's medium; EDTA, disodium salt of ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid; PBS, phosphate-buffered saline (0.01 M phosphate buffer at pH 7.2 containing 0.15 M NaCl); PC, phosphatidylcholine; SUV, small unilamellar vesicle(s).

S0006-2960(96)02066-1 CCC: $14.00

the BBM, e.g. BBMV and Caco-2 cells, information pertinent to the mechanism of the discrimination is hoped to be gained.

The terms lipid uptake and lipid absorption are often used synonymously. When working with different BBM models, particularly in vivo ones, we use these terms in the following way. Lipid uptake is the transfer of lipid from the donor to the BBM, while absorption refers to the transfer of lipid from the donor to the lymph. Absorption in Caco-2 cells is accordingly defined as the lipid transfer from the donor to the basolateral compartment.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine (PC) and egg phosphatidic acid were purchased from Lipid Products (South Nutfield, Surrey, U.K.), and cholesterol, β-sitosterol (24β-ethyl-Δ^5-cholesten-3β-ol), and sodium taurocholate were purchased from Fluka (Buchs, Switzerland). The sodium salt of oleic acid was purchased from Applied Sciences (State College, PA) or Sigma (St. Louis, MO) and lyophilized proteinase K from *Tritirachium album* from Boehringer (Mannheim, Germany). [1,2-$^3H_2$(N)]Cholesterol (50 Ci/mmol), [24-^{14}C]taurocholic acid (46 Ci/mol), [1,2-^{3}H]polyethylene glycol (2 mCi/g) (average $M_r = 900$), and inulin[^{14}C]carboxylic acid (2 mCi/g) were obtained from Du Pont-NEN (Regensdorf, Switzerland), and β-[4-^{14}C]sitosterol (55.4 Ci/mol) was obtained from Amersham (U.K.). All lipids used in this study were pure by thin-layer chromatography standards.

Methods

Preparation of BBMV and Various Donor Particles. BBMV were prepared routinely from rabbit duodenum and jejunum according to Hauser et al. (1980). As donor particles, mixed sodium taurocholate (5 mM) micelles containing up to 4 mol % cholesterol or sitosterol were used, and these micelles were supplemented with either 0.6 mM sodium oleate or 0.5 mM egg PC. Alternatively, egg PC SUV containing various amounts of sterols were used as donor particles.

Lipid Uptake by BBMV. Lipid uptake by BBMV was measured as described previously (Thurnhofer et al., 1991; Schulthess et al., 1994; Compassi et al., 1995). Mixed bile salt micelles or egg PC SUV containing the radiolabeled lipids were used as the donor particles. At time zero, donor and acceptor both dispersed in buffer A [10 mM HEPES (pH 7.2), 0.15 M NaCl, and 5 mM EDTA] were mixed at appropriate concentrations and incubated at 23 °C. After timed intervals, BBMV were separated from the donor by centrifugation at 115000*g* for 2 min in a Beckman airfuge. The radioactivity present in both donor and BBMV was determined. In one series of experiments, sterol uptake was measured at 37 °C using egg PC SUV as the donor and BBMV as the acceptor. Published procedures were used to measure rates of exchange of sitosterol and cholesterol between two populations of SUV (Thurnhofer & Hauser, 1990b; Thurnhofer et al., 1991). SUV of egg PC containing [^{3}H]cholesterol or [^{14}C]sitosterol as the donor and SUV of egg PC/egg phosphatidic acid (85:15 mole ratio) as the acceptor, both suspended in buffer A at 1 and 5 mg of total lipid/mL, respectively, were incubated at 23 °C in the absence and presence of supernatant proteins (see below). After timed intervals, aliquots of 0.1 mL of the incubation mixture were filtered through DEAE-Sepharose CL-6B columns (2 × 0.6 cm, from Pharmacia) which retained the negatively charged acceptor vesicles. The radioactivity in the donor vesicles eluted from the column was determined.

Proteolytic Treatment of BBMV and Production of Supernatant Proteins. The proteolytic treatment of BBMV with proteinase K or papain was carried out as described by Thurnhofer and Hauser (1990b). The liberation of water-soluble lipid exchange proteins from BBMV by autoproteolysis was induced by subjecting BBMV suspended in buffer A to freeze−thaw cycles as described previously (Thurnhofer & Hauser, 1990b; Thurnhofer et al., 1991). Proteins thus liberated were separated from BBMV by centrifugation at 115000*g* for 30 min. The pelleted BBMV were resuspended in buffer A, and the centrifugation−resuspension was repeated twice in order to completely extract supernatant proteins. Supernatant proteins thus produced were concentrated in dialysis bags (Union Carbide, Type 18/32 with a cutoff molecular weight of 5K to 8K) using polyethylene glycol externally ($M_r = 12K$). The supernatant proteins exhibiting lipid exchange activities were purified in a two-step procedure as described previously (Compassi et al., 1995) using gel filtration on Sephadex G75 SF and anion exchange chromatography on a Mono Q HR 5/5 FPLC column (Pharmacia LKB). The purified proteins were used to raise polyclonal antibodies in sheep. Sheep preimmune sera and antisera were purified by chromatography on CM Affi-Gel Blue followed by ammonium sulfate precipitation (Gee et al., 1979), and the resulting IgG solutions were stored at 4 °C and used within 2 weeks after preparation.

Lipid Absorption Measurements Using Caco-2 Cells. Caco-2 cells were a generous gift of H. P. Merkle of the Department of Pharmacy of the Swiss Federal Institute of Technology (ETH) Zurich. The passages of the cells used here ranged between 44 and 48. The cells were routinely grown in 80 cm^2 plastic flasks (NUNC, Roskilde, Denmark) in Dulbecco's modified Eagle's medium (DMEM) (4.5 g/L D-glucose, 3.7 g/L sodium bicarbonate, and 0.11 g/L sodium pyruvate, from Life Technologies, Basel, Switzerland) at 37 °C in a 95% air/5% CO_2 atmosphere. The medium was supplemented with 20% fetal calf serum, 1% L-glutamine, 1% nonessential amino acids, penicillin (500 IU/mL), and streptomycin (500 μg/mL) (all from Life Technologies). The culture medium was changed every other day and the day before the experiment. For subculture, the medium was removed and the cells were washed twice with Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline and then detached from the culture flask with 0.25% trypsin in a 1 mM EDTA solution. Culture medium with fetal calf serum was added to stop trypsinization. Cells were grown to confluency in 1.5 mL of complete DMEM containing approximately 0.325×10^6 cells plated on the apical side of presoaked, collagen-topped polycarbonate filters with 3.0 μm pore size and 24.5 mm diameter (Transwell-COL, Costar). The lower well contained 2.6 mL of complete DMEM. The experiments were performed 2 weeks after the cells had reached confluency. This setup allows the measurement of exchange of substances across the apical and basolateral borders of confluent Caco-2 cells as well as the measurement of polarized transport (Ranheim et al., 1994a,b; Levy et al., 1995; Trotter et al., 1996).

Mixed bile salt micelles (5 mM sodium taurocholate, 0.6 mM sodium oleate, and 0.1 mM radiolabeled cholesterol or sitosterol) were dispersed in buffer B [8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 (pH 7.2) containing 137 mM NaCl and 2.7 mM KCl] and preincubated at 37 °C. The Caco-2 cells were washed three times with this buffer, and then 1.5 mL of the dispersion of mixed bile salt micelles in buffer B was added to the apical compartment and 2.6 mL of DMEM free of fetal calf serum to the basolateral compartment to start the absorption measurement. At timed intervals, 100 μL was removed from both the apical and basolateral compartment and the radioactivity of 30 μL aliquots was determined. The volume (100 μL) removed from the apical compartment was replaced by the same volume of the original micellar dispersion. The viability of the cells was checked for each new micellar composition by Trypan Blue (Sigma, Buchs) staining. For these experiments, Transwell-Clear plates from Costar were used. Cells were grown on these plates as described before. After incubation of the cells with the micellar dispersion for 24 h, Trypan Blue staining was performed. The viability of the cells was >99%.

