A comparative study of sterol absorption in different small-intestinal brush border membrane models

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Abstract We reported previously that the absorption of cholesterol and long-chain cholesteryl esters by rabbit small-intestinal brush border membranes (BBMV) is protein-mediated (Thurnhofer, H., and H. Hauser. 1990. *Biochemistry.* **29:** 2142–2148; Compassi, S., M. Werder, D. Boffelli, F. E. Weber, H. Hauser, and G. Schulthess. 1995. *Biochemistry.* **34:** 16473–16482). Evidence is presented for similar cholesterol transport activities in rabbit, pig, and human BBMV. As BBMV are subject to a number of limitations and the influence of these on sterol absorption is unknown, it is desirable to verify results obtained with this model system in other brush border membrane models more closely related to the in vivo situation. Sterol absorption in intact enterocytes parallels the absorption measured in BBMV, provided that both model systems are normalized to equal sucrase activity. The parallel behavior of the two brush border membrane models lends support to our previous conclusion that the brush border membrane takes up free and esterified cholesterol in a facilitated and energy-independent process. The absorption of sterols in small-intestinal segments mounted in the Ussing chamber is shown to be a complex process in which the diffusion of the bile salt micelles to the brush border membrane is rate-limiting. All brush border membrane models share the disadvantage of being unstable and subject to degradation. The seriousness of the problem increases apparently with the complexity of the model, i.e., in the order BBMV → enterocytes → intestinal segments. One main conclusion of this study is that no brush border membrane model is sufficient and satisfactory, therefore conclusive work in lipid absorption can never be based on a single brush border membrane model.—**Schulthess, G., S. Compassi, D. Boffelli, M. Werder, F. E. Weber, and H. Hauser.** A comparative study of sterol absorption in different small-intestinal brush border membrane models. *J. Lipid Res.* 1996. **37:** 2405–2419.

Supplementary key words cholesterol absorption • cholesteryl ester absorption • brush border membrane vesicles • intact enterocytes • Ussing chamber

Fats and products of fat hydrolysis are dispersed in the lumen of the small intestine and transported in this form to the site of absorption (3, 4). Mixed bile salt micelles and small unilamellar vesicles (SUV) are supposed to be the natural carriers from which the lipids are absorbed (5). In the absorption process the lipids are presumably incorporated in the external lipid monolayer of the brush border membrane (BBM). Subsequently they diffuse across the BBM and are eventually released into the cytosol of the enterocyte. There the lipids are processed by the cell's internal machinery which is characterized by a highly developed metabolic capacity. For example, cholesterol is esterified by microsomal acylcoenzyme A:cholesterol acyltransferase (ACAT) and cytosolic cholesterol esterase and eventually incorporated in lipoprotein particles.

We reported that the absorption of cholesterol and long-chain cholesteryl esters by small-intestinal BBMV is protein-mediated. This is true for both mixed bile salt micelles and phospholipid unilamellar vesicles used as donor particles (1, 2, 6). The drawbacks of BBMV prepared from fresh or frozen small intestines are well known and have been discussed in a number of review articles (e.g., ref. 7). Due to the inherent instability of the BBMV, results obtained with this model should be treated cautiously. In light of this fact it is desirable to verify results obtained with BBMV in other BBM models more closely related to the in vivo situation. To this end we have undertaken a comparative study of cholesterol and cholesteryl ester absorption using BBMV, intact enterocytes, and small-intestinal segments mounted in the Ussing chamber. These models have been used by many researchers in the past, but usually in isolation rather than in combination. Results obtained with these differ-

Abbreviations: BBM, brush border membrane(s); BBMV, brush border membrane vesicle(s); EDTA, disodium salt of ethylenediaminetetraacetic acid; HEPES, N-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid; PC, phosphatidylcholine; SUV, small unilammelar vesicle(s).

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ent models will be compared and the usefulness and drawbacks of these models as to the study of lipid absorption will be discussed. It is encouraging and reassuring that cholesterol and cholesteryl ester absorption in intact enterocytes parallels the absorption measured in BBMV. The absorption in small-intestinal segments is shown to be a complex process in which the diffusion of bile salt micelles to the BBM is rate-limiting.

MATERIALS AND METHODS

Materials

Cholesterol, D-glucose, Glucotest 12193, and Merckotest 3359 were purchased from Merck (Darmstadt, Germany), egg PC from Lipid Products (South Nutfield, Surrey, U.K.), the sodium salt of taurocholate, 1-*cis*-9-octa-decenoyl-*sn*-glycerol, the sodium salt of oleic acid, the trypan blue solution (0.4%), hyaluronidase from bovine testes, and soybean trypsin inhibitor (both enzymes type I-S) from Sigma (St. Louis, MO), proteinase K from tritirachium album and papain from papaya latex from Boehringer (Mannheim, Germany), [1,2-^{3}H(N)]cholesterol (53.3 Ci/mmol) and [1,2,6,7-^{3}H(N)]cholesteryl oleate (78 Ci/mmol) from DuPont-NEN Products (Boston, MA), phenylmethylsulfonyl fluoride, 2,3-dihydroxy-1,4-dithiolbutane, maleic acid, and sucrose from Fluka (Buchs, Switzerland), 3-doxyl-5α-cholestane from Aldrich (Steinheim, Germany), DMEM buffer from Gibco BRL (Basel, Switzerland), the sodium salt of tauro[carbonyl-^{14}C]cholic acid, inulin (^{14}C)carboxylic acid (approximate molecular weight 5200), D-[6-^{3}H]glucose, [1,2-^{3}H(N)]cholesteryl oleyl ether (37 Ci/mmol), and NCS^R tissue solubilizer from Amersham International, U.K. All lipids used in this work were pure as judged by thin-layer chromatography standard.

Preparation of mixed bile salt micelles and egg PC SUV

Mixed bile salt micelles and egg PC SUV were prepared by dissolving the lipid constituents in $CHCl_3$–CH_3OH 2:1 (by volume) and evaporating the solvent by rotary evaporation. The resulting film of mixed lipids was dried in vacuo and the dry residue was dispersed in buffer by vortexing for 2 min at room temperature. Dispersions of small unilamellar phospholipid vesicles were made by subjecting 2 ml of the lipid dispersion to tip sonification.

Preparation and characterization of BBMV

BBMV were prepared according to Hauser et al. (8) using either 1.5 m of proximal rabbit small intestine (comprising duodenum and jejunum) or 3 m of upper pig jejunum. BBMV were dispersed in 0.01 M HEPES, pH 7.2, 0.15 M NaCl, 5 mM EDTA, 0.02% NaN_3. The specific sucrase activity and leucine aminopeptidase activity of BBMV were determined using Glucotest 12193 and Merckotest 3359, respectively. Digestion of BBMV with papain or proteinase K was carried out as described before (3, 9). The size and size distribution of BBMV was monitored by quasi-elastic light scattering (10). Human BBMV were prepared as described by Lipka et al. (11). CaCo-2 cells were grown on Optilux petri dishes (Falcon Laboratories, Oxnard, CA) and BBMV from CaCo-2 cells were prepared according to Stieger et al. (12).

Preparation and characterization of intact enterocytes isolated from pig jejunum

Preparation. Intact enterocytes were prepared routinely from pig jejunum as described previously (9) following essentially published procedures (13–16). Briefly, a 7-cm-long piece of upper jejunum obtained from pig at the slaughterhouse in Zurich was incubated in buffer A (0.01 M HEPES, pH 7.4, 0.14 M NaCl, 5.9 mM KCl, 1.2 mM Na_2SO_4, 2.4 mM Na_2HPO_4) containing 1 mg hyaluronidase/ml and 0.13 mg trypsin inhibitor/ml at 35°C for 20 min under continuous gassing with pure oxygen (9). We found it easier to prepare enterocytes from pig rather than rabbit jejunum, and the resulting cells turned out to be more stable than rabbit enterocytes.

Integrity and viability of intact enterocytes. The integrity of isolated enterocytes was assessed by light microscopy using a 0.4% solution of trypan blue according to the supplier's instruction. By this dye exclusion test the number of intact cells was determined before and after the sterol absorption measurements. The effect of bile salts on the integrity was determined by incubating enterocytes suspended in buffer A at 23°C with mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol). After timed intervals aliquots of the suspension were subjected to the trypan blue exclusion test. Further aliquots were used to pellet enterocytes by centrifugation at 12000 *g* for 5 s and to determine the amount of protein in the supernatant and the pellet. The viability of intact enterocytes was assessed by measuring D-[6-^{3}H]glucose uptake (9).

Determination of the total cell volume of suspended enterocytes. A trace amount of inulin [^{14}C]carboxylic acid was dissolved in buffer A and the radioactivity per unit volume R_1 of this solution was determined. Aliquots of the inulin solution were mixed with equal volumes of the pig enterocyte suspension so that the enterocyte concentration in the total volume V_T varied between 1 and

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12 mg cell protein/ml. After 30 s of incubation the enterocytes were pelleted by centrifugation at 12000 *g* for 5 s and the radioactivity per unit volume in the supernatant R_2 was determined. The cell volume V_E occupied by the enterocytes in the total volume V_T of the suspension of enterocytes is

$$V_E = V_T [(2R_2 - R_1)/2R_2]$$

The cell volume V_E was determined at 23°C for two populations of pig enterocytes that differed in the trypan blue exclusion test yielding 90% and 70% of intact cells. The number of enterocytes per unit volume was determined in an improved Neubauer chamber, and the cell count was normalized to the protein concentration. Contaminant cells amounting to less than 20% were identified by light microscopy as mainly lymphocytes.

Absorption measurements using BBMV and enterocytes

Sterol absorption by BBMV from various donor particles was measured as described in previous publications from this laboratory (1, 2, 6, 9). For the uptake of sodium taurocholate by pig enterocytes, cells suspended in buffer A to 12.1 mg protein/ml were incubated at 23°C with either a monomeric solution or a micellar dispersion of sodium taurocholate. The monomeric solution was 5 μM and the micellar dispersion was 4.8 mM radiolabeled with [^{14}C]taurocholate, containing 0.3 mM 1-monooleoyl-*sn*-glycerol and 0.1 mM cholesterol. In the same series of experiments micelles of this composition but containing additionally 0.1 mM or 0.5 mM egg PC were used. After timed periods of incubation the enterocytes were spun down at 12000 *g* for 5 s, solubilized in 1% SDS, and radioactivity and protein contents of the resulting solution were determined. Enterocytes of the same suspension were mechanically damaged by homogenization with about 10 strokes in a Teflon–glass homogenizer prior to the determination of taurocholate absorption. After this treatment more than 95% of the cells were susceptible to trypan blue staining. Such a mechanically damaged cell suspension served as a reference. The absorption of free and esterified cholesterol by intact enterocytes was measured at 23°C. Mixed bile salt micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol and 0.1 mM cholesterol labeled with [^{3}H]cholesterol or 3.5 μM [^{3}H]cholesteryl oleyl ether) were used as the donor. After timed intervals bile salt micelles and enterocytes were separated by centrifugation at 12000 *g* for 5 s and the radioactivity remaining in the donor was determined. The following centrifugation conditions were tested: 100 *g* for 2 min, 400 *g* for 2 min, 900 *g* for 5 min, 1400 *g* for 10 min, 5900 *g* for 15 min, 12000 *g* for 5 s and 2 min, 115000 *g* for 10 min. Centrifugation at 12000 *g* for 5 s or 2 min produced the same result within experimental error as centrifugation at 100 *g* for 2 min. Only after centrifugation at 115000 *g* for 10 min, i.e., at centrifugal fields ten times larger than the one routinely used here (12000 *g* for 5 s), did the radioactivity in the donor drop significantly below the average level obtained under standard conditions. This result was interpreted to indicate that by and large whole cells and not cell debris interacted with bile salt micelles.

Absorption measurements with segments of rabbit jejunum

Lipid absorption measurements on intestinal segments were carried out in a modified Ussing chamber (Fig. 8). Nonfasting albino rabbits were killed in our laboratory and a 10- to 20-cm-long piece of proximal jejunum was immediately excised, opened along the mesenteric border, and rinsed with buffer A. Such fast processing was not possible with pig small intestine and therefore these experiments were carried out with rabbit small intestine. The tissue was stored in ice-cold buffer A for 10 to 20 min during further preparation and prior to its use. A circular piece of a diameter of ~1.5 cm was cut from the intestine, and the serosa was removed from the intestinal piece using a fine pair of scissors and tweezers. The intestinal piece was mounted in the Ussing chamber such that the BBM faced the mucosal compartment. Both the mucosal and the serosal compartment were accessible from the outside as shown in Fig. 8. The BBM was exposed to 0.6 ml of a dispersion of mixed taurocholate micelles in buffer A labeled with trace amounts of [^{3}H]cholesterol and inulin [^{14}C]carboxylic acid at 23°C. The content of the mucosal compartment was continuously gassed with pure oxygen and thoroughly mixed every 30 s by aspirating and reinjecting the micellar dispersion. The serosal compartment was filled with Dulbecco's Modified Eagle Medium (Cat. No. 41966-029, from Life Technologies) containing both Ca^{2+} and Mg^{2+}. At timed intervals the incubation was terminated by aspirating the micellar dispersion from the mucosal compartment and the intestinal segment and the mucosal compartment were rinsed with buffer A. The circular piece of the intestinal segment (about 0.8 cm^2) exposed to the micellar dispersion was cut out from the intestinal segment and dissolved in 2 ml of tissue solubilizer (NCS^R) at 45°C. Aliquots of this solution were mixed with 4 ml scintillation cocktail (Packard, Emulsifier Save™) 24 h prior to counting [^{3}H]cholesterol and inulin [^{14}C]carboxylic acid radioactivities. Tissue dry weights were determined by drying the circular pieces of an area of about 0.8 cm^2 to constant weight at 45°C. The micellar dispersion and the washings were combined and centrifuged at 12000 *g* for 2 min to pellet cells and cell debris. The pellet

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thus obtained was dissolved in 1% SDS, and radioactivities and protein content of the resulting solution were determined.

In order to relate the cholesterol absorption by enterocytes to the cholesterol absorption measured in intestinal segments, another series of experiments was conducted. At the end of the lipid absorption experiment in the Ussing chamber some mucosa (0.5–1.5 mg protein) was scraped from the circular segment that was exposed to the micellar dispersion. The scraping was dissolved in 1% SDS, and the protein content and the amount of lipid taken up by the mucosa were determined by measuring radioactivity. The cholesterol absorption measured in intestinal scrapings was corrected for adherent fluid as described in the legends to Fig. 9.

Analytical methods

Protein concentrations were determined by the bicinchoninic acid (BCA) method following the instructions of the manufacturer (Pierce, Europe) (9). Lipid phosphorus was determined according to Chen, Toribara, and Warner (17). Radioactivities were determined by counting 2–4 aliquots in a Beckman LS 7500 liquid scintillation counter. Total lipids were extracted from BBMV according to Bligh and Dyer (18), total lipid and phospholipid of BBMV were determined as described by Hauser et al. (8) and cholesterol by using the Merckotest.