Analytical Methods. The kinetic curves of sterol uptake by BBMV and sterol absorption by Caco-2 cells were fitted as described before (Compassi et al., 1995) using the software Origin (Microcal Software, Inc., Northampton, MA). Lipids were extracted from Caco-2 cells after 24 h of incubation with mixed sodium taurocholate micelles according to Hauser et al. (1980). Aliquots of the lipid extract were applied to a silica gel 60 F254 plate (20 × 20 cm) from Merck (Darmstadt, Germany) as a 1.5 cm wide band, and the chromatogram was developed with cyclohexane/diethyl ether/acetic acid (50:50:1 by volume). The R_f value of cholesterol and β-sitosterol was ~0.28 and that of cholesteryl oleate ~0.65. Radioactive bands were made visible by storage phosphor imaging. Sitosterol was separated from cholesterol by thin-layer chromatography on RP-18 plates (5 × 10 cm) from Merck. After the chromatography tank was carefully saturated with ethanol, the chromatogram was developed with the same solvent.

The octanol/water partition coefficient of sterols was determined according to Lepetit (1977) and Cohnen et al. (1978). Briefly, solutions of the radiolabeled sterols of different concentrations in octanol were prepared and shaken with identical volumes of buffer A at 37 °C for 45 min. The radioactivities present in the water and octanol phase were measured. To correct for water present in the octanol phase, the distribution of inulin[^{14}C]carboxylic acid between the two phases was determined. The concentrations of the sterols in the octanol phase were chosen such that after equilibration with buffer the sterol concentration was well below that of a saturated solution of cholesterol in buffer. This concentration was reported to be 26 nM (Saad & Higuchi, 1965).

The concentration of radiolabeled lipids was determined by counting three or four aliquots of the lipid dispersion in a Beckman LS 7500 liquid-scintillation counter. Protein concentrations were determined using the bicinchoninic acid method of Smith et al. (1985). SDS−PAGE was carried out in a Mini-Protean II dual-slab cell from Bio-Rad according to the Bio-Rad instruction manual using Bio-Rad molecular weight markers. Protein bands were visualized by Coomassie brilliant blue and/or silver staining.

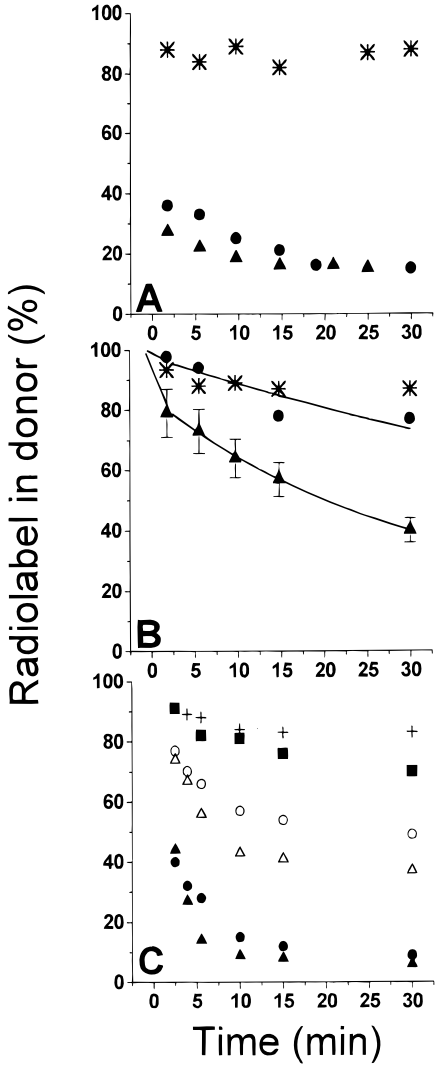

FIGURE 1: Kinetics of sitosterol and cholesterol uptake by rabbit jejunal BBMV from mixed sodium taurocholate micelles. Mixed sodium taurocholate micelles (5 mM) containing either 1 μM sitosterol or 1 μM cholesterol were dispersed in buffer A and incubated at 23 °C with BBMV at 10 mg of protein/mL. The radioactivity remaining in the donor was determined (see Methods) and plotted as a function of time. Usually, the standard deviation was less than 6%, in which case it was not included in the diagram. The error bars represent the standard deviation of four measurements. (A) Uptake of sitosterol (circles), cholesterol (triangles), and sodium taurocholate (stars) using native BBMV as the acceptor. (B) Uptake of sitosterol (circles), cholesterol (triangles), and sodium taurocholate (stars) using proteinase K-treated BBMV as the acceptor. (C) Uptake of sitosterol and cholesterol from mixed sodium taurocholate micelles with the composition described above, containing additionally 0.5 mM egg phosphatidylcholine. BBMV were used at concentrations of 1 mg of protein/mL (sitosterol, crosses; cholesterol, squares), 3 mg of protein/mL (sitosterol, open circles; cholesterol, open triangles), and 10 mg of protein/mL (sitosterol, closed circles; cholesterol, closed triangles).

RESULTS

Sterol Uptake by Rabbit Jejunal BBMV. Evidence is presented in Figure 1 showing that similar quantities of cholesterol and sitosterol are taken up by intact BBMV using mixed taurocholate micelles as the donor. The kinetic curves in Figure 1A are biphasic. Since the time resolution of these experiments was limited to 2 min, which is the time required to separate donor and acceptor by centrifugation, we refrained from curve fitting. Included in Figure 1A is the fraction of

Table 1: Uptake of Sitosterol and Cholesterol by Rabbit Jejunal BBMV from Mixed Taurocholate Micelles

donor	BBMV protein concentration (mg/mL)	sterol uptake[a] [nmol/(mg of protein)]	
		sitosterol	cholesterol
5 mM taurocholate, 1 μM sterol	1	0.36 ± 0.04	0.45 ± 0.05
	10	0.082 ± 0.005	0.082 ± 0.003
5 mM taurocholate, 0.5 mM egg PC, 1 μM sterol	1	0.15 ± 0.01	0.30 ± 0.01
	3	0.16 ± 0.02	0.27 ± 0.03
	10	0.090 ± 0.005	0.090 ± 0.002
5 mM taurocholate, 0.6 mM sodium oleate, 1 μM sterol	1	0.58 ± 0.02	0.58 ± 0.03
5 mM taurocholate, 0.6 mM sodium oleate, 1 μM sterol	3	0.30 ± 0.01	0.31 ± 0.01
5 mM taurocholate, 0.6 mM sodium oleate, 1 μM sterol	10	0.096 ± 0.002	0.095 ± 0.003
5 mM taurocholate, 0.6 mM sodium oleate, 20 μM sterol	1	10.6 ± 0.1	13.1 ± 0.1
5 mM taurocholate, 0.6 mM sodium oleate, 40 μM sterol	1	23.0 ± 0.2	25.1 ± 0.1
5 mM taurocholate, 0.6 mM sodium oleate, 60 μM sterol	1	34.6 ± 0.1	40.5 ± 0.1
5 mM taurocholate, 0.6 mM sodium oleate, 100 μM sterol	1	54.8 ± 0.2	66 ± 4
5 mM taurocholate, 0.6 mM sodium oleate, 200 μM sterol	1	128 ± 4	143 ± 3

[a] Sterol uptake was measured after 30 min of incubation of donor and acceptor at 23 °C.

taurocholate remaining in the donor which differed significantly from that of the sterols. Furthermore, taurocholate uptake seems to occur instantaneously upon mixing of donor and acceptor and does not increase with time. It probably reflects the equilibration of the bile salt with the BBM. After treatment of the BBMV with proteinase K, the uptake of the sterols was significantly slowed, that of sitosterol even more so than that of cholesterol (Figure 1B). The solid lines represent single-exponential fits to the experimental data yielding half-times of about 30 min and 1 h for cholesterol and sitosterol, respectively. These values are characteristic of passive exchange of the sterols between bile salt micelles and lipid bilayers. In contrast, the taurocholate uptake remained unchanged after proteinase K treatment, supporting the interpretation in terms of a passive equilibration procedure.