RESULTS

Characterization of pig BBMV and pig enterocytes

The physico-chemical properties listed in **Table 1** were used as a quality control of BBMV. They are crucial in assessing the stability and integrity of BBMV. The instability of BBMV manifests itself in the release of proteins and the loss in enzymatic activities. Prior to lipid uptake measurements, the properties of BBMV listed in Table 1 were checked routinely. If one of these properties was significantly outside the range given in Table 1 the BBMV preparation was discarded. Most of the properties of pig small-intestinal BBMV listed in Table 1 agreed within the error of the measurement with those of rabbit small-intestinal BBMV. Since the routine procedures for preparing and characterizing BBMV were published (8) we have observed a much greater variability in the cholesterol content than given in the 1980 paper (cf. Table 1). Laser light scattering showed that pig BBMV are fairly homogeneous with respect to size with an average diameter of 170 nm and a size range of 50–500 nm, which is entirely consistent with the average vesicle size and the size range of rabbit BBMV determined by laser light scattering, freeze-fracture EM, and gel filtration on Sephracyl S-1000 (19). The properties of BBMV summarized in Table 1 were also in good agreement with published data (8, 20–25).

TABLE 1. Comparison of BBMV prepared from pig and rabbit small intestine

Physico-Chemical Properties	Rabbit BBMV	Pig BBMV
Lipid/protein weight ratio	0.65 ± 0.15	0.56 ± 0.07
Cholesterol content (μg/mg protein)	120 ± 50	100 ± 20
Phospholipid content (mg/mg protein)	0.23 ± 0.04	0.28 ± 0.05
Cholesterol/phospholipid mole ratio	0.50 ± 0.05	0.56 ± 0.10
Specific sucrase activity (U/mg protein)	1.5 ± 0.3	1.2 ± 0.2
Specific leucine aminopeptidase activity (U/mg protein)	2.6 ± 0.4	3.5 ± 0.6
Average vesicle size (nm)	170 ± 30	170 ± 30

Intact enterocytes were used in this study as another model for the BBM. In **Fig. 1** an electron micrograph is shown of an enterocyte isolated routinely from pig jejunum. Intact enterocytes were characterized and tested as to their suitability for lipid absorption measurements using the dye exclusion test and the release of protein. Enterocytes suspended in buffer A at 23°C remained impermeable to trypan blue for about 1–2 h and the release of cell protein into the supernatant in that period of time did not increase significantly on top of the control value measured within the first few minutes (**Table 2**). In the presence of mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol) enterocytes were less stable as judged by the dye exclusion test and the release of cell protein (Table 2). The amount of protein released within the first few minutes was almost doubled and increased steadily with time. From an inspection of Table 2 and **Fig. 2** (see below) it is clear that lipid absorption measurements in the presence of bile salt micelles should be limited to 10–20 min. The results in Table 2 were obtained with enterocytes at ~12 mg total cell protein/ml; with lower concentrations, e.g., at ~3.5 mg protein/ml, the barrier properties of the plasma membrane of enterocytes appeared to be seriously affected after incubation with bile salt for only 5 min (data not shown). Similar results were reported by Hoffman, Child, and Kuksis (26).

In further control experiments, enterocytes suspended in buffer A were incubated with either a 5 μM solution of sodium taurocholate or with aqueous dispersions of sodium taurocholate micelles of different composition. The time course of taurocholate uptake by enterocytes is depicted in Fig. 2. The uptake remained constant within the experimental error up to ~10 min. This uptake very likely represents the equilibration of taurocholate with the plasma membrane of enterocytes. After incubation times longer than 10 min, the tauro-

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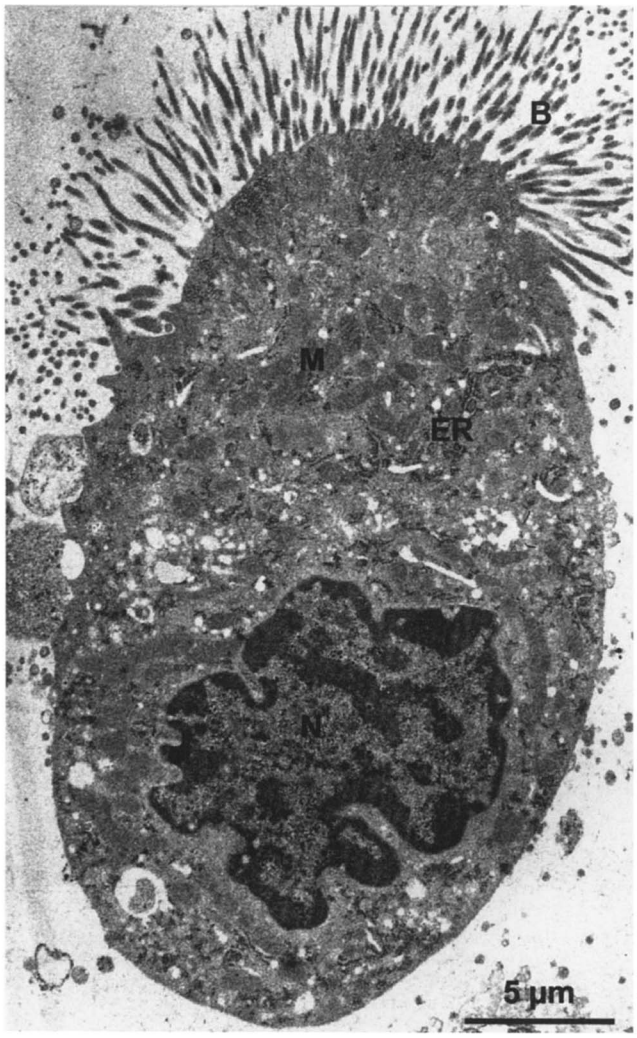

Fig. 1. Electron micrograph of a single enterocyte isolated from pig jejunum. A suspension of enterocytes in buffer A was pelleted; the pellet was fixed with paraformaldehyde (2%) and glutaraldehyde (0.5%) and embedded in Epon 812. Sections were stained with uranylacetate and lead citrate and observed with a Philips EM 301 microscope. B, brush border membrane, M, mitochondrion, ER, endoplasmic reticulum, N, nucleus.

cholate uptake increased significantly, eventually reaching a level similar to that of lysed cells. The taurocholate uptake by cells, which were deliberately damaged by mechanical agitation as described under Methods, is included on the right of Fig. 2 (labeled reference). In the trypan blue exclusion test, more than 95% of these cells were permeable to the dye. Concomitantly, the uptake of taurocholate from both monomeric solutions and mixed bile salt micelles increased by a factor of 3–5 relative to intact enterocytes (Figs. 2A and B). The level of taurocholate uptake by lysed cells in 5 min is the same within experimental error as that by intact enterocytes after incubation with mixed bile salt micelles at 23°C for 4 h or longer. As evident from Fig. 2 the taurocholate uptake from a 5 µM solution is approximately a thousand times smaller than that from mixed taurocholate (4.8 mM) micelles, implying that the critical micellar concentration of taurocholate is close to 5 mM. This observation is in good agreement with published values for the cmc of taurocholate (27–30). Figure 2B suggests that mixed taurocholate micelles (black bars) are more aggressive than the same micelles containing egg PC.

Figure 3 gives the total cell volume of two different populations of pig enterocytes that differed significantly in the trypan blue exclusion test yielding 90% (squares) and 70% (triangles) intact cells. The values of the cell volume derived from the slope of the two straight lines in Fig. 3 correlated reasonably well with the fraction of intact cells. The cell count of 2.34×10^6 cells per mg protein corresponding to about 1.9×10^6 enterocytes per mg protein was higher than the values of $1.0–1.5 \times 10^6$ enterocytes per mg protein reported previously (16, 31).

Cholesterol absorption by BBMV of different sources

Cholesterol absorption by rabbit BBMV was shown to be protein-mediated from egg PC SUV and from bile salt micelles as the donor (1, 2). Here we compare the kinetics of cholesterol absorption using BBMV of different sources. SUV of egg PC with 1 mol % [^{3}H]cholesterol were used as the donor because cholesterol absorption from SUV is considerably slower than from bile salt micelles and allows the evaluation of the kinetic curves in terms of pseudo-first-order rate constants k_1 and half times $t_{1/2}$. These kinetic parameters were determined under identical experimental conditions. The radioactivity in the donor decreased exponentially with time and the values for k_1 and $t_{1/2}$ derived from curve fitting are summarized in **Table 3.** Identical k_1 and $t_{1/2}$ values within experimental error were obtained for all kinds of BBMV. After papain treatment of BBMV the k_1 values were reduced by a factor of 4 to 5 approaching the values characteristic of cholesterol exchange between two populations of phospholipid SUV (Table 3).

The initial uptake of cholesterol of BBMV from mixed bile salt micelles is so fast that the evaluation of the kinetic data in terms of k_1 and $t_{1/2}$ values is not possible (cf. Fig. 4). However, it is possible to determine the amount of cholesterol taken up during the initial kinetic phase. These values and the rate constants of the second kinetic phase are included in Table 3. Both these values characterizing cholesterol absorption from mixed bile salt micelles were found to be similar for rabbit and pig BBMV.

Comparison of the cholesterol absorption by BBMV and intact enterocytes

The kinetics of cholesterol absorption were compared using enterocytes and BBMV as the acceptor, both prepared from pig upper jejunum. Mixed tauro-

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TABLE 2. Trypan blue dye exclusion test and protein release from pig enterocytes incubated in the absence (control) and presence of mixed taurocholate micelles

Time	Trypan Blue Dye Exclusion Test		Cell Proteins Released to the Supernatant	
	Control	Incubated with Mixed Bile Salt Micelles	Control	Incubated with Mixed Bile Salt Micelles
min	*% intact cells*		%	
0.5			4.2 ± 0.5	7.2 ± 1.2
1			4.0 ± 0.4	7.8 ± 1.5
2			4.6 ± 0.4	8.1 ± 1.1
5	86 ± 3	85 ± 7	4.6 ± 0.4	7.7 ± 0.5
10	82 ± 4	80 ± 6	4.5 ± 0.9	8.5 ± 1.6
15	82 ± 4	75 ± 4	4.6 ± 0.9	9.2 ± 1.7
20	80 ± 2	75 ± 4	4.7 ± 1.1	9.5 ± 1.4
25	80 ± 2	73 ± 4	4.6 ± 0.9	9.6 ± 1.2
30	75 ± 7	68 ± 4	4.9 ± 1.1	10.3 ± 1.4
60	80 ± 2	60 ± 7	5.3 ± 1.4	11.6 ± 1.8
120	77 ± 4	62 ± 4	5.8 ± 1.9	12.8 ± 3.4

Enterocytes isolated from pig jejunum were suspended at 12.2 mg protein/ml in buffer A at 23°C in the absence and presence of mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol). After timed intervals, aliquots of the cell suspension were assessed by the dye exclusion test and for proteins released into the supernatant. The trypan blue exclusion test was carried out according to the supplier, and the results obtained by two independent experimentalists are presented as the mean ± standard deviation.

cholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol) were used as the donor. The concentrations of enterocytes and BBMV were chosen such that the sucrase activities measured per unit volume were similar, i.e., the concentrations of enterocytes and BBMV were normalized to equal sucrase activity. Under these conditions similar kinetic curves for cholesterol absorption were obtained with both models of acceptor membranes as shown in **Fig. 4.** The kinetics of cholesterol absorption by both pig enterocytes and pig BBMV were biphasic. The time resolution of these kinetic curves was limited by the time required to separate donor and acceptor by centrifugation. Due to this limitation, half times for the first fast phase of cholesterol absorption could not be determined. However, the amount of cholesterol absorbed during the first phase was derived from the computer analysis of the experimental curves in Fig. 4 (cf. Table 4 and legends to this table). For both acceptor systems linear relationships were obtained between this amount of cholesterol absorbed and the acceptor concentration. Values for the acceptor pool a_1 available during the first phase of cholesterol absorption varied between 21% and 48% (**Table 4**), reflecting the low precision of this determination. Similar half times were obtained for the second slower phase of cholesterol absorption for both enterocytes and BBMV, and within experimental error these half times were independent of the acceptor concentration (Table 4). The average half times obtained for the second phase of cholesterol absorption by enterocytes and BBMV were 3.8 ± 1.0 and 4.1 ± 0.6 min, respectively. As shown in the inset of Fig. 4 most of the taurocholate absorbed by BBMV was taken up within the first minute. The uptake of taurocholate by enterocytes was similar to that of BBMV under normalized conditions. Proteinase K treatment of BBMV led to a loss in total membrane protein and in sucrase activity of ~60% each. After this treatment the absorption of cholesterol was significantly slowed down and the amount of cholesterol absorbed was reduced to about 1/10 of that normally taken up in the first fast phase of lipid absorption (Table 4).

As would be expected from Fig. 4, there is a good correlation between the sucrase activity and the capacity of the membrane to take up cholesterol. Plotting the amount of cholesterol absorbed by enterocytes and BBMV in the first fast phase (**Fig. 5**) as a function of the sucrase activity gave a straight line with a correlation coefficient of $r^2 = 0.982$ (Fig. 5C). The specific sucrase activity was found to be 0.09 ± 0.04 U/mg cell protein for pig enterocytes and 1.2 ± 0.2 U/mg protein for pig BBMV. Apparently the specific sucrase activity of BBMV was augmented by a factor of ~13.

To shed light on the first fast phase of cholesterol absorption, the uptake of cholesterol by BBMV was measured using a rapid filtration method to separate donor and acceptor described in detail previously (6). The kinetic curve obtained with this method was significantly improved in its time resolution (**Fig. 6**) and was best fitted by the sum of three exponentials yielding the following half times: $t_{1/2} = 0.14$ s, first intermediate state: 65%; $t_{1/2} = 3.2$ s, second intermediate state 24%; and

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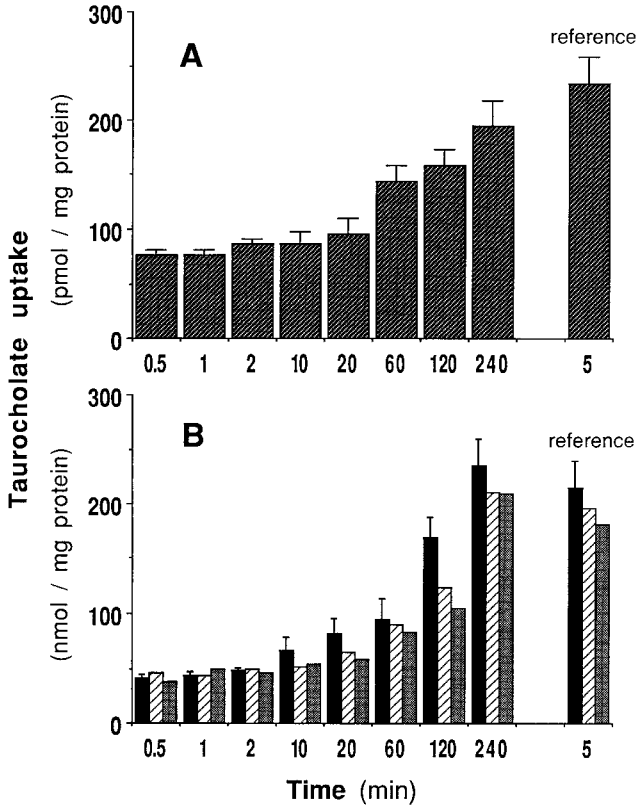