In the following series of experiments, the uptake kinetics of sitosterol are compared with those of cholesterol using mixed taurocholate micelles with different compositions as the donor and rabbit BBMV of different concentrations as the acceptor. Mixed sodium taurocholate micelles with the same composition as those in Figure 1A were used in these experiments as well as mixed sodium taurocholate micelles containing additionally either 0.5 mM egg PC or 0.6 mM sodium oleate. Representative kinetic curves are shown in Figure 1C. For reasons mentioned above, we determined the amount of sterol taken up by the BBMV in 30 min of incubation (Table 1) instead of deriving kinetic parameters. The following conclusions can be drawn from the kinetic curves (cf. Figure 1) and the data compiled in Table 1. (I) Within experimental error, identical amounts of cholesterol and sitosterol were taken up from mixed taurocholate micelles (Figure 1A), i.e. micelles containing neither egg PC nor oleate. (II) The addition of egg PC to bile salt micelles slowed sterol uptake, while the addition of oleate led to an augmentation. (III) There was some discrimination between the two sterols if bile salt micelles contained egg PC. Under these conditions, cholesterol was preferentially taken up, at best by a factor of about 2. The discrimination was apparent at low BBMV concentrations but disappeared at BBMV concentrations of about 10 mg of protein/mL (Figure 1C); the discrimination was less apparent for micelles containing 0.6 mM oleate. (IV) The amount of sterol taken up appeared to be directly proportional to the sterol content of the bile salt micelle keeping the acceptor concentration constant (Table 1). (V) For both cholesterol and sitosterol, the rate of uptake increased with increasing BBMV concentration (Figure 1C).

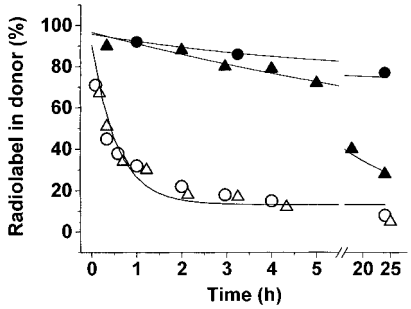

FIGURE 2: Kinetics of sitosterol (circles) and cholesterol (triangles) uptake by rabbit BBMV from egg PC SUV as the donor. BBMV (open symbols) were compared to BBMV treated with proteinase K (closed symbols). SUV of 1 mg of total lipid/mL containing 1 mol % [^{14}C]sitosterol or [^{3}H]cholesterol were incubated at 23 °C with BBMV (10 mg of protein/mL) dispersed in buffer A. The experimental data were fitted best by a single-exponential function yielding the solid lines.

Identical "uptake" kinetics within experimental error were observed for cholesterol and sitosterol using SUV of egg PC as the donor and BBMV as the acceptor (Figure 2). Sterol uptake from this donor was markedly slower than that from bile salt micelles, consistent with published data (Thurnhofer et al., 1991). This is immediately apparent from a comparison of the time axes of Figures 1 and 2. The kinetic curves shown in Figure 2 are monophasic and were fitted best by a single-exponential curve. Pseudo-first-order rate constants k_1 and half-times $t_{1/2}$ derived from curve fitting are summarized in Table 2. For both sterols, the pseudo-first-order rate constants k_1 increased approximately linearly with the acceptor concentration. Linear regression analysis of both plots (not shown) yielded straight lines that were identical within the error of the measurement: $y = (-0.12 \pm 0.08) + (0.16 \pm 0.02)x$ and $r^2 = 0.964$ for sitosterol and $y = (-0.03 \pm 0.03) + (0.13 \pm 0.01)x$ and $r^2 = 0.992$ for cholesterol.

The treatment of BBMV with papain or proteinase K resulted in a significant reduction in sterol uptake as shown in Figure 2. Accordingly, the pseudo-first-order rate constants k_1 obtained after proteolysis were markedly reduced. The half-times of 7−10 h measured for cholesterol uptake after proteolysis of the BBMV (Table 2) are characteristic of passive exchange of cholesterol between two populations of SUV (McLean & Phillips, 1981). The passive exchange

Table 2: Sitosterol and Cholesterol Uptake by Rabbit Jejunal BBMV from Egg PC SUV[a]

	donor		acceptor			kinetics of absorption		
radiolabeled sterol	lipid pool (mg/mL)	sterol content	BBMV	lipid pool (mg/mL)	temp (°C)	kinetics	k_1 (h^{-1})	half-time $t_{1/2}$ (h)
sitosterol	1	1 mol % (13.2 mM)	native	3	23	monophasic	1.6 ± 0.25	0.33 ± 0.05
sitosterol	1	1 mol % (13.2 mM)	native	3	37	monophasic	5.0 ± 1	0.11 ± 0.02
sitosterol	1	5 mol % (67.4 mM)	native	3	23	monophasic	1.0 ± 0.2	0.52 ± 0.1
sitosterol	1	10 mol % (138 mM)	native	3	23	monophasic	1.5 ± 0.3	0.46 ± 0.09
sitosterol	1	1 mol % (13.2 mM)	native	1	23	monophasic	0.24 ± 0.02	1.4 ± 0.2
sitosterol	1	1 mol % (13.2 mM)	native	0.5	23	monophasic	0.07 ± 0.01	3.3 ± 0.5
sitosterol	1	1 mol % (13.2 mM)	native	0.2	23	monophasic	0.03 ± 0.01	4 ± 1
sitosterol	1	1 mol % (13.2 mM)	proteinase K-treated	3	23	monophasic	0.013 ± 0.004	40 ± 12
sitosterol	1	1 mol % (13.2 mM)	proteinase K-treated	3	37	monophasic	0.077 ± 0.008	9.0 ± 1.0
sitosterol	1	1 mol % (13.2 mM)	papain-treated	3	23	monophasic	0.012 ± 0.005	43 ± 18
cholesterol	1	1 mol % (13.2 mM)	native	3	23	monophasic	1.2 ± 0.2	0.42 ± 0.07
cholesterol	1	1 mol % (13.2 mM)	native	3	37	monophasic	5.0 ± 1	0.11 ± 0.02
cholesterol	1	5 mol % (67.4 mM)	native	3	23	monophasic	0.8 ± 0.2	0.64 ± 0.15
cholesterol	1	10 mol % (138 mM)	native	3	23	monophasic	1.0 ± 0.2	0.5 ± 0.1
cholesterol	1	1 mol % (13.2 mM)	native	1	23	monophasic	0.34 ± 0.04	1.0 ± 0.1
cholesterol	1	1 mol % (13.2 mM)	native	0.5	23	monophasic	0.06 ± 0.01	3.9 ± 0.7
cholesterol	1	1 mol % (13.2 mM)	native	0.2	23	monophasic	0.03 ± 0.02	4 ± 3
cholesterol	1	1 mol % (13.2 mM)	proteinase K-treated	3	23	monophasic	0.05 ± 0.02	10 ± 4
cholesterol	1	1 mol % (13.2 mM)	proteinase K-treated	3	37	monophasic	0.29 ± 0.01	2.4 ± 0.07
cholesterol	1	1 mol % (13.2 mM)	papain-treated	3	23	monophasic	0.08 ± 0.05	7 ± 4

[a] The experimental conditions are given in the legend to Figure 2. The k_1 values of both cholesterol and sitosterol uptake depended linearly on the BBMV concentration. Linear regression analysis yielded identical straight lines within the error of the measurements: $y = (-0.12 \pm 0.08) + (0.16 \pm 0.02)x$ and $r^2 = 0.964$ for sitosterol and $y = (-0.03 \pm 0.03) + (0.13 \pm 0.01)x$ and $r^2 = 0.992$ for cholesterol.