Fig. 2. (A) Bar histogram of [^{14}C]taurocholate uptake by pig enterocytes from a 5 μM sodium taurocholate solution as a function of time. Enterocytes from pig jejunum at 12.1 mg protein/ml suspended in buffer A were incubated with a 5 μM taurocholate solution in the same buffer at 23°C. After timed intervals given on the x-axis, the enterocytes were pelleted by centrifugation at 12000 *g* for 5 sec, the pellet was solubilized in 1% SDS, and protein content and radioactivity of the resulting solution were determined. (B) Bar histogram of [^{14}C]taurocholate uptake by pig enterocytes from mixed bile salt micelles as a function of time. Mixed bile salt micelles of different composition were used: 4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol (black), micelles of this composition containing additionally 0.1 mM PC (hatched) and 0.5 mM PC (shaded). Experimental details as in (A). The error bars represent the standard deviation of 2 measurements using enterocytes of one preparation. The bars on the right of both panels labeled "reference" represent the taurocholate absorption by damaged enterocytes after 5 min incubation at 23°C. Enterocytes were damaged by mechanical agitation as described under Methods.

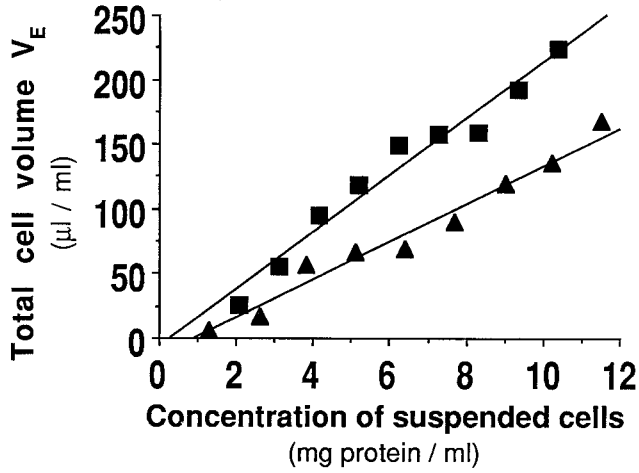

Fig. 3. Total cell volume of pig enterocytes as a function of the cell concentration. Enterocytes suspended in buffer A at various concentrations were incubated with inulin-[^{14}C]carboxylic acid at 23°C for 30 sec, pelleted by centrifugation at 12000 *g* for 5 s, and the radioactivity in the supernatant was determined. The total cell volume V_E was calculated for two populations of enterocytes as described under Methods. The solid lines were fitted to the experimental data points by linear regression analysis yielding the equations: $y = -6.1 + 21.9\,x$ ($r^2 = 0.964$) (■), and $y = -13.4 + 14.6\,x$ ($r^2 = 0.976$) (▲). The barrier properties of the two populations of pig enterocytes were examined by the trypan blue dye exclusion test, and the percentages of intact cells were found to be 90% (■) and 70% (▲).

$t_{1/2}$ = 2.5 min, final equilibrium 7%. Whatever the origin of the first two phases of cholesterol absorption might be, these data indicate that three quarters of cholesterol are transferred to the acceptor with a half time on the order of seconds. This result is entirely consistent with a previous report (6).

Cholesteryl oleyl ether absorption by BBMV and intact enterocytes

The absorption kinetics of cholesteryl oleyl ether were compared using pig enterocytes at 6.9 mg protein/ml and pig BBMV at 0.25 mg protein/ml as the acceptor (**Fig. 7**). As discussed for the absorption of free cholesterol (Fig. 4), the concentrations of enterocytes and BBMV were chosen such that approximately equal sucrase activities were present per unit volume. Mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol) were used as the donor containing either 0.1 mM [^{3}H]cholesterol or 3.5 μM [^{3}H]cholesteryl oleyl ether. The ether was used as a nonhydrolyzable analogue of cholesteryl esters, its concentration of 3.5 μM being dictated by the low solubility of this compound in bile salt micelles. In control experiments we showed this concentration of cholesteryl oleyl ether to be soluble in the mixed taurocholate micelles and pure taurocholate micelles (4.8 mM), and that the kinetics of cholesterol absorption by pig enterocytes are similar to mixed taurocholate micelles containing 0.1 mM and 3.5 μM free cholesterol. As observed with free cholesterol (Fig. 4), cholesteryl oleyl ether also shows similar absorption kinetics in intact enterocytes and BBMV under conditions of normalized sucrase activity (Fig. 7). However, the absorption kinetics of cholesteryl oleyl ether differed significantly from those of free cholesterol after about 1 min. With cholesteryl oleyl ether, the second kinetic phase appears to approach an equilibrium of about 75%, significantly higher than that of free cholesterol of 54 ± 3% (Fig. 7).

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TABLE 3. Cholesterol absorption by BBMV from different sources

Acceptor[a]	Donor	k_1	$t_{1/2}$
		h^{-1}	h
BBMV from rabbit small intestine	egg PC SUV	0.49	1.1
Papain-treated BBMV from rabbit small intestine	egg PC SUV	0.10	5.2
BBMV from pig small intestine	egg PC SUV	0.43	1.2
Papain-treated BBMV from pig small intestine	egg PC SUV	0.09	5.8
BBMV from human small intestine	egg PC SUV	0.41	1.3
Papain-treated BBMV from human small intestine	egg PC SUV	0.10	5.2
BBMV from CaCo-2 cells	egg PC SUV	0.65	0.80
Papain-treated BBMV from CaCo-2 cells	egg PC SUV	0.12	4.5

Acceptor[b]	Donor	Cholesterol Absorption	Half-time
		nmol/mg protein	*min*
BBMV from rabbit small intestine	bile salt micelles	8.0 ± 0.6	3.8 ± 0.6
BBMV for pig small intestine	bile salt micelles	8.8 ± 0.3	4.3 ± 0.9

[a]The kinetics of cholesterol absorption by BBMV from different sources were measured at room temperature. SUV of egg PC containing 1 mol % of [^{3}H]cholesterol were used as the donor. Donor and acceptor were mixed at time zero so that their final concentrations were 0.2 mg lipid/ml and 2 mg protein/ml, kinetic lipid pool 0.6 mg lipid/ml, respectively. Kinetic curves were fitted by a single exponential function and pseudo-first-order rate constants k_1 and half-times $t_{1/2}$ were derived from curve fitting. The error is estimated to be ±10%.

[b]The amount of cholesterol taken up during the first kinetic phase of absorption and the half-times of the second kinetic phase are given. The concentration of the BBMV was 10 mg protein/ml. The composition of the mixed bile salt micelles was 4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol. The experimental conditions were the same as for Fig. 4 (cf. Methods).

Cholesterol and cholesteryl oleyl ether absorption by intestinal segments

In control experiments the stability of the intestinal segments was assessed. For this purpose intestinal segments were incubated with mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol), and the amount of cells and cell debris shed from the tissue as well as the amount of cholesterol absorbed were determined as described under Methods. As shown in **Table 5** the shedding of cells and cell debris varied greatly from sample to sample and so did the absorption of cholesterol by these cells and cell debris.

The BBM of segments of rabbit upper jejunum were incubated in a modified Ussing chamber (**Fig. 8**) with mixed taurocholate micelles double-labeled with [^{3}H]cholesterol and inulin [^{14}C]carboxylic acid. The latter compound was used to determine the volume of adherent fluid according to Lukie, Westergaard, and Dietschy (32). Assuming that inulin is not bound to the BBM, a value of 29 µl per 100 mg dry weight of tissue was obtained in good agreement with published data (open triangles, **Fig. 9A**) (32). The absorption of cholesterol by the intestinal segment (closed circles, Fig. 9A) was corrected for adherent fluid (open triangles, Fig. 9A) yielding the straight line relationship shown in Fig. 9B. The amount of cholesterol absorbed over 30 min was 5.8 ± 1.7 nmol per 100 mg dry weight of tissue. At the end of the lipid absorption experiment carried out in the Ussing chamber, part of the intestinal segment was scraped and the absorption of cholesterol by the scraped mucosa was determined. The results for the cholesterol absorption corrected for adherent fluid are shown in Fig. 9C. The kinetics are linear, similar to those shown in Fig. 9B, suggesting that the kinetics of cholesterol absorption measured with intestinal segments are representative of the mucosa. In the scrapings the amount of cholesterol absorbed over 30 min was 0.74 nmol cholesterol/mg protein. This is about one-eighth of the cholesterol absorbed by intact enterocytes at the equilibrium reached after ~20 min. The amount of cholesterol absorbed by intact enterocytes was 6.2 ± 0.7 nmol/mg protein.

The absorption of cholesteryl oleyl ether by small-intestinal segments mounted in the Ussing chamber was determined from mixed bile salt micelles (4.8 mM taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol) double-labeled with [^{3}H]cholesteryl oleyl ether and [^{14}C]cholesterol. The fraction of cholesteryl oleyl ether absorbed within 30 min amounted to 69 ± 21% of that of free cholesterol (4 experiments, data not shown). Correcting these values for adherent fluid, cholesteryl oleyl ether absorption was about half of the cholesterol absorption.

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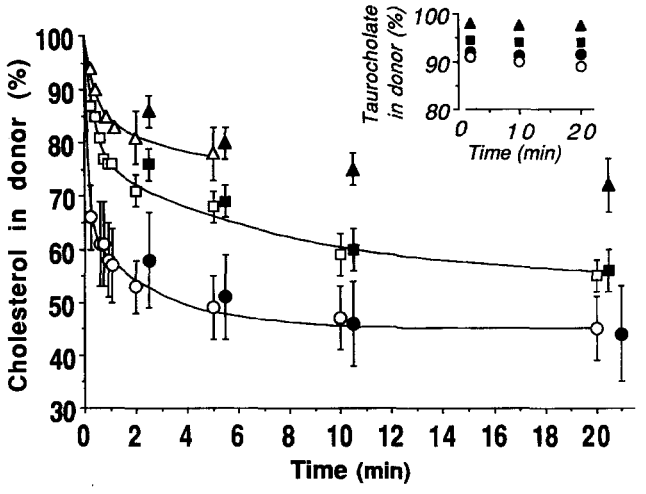

Fig. 4. Comparison of the kinetics of [^{3}H]cholesterol absorption by pig enterocytes and pig BBMV. Enterocytes (open symbols) and BBMV (closed symbols), both prepared from pig upper jejunum, were compared as to their capacity to absorb free cholesterol from mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol). Donor and acceptor both suspended in buffer A were incubated at 23°C. The donor concentration was kept constant at 2.9 mg total lipid/ml; the concentrations of enterocytes were: 3.6 ± 0.1 (△), 6.9 ± 0.6 (□), and 12.8 ± 1.4 (○) mg cell protein/ml. The solid lines represent biexponential computer fits to the kinetic data using equation 1 of the Appendix. The concentration of BBMV varied between 0.13 (▲), 0.25 (■), and 0.5 (●) mg protein/ml. After timed intervals donor and acceptor were separated by centrifugation at 12000 *g* for 5 s and 115000 *g* for 2 min for enterocytes and BBMV, respectively, and the radioactivity remaining in the donor was determined. For cholesterol absorption by BBMV at 0.13 and 0.25 mg protein/ml, a correction had to be made taking care of the partial solubilization of the BBMV in the presence of mixed bile salt micelles amounting to 40% and 20% (referred to the fraction of solubilized membrane proteins), respectively. The data points represent the mean ± the standard deviation (see error bars) of 3 measurements. The standard deviations of the absorption by enterocytes at 3.6 and 6.9 mg protein/ml for incubation times <2 min were omitted for the sake of clarity. Inset: Sodium taurocholate uptake by BBMV at 0.13, 0.25, and 0.5 mg protein/ml (closed symbols) and enterocytes at 12.8 mg cell protein/ml (open symbols) from mixed taurocholate micelles of composition given above labeled with [^{14}C]taurocholate.

DISCUSSION

BBMV have been widely used as a model in transport studies such as the uptake of ions and small molecules, e.g., D-glucose, L-amino acids, and other nutrients present in the small intestine. As BBMV are subject to a number of limitations (see Discussion below), it is desirable to verify results obtained with this model system in other BBM models more closely related to the in vivo situation. This is particularly true for lipid absorption measurements.

The small-intestinal epithelium is characterized by highly developed digestive, absorptive, and metabolic functions. The natural lifetime of enterocytes is only about 48–72 h (33, 34). A manifestation of this natural instability is the high tendency of the small-intestinal epithelium to shed cells and cell debris. So it is not surprising that all small-intestinal in vitro models are troubled by limited stability and degradation processes. For instance, we and other groups have observed that the tendency of the small-intestinal tissue to shed enterocytes varied greatly from animal to animal and increased with time elapsed after death of the animal. As a consequence it has become common practice to excise and to process the small intestine post mortem as fast as possible (31).

Advantages and disadvantages of various brush border membrane models

BBMV. BBMV are a simple and often useful model of the apical part of the plasma membrane of enterocytes. BBMV are relatively pure and essentially free of nuclear, mitochondrial, and microsomal membranes (3), but may be contaminated by about 10% with basolateral membranes. They are oriented right-side-out to more than 90% (35, 36) and impermeable to particles of the size of lipid micelles and SUV; hence only the external surface of the BBMV will be exposed to donor particles and accessible for collision-induced uptake of dietary lipids. One major advantage is that BBMV allow us to investigate the event of lipid absorption at the membrane level independently from metabolic processes taking place in the interior of the epithelial cell (enterocyte). BBMV can be stored for months in the frozen state without significant loss in transport and enzymatic activities, but they are known to become unstable upon being subjected to freeze–thaw cycles. This instability is probably due to the presence of hydrolases, the control and regulation of which are still unknown. The inherent instability of the BBMV depends sensitively on experimental conditions such as pH, ionic strength, and content, the presence of detergents (37), the temperature, and other parameters. As to possible contaminations, it should be borne in mind that in the course of the preparation of BBMV, epithelial cells are homogenized and lysed. As a result, components of the cytosol and cell organelles may interact with the BBM and could be absorbed to the membrane surface. This process may give rise to artifacts. Some advantages and limitations of BBMV in studying the molecular mechanism of transport systems of this membrane were discussed by Bertheloot and Semenza (7). The conclusion was that adequate caution must be exerted in the interpretation of data obtained with this BBM model.

Intact enterocytes. Intact enterocytes have the advantage that they are metabolically active and therefore closer to the in vivo situation than BBMV. Active transport of glucose and respiratory metabolism were demonstrated with enterocytes in aqueous dispersion (13).