Table 3: Effect of Increasing Sterol Concentration on Sitosterol and Cholesterol Uptake by Rabbit Jejunal BBMV from Egg PC SUV[a]

	donor			kinetics of absorption	
radiolabeled sterol	concentration of radiolabeled sterol	cold sterol	concentration of cold sterol	k_1 (h^{-1})	half-time $t_{1/2}$ (h)
[^{14}C]sitosterol	1 mol % (13.2 μM)	–	–	0.23 ± 0.03	1.9 ± 0.25
[^{14}C]sitosterol	1 mol % (13.2 μM)	cholesterol	30 mol % (396 μM)	0.13 ± 0.01	3.3 ± 0.3
[^{14}C]sitosterol	1 mol % (13.2 μM)	sitosterol	30 mol % (396 μM)	0.15 ± 0.02	2.9 ± 0.4
[^{3}H]cholesterol	1 mol % (13.2 μM)	–	–	0.27 ± 0.04	1.6 ± 0.2
[^{3}H]cholesterol	1 mol % (13.2 μM)	sitosterol	30 mol % (396 μM)	0.14 ± 0.02	3.1 ± 0.4
[^{3}H]cholesterol	1 mol % (13.2 μM)	cholesterol	30 mol % (396 μM)	0.14 ± 0.01	3.1 ± 0.2

[a] The experimental conditions are in the legend to Figure 3.

of sitosterol between SUV of egg PC and proteolytically treated BBMV was about 4 times slower, characterized by half-times of about 40 h (Table 2). This is in good agreement with published data on cholesterol and sitosterol exchange between SUV (Kan & Bittman, 1990, 1991).

In one series of experiments, the kinetics of sterol uptake were measured at 37 °C using egg PC SUV as the donor and BBMV as the acceptor. As at 23 °C, identical uptake kinetics were observed for cholesterol and sitosterol. The pseudo-first-order rate constants k_1 obtained at 37 °C exceeded those measured at room temperature by a factor of about 4 (Table 2). The passive sterol exchange between egg PC SUV and proteinase K-treated BBMV at 37 °C was accelerated by a factor of about 6 compared to the passive exchange at 23 °C (Table 2). As observed for the passive exchange at 23 °C, the exchange of sitosterol was about 4 times slower than that of cholesterol.

The competition experiments presented in Figure 3 addressed the question of whether plant sterols interact competitively with the protein(s) of the BBM catalyzing cholesterol uptake. The kinetic curves of Figure 3 are monophasic, and pseudo-first-order rate constants (half-times) derived from curve fitting were compiled in Table 3. Table 3 and Figure 3 confirm the conclusion that the uptake kinetics of both cholesterol and sitosterol are identical within experimental error. This was shown to be true up to sterol contents in the donor vesicle of about 30 mol %. The uptake of both sterols was significantly slowed by increasing the total sterol content in the donor (Figure 3). The pseudo-first-order rate constants k_1 were halved ($t_{1/2}$ values doubled) as the total sterol content in the donor increased from 1 to 30 mol % (Table 3). The comparison of panels A and B of Figure 3 reveals that the slowing effects of 30 mol % sitosterol on cholesterol uptake (closed symbols, Figure 3A) and of 30 mol % cholesterol on sitosterol uptake (closed symbols, Figure 3B) are similar. This indicates that there is no discrimination between cholesterol and sitosterol at the level of the small-intestinal BBMV, at least not with egg PC SUV as the donor.

Sterol Exchange between Two Populations of SUV. The exchange of sitosterol between two populations of SUV in the absence and presence of supernatant proteins is shown in Figure 4. The passive sitosterol exchange in the absence of supernatant protein was slower than that of cholesterol, characterized by a half-time of about 20 h (Table 4). The kinetic curves of Figure 4 were monophasic, and for sitosterol exchange, the pseudo-first-order rate constants k_1 derived from curve fitting increased approximately linearly with protein concentration (data not shown), yielding a straight line by linear regression analysis: $y = (-0.06 \pm 0.07) + (0.71 \pm 0.08)x$ and $r^2 = 0.95$. As shown in Figure 4 and Table 4, supernatant proteins catalyzed equally well the

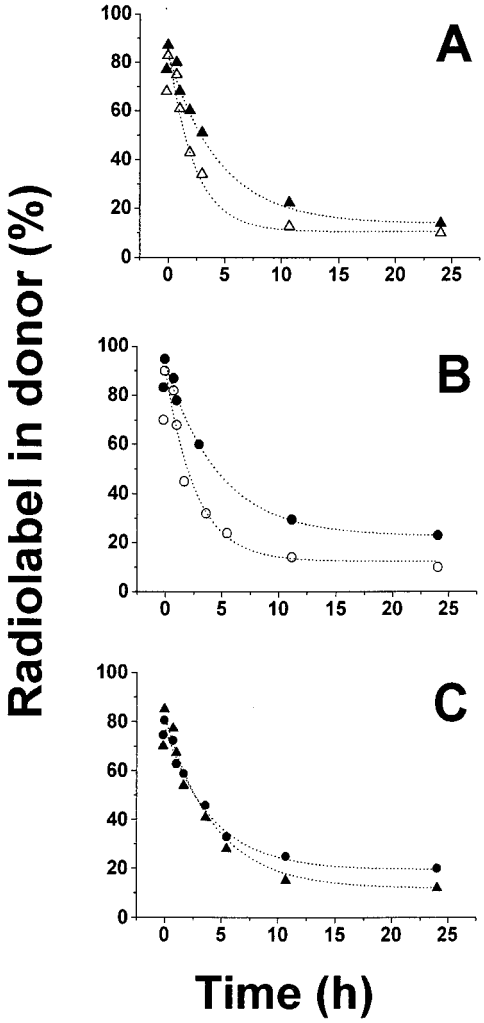

FIGURE 3: Kinetics of cholesterol (triangles) and sitosterol (circles) uptake by rabbit BBMV from egg PC SUV containing the two sterols at different concentrations. Egg PC SUV of 1 mg of total lipid/mL containing the radiolabeled sterols were incubated at 23 °C with BBMV at 5.5 mg of protein/mL in buffer A. The data points represent the mean of four measurements, and the standard deviations were less than 6%. The dotted lines were obtained by curve fitting using a single-exponential function. The sterol concentrations in the SUV (donor) were as follows: (A) 1 mol % (13.2 μM) cholesterol labeled with [^{3}H]cholesterol (△) and 1 mol % labeled cholesterol (▲) together with 30 mol % (396 μM) sitosterol; (B) 1 mol % (13.2 μM) sitosterol labeled with [^{14}C]sitosterol (○) and 1 mol % labeled sitosterol (●) together with 30 mol % (396 μM) cholesterol; and (C) 31 mol % (409 μM) labeled cholesterol (▲) and 31 mol % (409 μM) labeled sitosterol (●).