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TABLE 4. [^{3}H]cholesterol absorption by pig enterocytes and pig BBMV from mixed bile salt micelles as a function of the acceptor concentration[a]

Acceptor	Protein Content	Sucrase Activity	First Phase			Second Phase		
			Cholesterol Absorption[b]	Intermediate State $x_{1\infty}$	Kinetic Lipid Pool a_1	Half time $t_{1/2}$	Final Equilibrium x_∞	Kinetic Lipid Pool a_2
	mg/ml	*U/ml*	*nmol/ml*	%	%	*min*	%	%
BBMV	0.13	0.2 ± 0.1	9 ± 4	91 ± 4	24 ± 11	4.5 ± 1.3	71 ± 3	
BBMV	0.25	0.3 ± 0.1	14 ± 5	86 ± 5	21 ± 8	4.5 ± 1.1	56 ± 5	79 ± 8
BBMV	0.50	0.7 ± 0.1	31 ± 8	69 ± 8	35 ± 10	3.2 ± 1.2	44 ± 2	65 ± 10
BBMV	1.0	1.3 ± 0.2	77 ± 4	23 ± 4	46 ± 13	4.1 ± 0.8	12 ± 2	54 ± 13
BBMV	10.0	12 ± 1	88 ± 3	12 ± 3	31 ± 17	4.3 ± 0.9	4 ± 2	69 ± 17
Protease-treated BBMV	(10.0)[c]	5.1	9 ± 3	91 ± 3	0.9 ± 0.7	19 ± 3	8 ± 6	99 ± 1
Enterocytes	3.6 ± 0.1	0.3 ± 0.2	14 ± 7	86 ± 7	43 ± 28	3.5 ± 0.8	73 ± 10	57 ± 28
Enterocytes	6.9 ± 0.6	0.6 ± 0.3	23 ± 6	77 ± 6	35 ± 10	4.9 ± 1.8	54 ± 3	65 ± 10
Enterocytes	12.8 ± 1.4	1.0 ± 0.5	39 ± 8	61 ± 8	48 ± 14	3.0 ± 1.3	44 ± 6	52 ± 14

[a]The total lipid concentration of the mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol, labeled with [^{3}H]cholesterol) was kept constant at 2.9 mg total lipid/ml. Protein content, sucrase activity, and [^{3}H]cholesterol absorption at 23°C were determined as described under Methods. A biexponential computer fit (cf. equation 1 of the Appendix) was applied to the experimental data yielding the following terms of equation 1 (cf. Appendix): intermediate state $x_{1\infty}$, final equilibrium x_∞, and half time $t_{1/2}$ of the second phase. Lipid pools of the acceptor membrane available during the first phase a_1 and during the second phase a_2 of the kinetics were calculated from equations 5 and 6 of the Appendix. The average and standard deviation of three measurements are given.

[b]The amount of cholesterol absorbed during the first kinetic phase of the absorption was derived from the computer simulation of the kinetic curves of Fig. 4. This amount (in %) is given by $(x_0 - x_{1\infty})$, and as the total cholesterol content of the donor is known $(x_0 - x_{1\infty})$ can be expressed in nmol/ml.

[c]Protein content of BBMV before treatment with proteinase K.

We show here that intact enterocytes are a useful model for the BBM in sterol absorption studies provided that appropriate control experiments are carried out. The instability of enterocytes is probably the most serious drawback of this BBM model. An appreciation of this major disadvantage is obtained by noting that on the average 3 out of 4 preparations of enterocytes had to be discarded, i.e., only about one quarter of the preparations were sufficiently stable and suitable for lipid absorption studies. Even with these preparations, the stability in the presence of bile salts is limited to about 10–20 min so that lipid absorption measurements must be done within this time frame. With intact enterocytes the whole plasma membrane is exposed to the external medium. Bile salt micelles as the natural carrier of dietary lipids are not expected to destabilize the BBM. The basolateral membrane domain, however, may be less resistant to detergents and harsh conditions in general and confer instability to enterocytes. There is some evidence from light microscopy showing that the basolateral membrane and/or the domains of the tight junctions are vulnerable regions of the plasma membranes of enterocytes. Lysed enterocytes will release part of their content including cytosolic and cell organelle proteins that might interfere with lipid absorption measurements. Further, upon lysis of enterocytes, intracellular membranes might become accessible to cholesterol absorption leading to an increase in the specific absorption of cholesterol. In any case, the exact contribution of lysed cells and cells permeable to trypan blue to sterol absorption is unknown. Because of that, preparations of enterocytes with more than 20% of the cells being permeable to trypan blue were discarded.

Intestinal segments. The major advantage of intestinal segments is that this BBM model is closest to the physiological situation. It is the only model in which the exact morphology of the small-intestinal mucosa is preserved, i.e., the morphology in terms of foldings and extensions leading to a significant enlargement of the BBM surface. This model also mimics the in vivo situation in as much as the cells are oriented with the BBM towards the mucosal compartment. The major disadvantages of intestinal segments are the instability and wide variation in quality of the preparations. Huge variations from preparation to preparation as well as from animal to animal were observed (cf. Table 5). These properties of intestinal segments are manifested in the large error of the lipid absorption measurements in this BBM model.

CaCo-2 cells. BBMV prepared from CaCo-2 cells show identical behavior in terms of cholesterol uptake as BBMV prepared from small intestine of different origin (Table 3). This indicates that the protein(s) catalyzing cholesterol uptake is also expressed in this cell line derived from a human colorectal carcinoma. CaCo-2 cells undergo differentiation to polarized cells resembling enterocytes when grown on proper support such as polycarbonate filters. Under these conditions CaCo-2 cells aggregate forming a tightly packed cell monolayer. Such a monolayer represents a barrier separating mucosal (BBM) and serosal (basolateral membrane) com-

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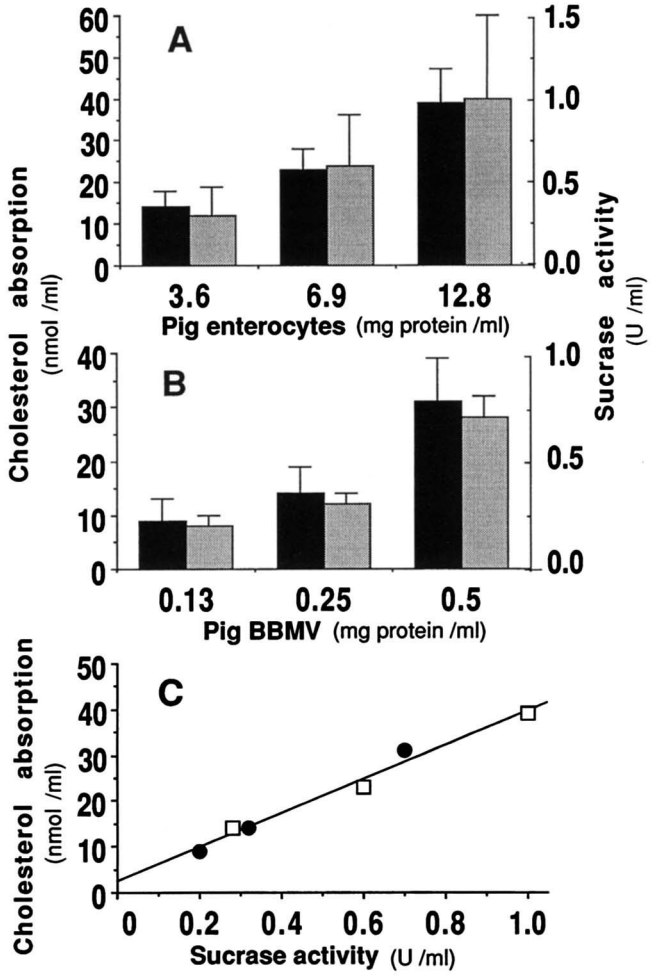

Fig. 5. Correlation between the sucrase activity of pig enterocytes and pig BBMV and the amount of cholesterol absorbed during the first kinetic phase. Biphasic computer fits (cf. eq. 1 of the Appendix) were applied to the absorption kinetics of Fig. 4 yielding the amount of cholesterol that was transferred by collisional contact from donor to acceptor during the first kinetic phase. The time points characterizing the end of the first kinetic phase amounted to about 1 min. Cholesterol absorption (black bars) is related to the sucrase activity (shaded bars) at three different concentrations of pig enterocytes (A) and pig BBMV (B): 3.6, 6.9, and 12.8 mg total cell protein/ml and 0.13, 0.25, and 0.5 mg protein/ml, respectively. (C) Correlation between sucrase activity and cholesterol absorption for pig enterocytes (open squares) and pig BBMV (filled circles).