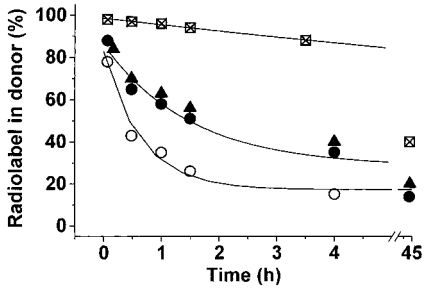

FIGURE 4: Sitosterol and cholesterol exchange between two populations of SUV as a function of the concentration of supernatant proteins. SUV of egg PC containing 1 mol % [^{14}C]sitosterol or 1 mol % [^{3}H]cholesterol were dispersed in buffer A at 1 mg of lipid/mL and incubated with SUV of egg PC/egg phosphatidic acid (85:15 mole ratio) at 5 mg of lipid/mL at 23 °C. Sitosterol exchange was measured in the absence of supernatant proteins (□) and with 1.8 mg of supernatant protein after proteolysis with proteinase K (×) and in the presence of 0.7 mg (●) and 1.8 mg (○) of supernatant proteins. For comparison, cholesterol exchange was measured in the presence of 0.7 mg of supernatant proteins (▲). The dotted lines were obtained by curve fitting using a single-exponential function. The standard deviation of four measurements was less than 6% (not shown).

exchange of cholesterol and sitosterol. After digestion of the supernatant proteins with proteinase K, half-times were measured as in the absence of supernatant proteins (Figure 4 and Table 4).

The polyclonal antibodies raised against the protein purified as described in Methods inhibited both cholesterol and sitosterol exchange between two populations of SUV in a dose-dependent manner (data not shown). In these experiments, lipid exchange was mediated by either 0.75 mg of partially purified supernatant protein/mL or 20 μg of purified protein/mL. Both cholesterol and sitosterol exchange were totally blocked at about 1 and 6 mg of IgG/mL, respectively, depending on whether the exchange was mediated by the purified antigen or the partially purified supernatant proteins (data not shown).

Sterol Absorption by Caco-2 Cells. The kinetics of cholesterol and sitosterol removal from mixed taurocholate micelles in the presence of Caco-2 cells are compared in panels A and B of Figure 5. As shown in Figure 5A, with bile salt micelles containing small quantities of sterol (4 μM = 0.07 mol %), no difference in the kinetics of the two sterols was detected within experimental error. This result was contrasted by the kinetics of sterol removal from bile salt micelles containing 100 μM (~2 mol %); there was a clear-cut difference between the two sterols (Figure 5B). Under

Table 4: Sitosterol and Cholesterol Exchange between Two Populations of Small Unilamellar Phospholipid Vesicles at 23 °C as a Function of the Concentration of Supernatant Proteins Added to the System[a]

	donor	acceptor		kinetics of absorption		
lipid	lipid pool (mg/mL)	supernatant proteins (mg/mL)	lipid pool (mg/mL)	kinetics	k_1 (h^{-1})	half-time $t_{1/2}$ (h)
sitosterol	1	1.8	5	monophasic	1.3 ± 0.4	0.44 ± 0.1
sitosterol	1	0.7	5	monophasic	0.3 ± 0.1	1.9 ± 0.7
sitosterol	1	0.5	5	monophasic	0.14 ± 0.02	4 ± 1
sitosterol	1	0.15	5	monophasic	0.08 ± 0.01	7 ± 1
sitosterol	1	0.05	5	monophasic	0.05 ± 0.02	12 ± 4
sitosterol	1	proteinase K-treated	5	monophasic	0.03 ± 0.01	20 ± 4
sitosterol	1	0	5	monophasic	0.03 ± 0.01	17 ± 2
cholesterol	1	0.7	5	monophasic	0.30 ± 0.06	1.9 ± 0.4
cholesterol	1	proteinase K-treated	5	monophasic	0.05 ± 0.01	11 ± 2
cholesterol	1	0	5	monophasic	0.06 ± 0.01	11 ± 1

[a] The experimental conditions were described in the legend to Figure 4.

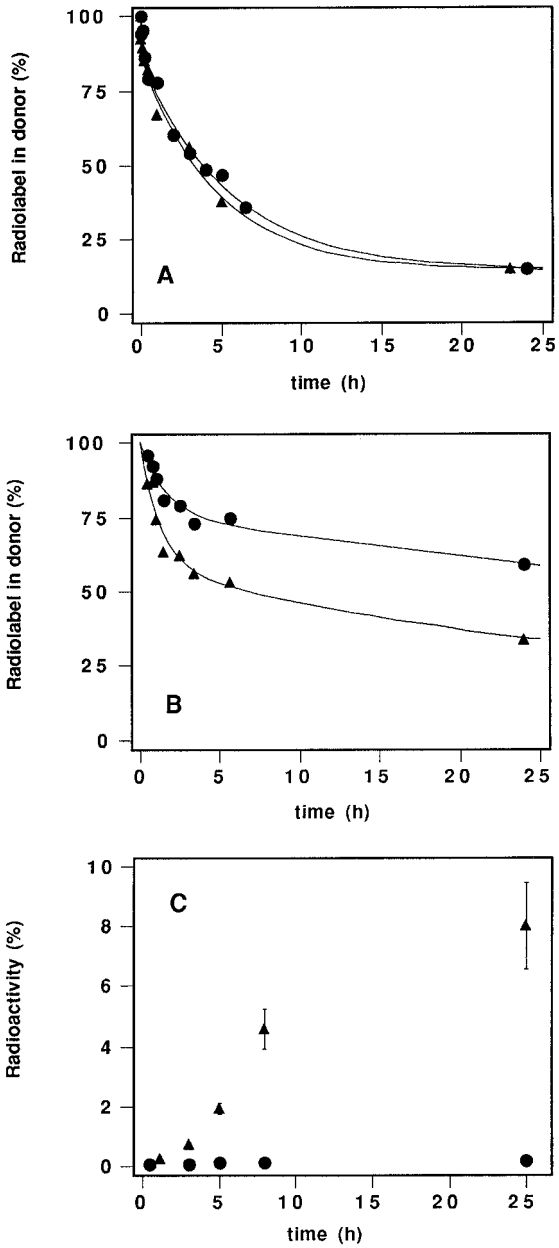

FIGURE 5: Kinetics of sterol absorption by Caco-2 cells. (A) Sitosterol (circles) and cholesterol (triangles) removal from mixed sodium taurocholate micelles (5 mM) containing 0.6 mM sodium oleate and 4 μM (0.07 mol %) sterol was measured in the presence of Caco-2 cells at 37 °C. The radioactivity (percent) remaining in the donor was determined as a function of time as described in Methods. (B) The kinetics of sitosterol (circles) and cholesterol (triangles) removal from mixed taurocholate micelles were measured as described in part A except that the mixed taurocholate micelles contained 100 μM sterol (~2 mol %). The solid lines in panels A and B represent curve fittings produced by using the sum of two exponential functions. (C) Secretion of sterols (cholesterol, triangles; β-sitosterol, circles) into the basolateral compartment. The radioactivity that is secreted by the Caco-2 cells into the basolateral compartment is expressed as the percentage of the activity applied to the apical compartment.

these conditions, approximately twice as much cholesterol was taken up by Caco-2 cells and this difference was observed over the total time course of the experiment.

The kinetic curves in panels A and B of Figure 5 were fitted best by the sum of two exponentials. Fitting of both curves shown in Figure 5A indicated that there is a fast initial phase accounting for ~20% of sterol removal characterized by a pseudo-first-order rate constant k_1 of 8.2 ± 0.5 h^{-1} ($t_{1/2}$ = 5.0 min) and a main second phase amounting to ~80% of sterol removal with a k'_1 of 0.28 ± 0.03 h^{-1} ($t_{1/2}$ = 2.5 ± 0.3 h). No clear-cut result could be derived from curve fitting of the data in Figure 5B, because the values of the final equilibrium were unknown.

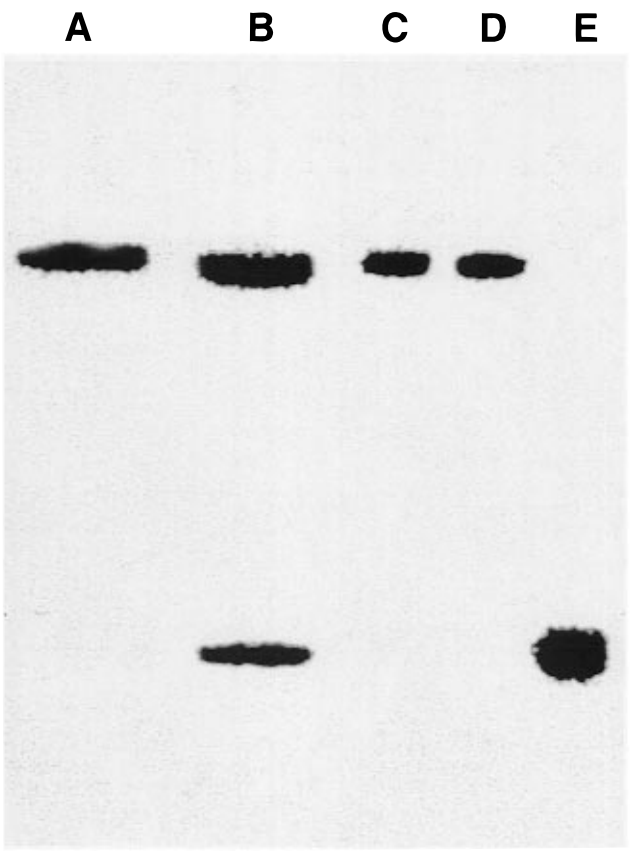

FIGURE 6: Thin-layer chromatograms of total lipids extracted from Caco-2 cells after incubation with mixed sodium taurocholate micelles containing 0.6 mM sodium oleate and 0.1 mM radiolabeled cholesterol or sitosterol. The lipid extract was applied as a 1.5 cm wide band to silica gel 60 F254 plates (20 × 20 cm) and the chromatogram developed with cyclohexane/diethyl ether/acetic acid (50:50:1 by volume). Radiolabeled lipids were made visible by storage phosphor imaging. Total lipid extracts from cells incubated with mixed taurocholate micelles containing either [^{14}C]sitosterol or [^{14}C]cholesterol were applied to lanes A and B, respectively. For comparison, chromatograms of [^{14}C]cholesterol, [^{14}C]sitosterol, and [^{14}C]cholesteryl oleate are included in lanes C−E, respectively.

Figure 5C illustrates the fact that a significant proportion of the radiolabeled cholesterol applied to the apical compartment is secreted from the Caco-2 cells into the basolateral compartment while less than 0.5% of the radiolabeled sitosterol appeared in this compartment. The quantity of radiolabeled sitosterol in the basolateral compartment was similar to that of [^{3}H]polyethylene glycol used to monitor the permeability and integrity of the Caco-2 cell monolayer. At the end of the absorption experiment, total lipids were extracted from Caco-2 cells and analyzed by thin-layer chromatography. The lipid extract of cells incubated with [^{14}C]sitosterol in the donor micelles contained only free sitosterol, while that from cells incubated with [^{14}C]cholesterol contained mainly free cholesterol but also 5−15% cholesteryl ester (Figure 6).

DISCUSSION

The capacity of the small-intestinal epithelium cells to discriminate between cholesterol and plant sterols (phy-

tosterols) is well-documented in the literature (Bruckdorfer et al., 1968; Borgström, 1969; Sylvén & Borgström, 1969; Battacharyya & Lopez, 1979; Hassan & Rampone, 1979; Battacharyya, 1981; Vahouny et al., 1981; Child & Kuksis, 1983; Ikeda et al., 1983, 1988a,b). This is true not only for plant sterols but also for some shellfish sterols (Connor & Lin, 1981).

We are presenting a comparative study of the uptake (absorption) of cholesterol and sitosterol using mixed bile salt micelles and egg PC SUV as donor particles and BBMV and Caco-2 cells as the acceptor. β-Sitosterol (24β-ethyl-Δ^5-cholesten-3β-ol) is employed here as a representative of the class of plant sterols. There are several lines of evidence indicating that the additional ethyl group on C atom 24 of the side chain makes sitosterol more hydrophobic than cholesterol. (I) The passive exchange of sitosterol between egg PC SUV and proteolytically treated BBMV is about 4−6 times slower than that of cholesterol (Table 2). (II) The rate of passive exchange of sitosterol between two populations of SUV is slower than that of cholesterol by a factor of ∼2 (Table 4). (III) The octanol/water partition coefficients were determined to be 2×10^3 and 6.3×10^4 for cholesterol and sitosterol, respectively. The clear preference of sitosterol for the organic (octanol) phase is yet another manifestation of the greater hydrophobicity of sitosterol.

The interpretation of the system egg PC SUV and BBMV is straightforward; no discrimination whatsoever was observed in the uptake properties of the two sterols. The interpretation of the sterol uptake from mixed bile salt micelles with different compositions is more complex. No discrimination between the two sterols was detectable for taurocholate micelles containing 1 μM sterol (0.02 mol %) (Figure 1A and Table 1). With taurocholate micelles containing egg PC, the amount of cholesterol taken up was about twice that of sitosterol uptake, provided that the concentration of BBMV was sufficiently low (Table 1). As shown in Table 1, the difference in the uptake of the two sterols disappeared as the BBMV concentration was raised. The actual difference depended on experimental conditions. This is true for both acceptors used here, BBMV (Figure 1C and Table 1) and Caco-2 cells (Figure 5A,B). Critical conditions are the sterol content in the donor, the acceptor membrane concentration, and, related to these, the ratio of substrate (sterol) to acceptor. High sterol concentrations in the donor, low acceptor concentrations, and therefore high ratios of sterol to acceptor have all the same effect of decreasing the rate of sterol uptake. The presence of egg PC in mixed bile salt micelles has the same effect (cf. Table 1). Under all these conditions, there is a discrimination in cholesterol and sitosterol uptake at the level of the BBM amounting at best to a factor of ∼2 in favor of cholesterol. Our results are consistent with published data, although some authors reported larger differences in the rates of uptake (absorption) of the two sterols (Sylvén & Nordström, 1970; Child & Kuksis, 1982, 1983; Ikeda et al., 1988a). For instance, when rats were given sodium taurodeoxycholate micelles (20 mM) containing monooleoylglycerol (20 mM) and trace quantities of either radiolabeled cholesterol or sitosterol, the weight ratio of cholesterol and sitosterol absorbed along the proximal part of the small intestine was 2.5 (Sylvén & Nordström, 1970). In comparing our results with published data, we have to bear in mind the fact that most of the uptake measurements with BBMV were carried out at room temperature. The main reason for this is that the kinetics of sterol transfer at physiological temperature were often too fast to be measurable. This is, for instance, true for the kinetics of sterol transfer from mixed bile salt micelles. Another reason for performing kinetic measurements at 23 °C rather than at 37 °C was the limited stability of BBMV at 37 °C. The one series of uptake measurements carried out at 37 °C supports the main conclusion derived from measurements at room temperature.

Caco-2 cells are a useful model for studying intestinal transport processes. They have also been used to study lipid absorption, although they exhibit some differences to enterocytes. Caco-2 cells lack the intestinal fatty acid binding protein; the apolipoprotein B-48/apolipoprotein B-100 resembles that of intestinal explants, and triacylglycerols are produced via the phosphatidic acid pathway [reviewed by Levy et al. (1995)]. All these known differences may lead to discrepancies between lipid absorption results obtained by in vivo studies and Caco-2 cells. To the best of our knowledge, no difference in the uptake behavior of lipids has been reported for in vivo models and Caco-2 cells. Qualitatively, a similar trend as with BBMV was observed with Caco-2 cells. In this case, the number of cells per well and hence the acceptor concentration are approximately constant, the only variable being the sterol content of the mixed bile salt micelles. At low sterol content in the donor (4 μM or 0.07 mol %), no discrimination between the two sterols was observed (Figure 5A). However, there was a clear difference in the kinetics when bile salt micelles containing 100 μM (∼2 mol %) sterol were used (Figure 5B). In the latter case, the amount of cholesterol taken up was on the average twice that of sitosterol. This result is in good qualitative agreement with those obtained with BBMV (cf. Figure 1C and Table 1). Taking then the uptake experiments on BBMV together with the measurements on Caco-2 cells, we can conclude that there is some discrimination in sterol uptake at the level of the BBM, depending on the experimental conditions, particularly the relative concentration of donor and acceptor. Presently, it is unknown if and to what extent differences in the physicochemical properties, e.g. differences in the solubilities in bile salt micelles, of the two sterols contribute to this discrimination (Armstrong & Carey, 1987).

However, this effect cannot account for the marked discrimination observed in the absorption of the two sterols by the small-intestinal epithelium. As mentioned before, the two sterols differ in their absorption behavior by a factor of at least 10 or more. The main part of this discrimination must therefore arise from differences in the intracellular processing of the two lipids. Evidence to this effect is presented in Figures 5 and 6. Thin-layer chromatography of the total lipid extract of Caco-2 cells after incubation with mixed bile salt micelles containing radiolabeled sitosterol shows that sitosterol is present in Caco-2 cells in its free form. No esterification of the plant sterol could be detected. Furthermore, practically no sitosterol was exported from the Caco-2 cells into the basolateral compartment (Figure 5C). In contrast, 5−15% of the radiolabeled cholesterol taken up by the Caco-2 cells were esterified (Figure 6), indicating that cholesterol is at least partially processed within the cell. The reason for the relatively low rate of esterification of cholesterol in Caco-2 cells is unknown, and this phenomenon is presently studied in our laboratory. Furthermore, 8−10%

of the radiolabeled cholesterol administered to Caco-2 cells were secreted from the cells (Figure 5C). The data obtained with Caco-2 cells suggest that the major discrimination observed in the absorption of cholesterol and sitosterol by the epithelium occurs at the level of sterol esterification and sterol secretion. In vivo studies have provided evidence for limited esterification of absorbed plant sterols compared to cholesterol that was esterified to 70−90% (Kuksis & Huang, 1962; Ikeda et al., 1988a). Furthermore, though both pancreatic cholesterol esterase (Swell et al., 1954) and intestinal acyl-CoA:cholesterol acyltransferase (Field & Mathur, 1983) have been shown to esterify some plant sterols in vitro, the rates of esterification are much lower for plant sterols than for cholesterol.

By and large, similar uptake kinetics are observed for both cholesterol and sitosterol. Therefore, it is likely that for the uptake of plant sterols the same mechanism holds (Mütsch et al., 1986) as for cholesterol uptake. The uptake of sitosterol probably involves the energy-independent, protein-facilitated transfer of sitosterol from the donor to the acceptor via collisional contact. The data presented in Figure 1A rule out the possibility that fusion of donor and acceptor contributes significantly to lipid uptake. The observation that under most conditions tested in this work identical or similar uptake kinetics are observed for both sterols is surprising in the light of the physicochemical differences between the two sterols. We interpret this to mean that proteins are at play because the two compounds differ significantly in their passive lipid transfer between two populations of SUV on the one hand and SUV and proteolytically treated BBMV on the other (cf. Tables 2 and 4). This argument is corroborated by the data in Figure 4 showing that lipid exchange proteins liberated from BBMV are unable to discriminate between the two sterols. Furthermore, the nonspecific lipid transfer protein (SCP2) was shown to mediate the exchange of both cholesterol and sitosterol between two populations of SUV with identical rate constants (Billheimer & Gaylor, 1990).

As shown in Figure 3, increasing the sterol content from 1 to 30 mol % leads to a significant reduction in the rate of sterol uptake by BBMV as is evident from an almost doubling of the half-time (Table 3). This observation could be rationalized in terms of a specific interaction between the sterol and the phospholipid possibly leading to domain formation (clustering) within the lipid bilayer and in turn to reduced off rates of the sterol (Nemecz & Schroeder, 1988; Nemecz et al., 1988). An alternative explanation of the data in Figure 3 is that the BBM protein(s) responsible for the facilitated sterol uptake becomes saturated at high sterol contents of the phospholipid bilayer. Increasing the sterol content of the donor to ∼10 mol % had no effect on the kinetic parameters (Table 2); only at sterol contents in the lipid bilayer higher than ∼20 mol % did the pseudo-first-order rate constants k_1 drop significantly (Table 3). A systematic study of the effect of increasing sterol content on the uptake kinetics, i.e. on the pseudo-first-order rate constants k_1, is hampered by the following problems. With bile salt micelles, the solubility of sterols is limited to a few mole percent (Small et al., 1966a,b; Carey & Small, 1978; Sömjen et al., 1995), so only a narrow concentration range can be tested. With egg PC SUV as the donor, increasing sterol concentrations in the lipid bilayer are known to decrease the bilayer fluidity or increase the microviscosity of the bilayer. Therefore, changes in k_1 values observed with increasing sterol content cannot be assigned. They may be due to changes in the physicochemical properties of the phospholipid bilayer or to competition of the two sterols or both. Increasing the cholesterol content of egg PC SUV from 1 to 40 mol % produced a decrease in the pseudo-first-order rate constant k_1 for passive cholesterol exchange between two populations of SUV by a factor of 2 (McLean & Phillips, 1982). It is unknown whether and to what extent this kind of physicochemical change in the bilayer fluidity affects the protein-mediated sterol uptake into the BBM. On the other hand, at high sterol contents of phospholipid bilayers, saturation of the protein(s) involved in lipid uptake and in turn competition between the two sterols is expected to occur which will manifest itself in diminishing k_1 values. Presently, we are unable to decide to what extent the two mechanisms contribute. At low sterol contents of a few mole percent, there is practically no competition between cholesterol and sitosterol. This result is consistent with published data [see, for instance, Sylvén and Borgström (1969)]. It implies that under these conditions the interaction of the sterols with any putative protein(s) involved in sterol uptake must be far from saturation. Our data with egg PC SUV as the donor indicate that large sitosterol contents in the donor are required before possible inhibitory effects on cholesterol uptake are detectable (cf. Table 3). This observation is in line with the fact that large quantities of sitosterol and plant sterols in general in the diet are necessary to reduce cholesterol absorption significantly and produce a hypocholesterolemic effect (Pollak, 1953; Grundy et al., 1969; Lees et al., 1977; Shidoji et al., 1980; Becker et al., 1993).

ACKNOWLEDGMENT

We thank Prof. Dr. H. P. Merkle of the Department of Pharmacy of the Swiss Federal Institute of Technology (ETH) Zurich for providing the Caco-2 cells.

REFERENCES

Armstrong, M. J., & Carey, M. C. (1987) *J. Lipid Res. 28*, 1144−1155.
Becker, M., Staab, D., & von Bergmann, K. (1993) *J. Pediatr. 122*, 292−296.
Bhattacharyya, A. K. (1981) *Am. J. Physiol. 240*, G50−G55.
Bhattacharyya, A. K., & Lopez, L. A. (1979) *Biochim. Biophys. Acta 574*, 146−153.
Billheimer, J. T., & Gaylor, J. L. (1990) *Biochim. Biophys. Acta 1046*, 136−143.
Borgström, B. (1969) *J. Lipid Res. 9*, 473−481.
Bruckdorfer, K. K., Graham, J. M., & Green, C. (1968) *Eur. J. Biochem. 4*, 512−518.
Carey, M. C., & Small, D. M. (1978) *J. Clin. Invest. 61*, 998−1026.
Child, P., & Kuksis, A. (1982) *Lipids 17*, 748−757.
Child, P., & Kuksis, A. (1983) *J. Lipid Res. 24*, 552−565.
Cohnen, E., Flasch, H., Heinz, N., & Hempelman, F. W. (1978) *Drug Res. 28*, 2179−2182.
Compassi, S., Werder, M., Boffelli, D., Weber, F. E., Hauser, H., & Schulthess, G. (1995) *Biochemistry 34*, 16473−16482.
Connor, W. E., & Lin, D. S. (1981) *Gastroenterology 81*, 276−284.
Crouse, J. R., & Grundy, S. M. (1971) *J. Lipid Res. 19*, 967−971.
Field, F. J., & Mathur, S. N. (1983) *J. Lipid Res. 24*, 409−417.
Gee, A. P., Borsos, T., & Boyle, M. D. P. (1979) *J. Immunol. Methods 30*, 119−126.
Grundy, S. M., Ahrens, E. H., & Davignon, J. (1969) *J. Lipid Res. 10*, 304−315.

Hassan, A. S., & Rampone, A. J. (1979) *J. Lipid Res. 20*, 646−653.
Hauser, H., Howell, K., Dawson, R. M. C., & Bowyer, D. E. (1980) *Biochim. Biophys. Acta 602*, 567−577.
Ikeda, I., Kawasaki, A., Samezima, K., & Sugano, M. (1983) *Biochim. Biophys. Acta 732*, 651−658.
Ikeda, I., Tanaka, K., Sugano, M., Vahouny, G. V., & Gallo, L. L. (1988a) *J. Lipid Res. 29*, 1583−1591.
Ikeda, I., Tanaka, K., Sugano, M., Vahouny, G. V., & Gallo, L. L. (1988b) *J. Lipid Res. 29*, 1573−1582.
Kan, C. Ch., & Bittman, R. (1990) *J. Am. Chem. Soc. 112*, 884−886.
Kan, C. Ch., & Bittman, R. (1991) *J. Am. Chem. Soc. 113*, 6650−6656.
Kuksis, A., & Huang, T. C. (1962) *Can. J. Biochem. Physiol. 40*, 1493−1504.
Lees, A. M., Mok, H. Y. I., Lees, R. S., McCluskey, M. A., & Grundy, S. M. (1977) *Atherosclerosis 28*, 325−338.
Lepetit, G. (1977) *Pharmazie 32*, 289−291.
Levy, E., Mehran, M., & Seidman, E. (1995) *FASEB J. 9*, 626−635.
McLean, L. R., & Phillips, M. C. (1981) *Biochemistry 20*, 2893−2900.
McLean, L. R., & Phillips, M. C. (1982) *Biochemistry 21*, 4053−4059.
Mütsch, B., Gains, N., & Hauser, H. (1986) *Biochemistry 25*, 2134−2140.
Nemecz, G., & Schroeder, F. (1988) *Biochemistry 27*, 7740−7749.
Nemecz, G., Fontaine, R. N., & Schroeder, F. (1988) *Biochim. Biophys. Acta 943*, 511−521.
Pollak, O. J. (1953) *Circulation 7*, 702−706.
Pollak, O. J., & Kritchevsky, D. (1980) in *Monographs on Atherosclerosis* (Clarkson, T. B., Kritchevsky, D., & Pollak, O., Eds.) pp 1−215, Karger, Basel.
Ranheim, T., Gedde-Dahl, A., Rustan, A., & Drevon, C. A. (1994a) *Biochem. J. 363*, 155−161.
Ranheim, T., Gedde-Dahl, A., Rustan, A., & Drevon, C. A. (1994b) *Biochim. Biophys. Acta 1212*, 295−304.
Saad, H. Y., & Higuchi, W. I. (1965) *J. Pharm. Sci. 54*, 1205−1206.
Schulthess, G., Lipka, G., Compassi, S., Boffelli, D., Weber, F. E., Paltauf, F., & Hauser H. (1994) *Biochemistry 33*, 4500−4508.
Schulthess, G., Compassi, S., Boffelli, D., Werder, M., Weber, F. E., & Hauser, H. (1996) *J. Lipid Res. 37*, 2405−2419.
Shidoji, Y., Watanabe, M., Oku, T., Muto, Y., & Hosoya, N. (1980) *J. Nutr. Sci. Vitaminol. 26*, 183−188.
Small, D. M., Bourges, M., & Dervichian, D. G. (1966a) *Nature 211*, 816−818.
Small, D. M., Bourges, M., & Dervichian, D. G. (1966b) *Biochim. Biophys. Acta 125*, 563−580.
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.
Sömjen, G. L., Lipka, G., Schulthess, G., Koch, M. H. J., Wachtel, E., Gilat, T., & Hauser, H. (1995) *Biophys. J. 68*, 2342−2349.
Subbiah, M. T. R. (1973) *Am. J. Clin. Nutr. 26*, 219−225.
Swell, L., Field, H., Jr., & Treadwell, C. R. (1954) *Proc. Soc. Exp. Biol. Med. 87*, 216−218.
Sylvén, C., & Borgström, B. (1969) *J. Lipid Res. 10*, 179−182.
Sylvén, C., & Nordström, C. (1970) *Scand. J. Gastroenterol. 5*, 57−63.
Thurnhofer, H., & Hauser, H. (1990a) *Biochemistry 29*, 2142−2148.
Thurnhofer, H., & Hauser, H. (1990b) *Biochim. Biophys. Acta 1024*, 249−262.
Thurnhofer, H., Schnabel, J., Betz, M., Lipka, G., Pidgeon, C., & Hauser, H. (1991) *Biochim. Biophys. Acta 1064*, 275−286.
Trotter, P. J., Ying Ho, S., & Storch, J. (1996) *J. Lipid Res. 37*, 336−346.
Vahouny, G. V., & Kritchevsky, D. (1981) in *Nutritional Pharmacology* (Spiller, G., Ed.) pp 32−72, Alan R. Liss, New York.
Vahouny, G. V., Connor, W. E., Roy, T., Lin, D. S., & Gallo, L. L. (1981) *Am. J. Clin. Nutr. 34*, 507−513.

BI9620666