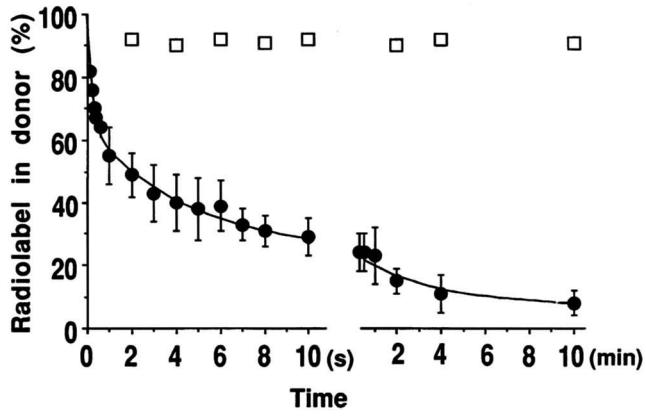

Fig. 6. Cholesterol (●) and taurocholate (□) absorption by rabbit brush border membrane vesicles from mixed bile sale micelles. Mixed bile salt micelles consisting of 4.8 mM taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.6 mM oleic acid, and 0.2 mM cholesterol double-labeled with [^{3}H]cholesterol and [^{14}C]taurocholate were dispersed at 3 mg total lipid/ml in 0.1 M HEPES adjusted with Tris to pH 7.3, 0.3 M D-mannitol, 5 mM EDTA, 0.02% NaN_3 and incubated with rabbit small-intestinal BBMV at 20 mg protein/ml at 23°C. The absorption process was stopped and the acceptor and donor particles were separated by a rapid filtration method as detailed previously (6). The amount of [^{3}H]cholesterol and [^{14}C]taurocholate remaining in the donor was determined by scintillation counting. The solid line represents a triexponential computer fit to the experimental data. The error bars represent the standard deviation of 3 measurements.

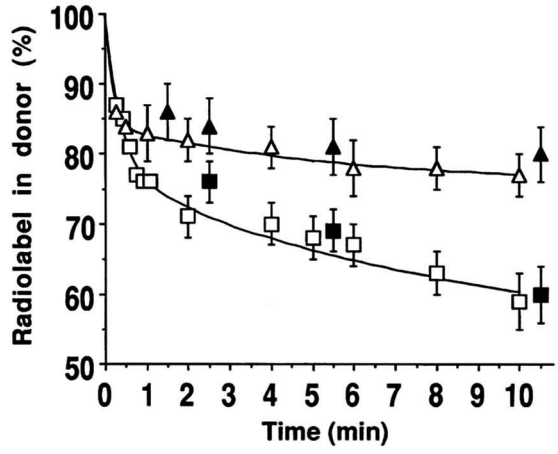

Fig. 7. Comparison of the kinetics of cholesterol and cholesteryl oleyl ether absorption by pig enterocytes (open symbols) and pig BBMV (closed symbols). Pig enterocytes at 6.9 ± 0.6 mg protein/ml suspended in buffer A were incubated at 23°C with mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, plus sterols) and the absorption of [^{3}H]cholesterol (□) and [^{3}H]cholesteryl oleyl ether (△) was determined. For comparison the absorption of [^{3}H]cholesterol (■) and [^{3}H]cholesteryl oleyl ether (▲) by pig BBMV at 0.25 mg protein/ml was included. Experimental details as discussed for cholesterol absorption in Fig. 4. The data points represent the mean ± standard deviation of 3 measurements. For short incubation times standard deviations were omitted for clarity's sake.

partments so that uptake by the BBM and secretion of compounds through the basolateral membrane and the polycarbonate filter can be monitored simultaneously in the mucosal and serosal compartments, respectively. However, it should be noted that the geometry of a monolayer of CaCo-2 cells grown on a flat piece of polycarbonate filter is significantly different from the morphology of the small-intestinal mucosa. Furthermore, CaCo-2 cells lack the surface layer of mucus and there are also differences in the enzymatic composition of CaCo-2 cells and enterocytes (38). Sterol absorption in

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TABLE 5. Shedding of cells and cell debris from intestinal segments in the Ussing chamber and the uptake of [^{3}H]cholesterol by these cells and debris

Time	Shedding Determined as Protein Released[a]	Cholesterol Uptake[b]
min	*mg/100 mg dry weight of tissue*	*nmol/100 mg dry weight of tissue*
1	0–0.03	0–0.05
2	0–0.04	0–0.29
10	0–0.07	0–9.5
30	0.03–0.17	1.2–29.3

[a]At the end of the incubation the micellar dispersion and the washings were collected from the mucosal compartment of the Ussing chamber and immediately centrifuged at 12000 *g* for 2 min. The pelleted cells and debris were dissolved in 1% SDS and protein as well as [^{3}H]cholesterol and inulin [^{14}C]carboxylic acid radioactivities were determined. A value of zero indicates that no cells and debris were pelleted by centrifugation at 12000 *g* for 2 min.

[b]The amount of cholesterol taken up by the shedding was corrected for the fluid entrapped in the pellet.

CaCo-2 cells will be the subject of a separate publication.

Cholesterol absorption

We reported previously that the absorption of cholesterol and long-chain cholesteryl esters by rabbit small-intestinal BBMV is protein-mediated (1, 2). The data summarized in Table 3 indicate that cholesterol absorption is facilitated and this function is conserved in BBMV prepared from pig and human small intestine. Table 3 shows that there is hardly any difference in cholesterol uptake using BBMV of different sources. Evidence for facilitated cholesterol uptake by BBMV of different origin is provided by the papain treatment of

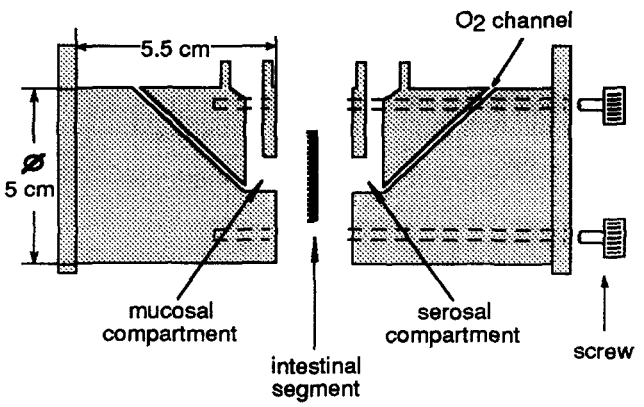

Fig. 8. Schematic of the modified Ussing chamber used in this study. A cross section of the horizontal, cylindric chamber milled from ply methyl methacrylate (Plexiglas) (diameter = 5 cm, length = 11 cm) is shown. A circular piece of intestinal tissue of diameter of ~1.5 cm was inserted between the two halves of the chamber and the two halves were tightened together by means of 4 screws. The circular piece of intestinal tissue was inserted such that the BBM represented by the saw-toothed line faced the mucosal compartment.

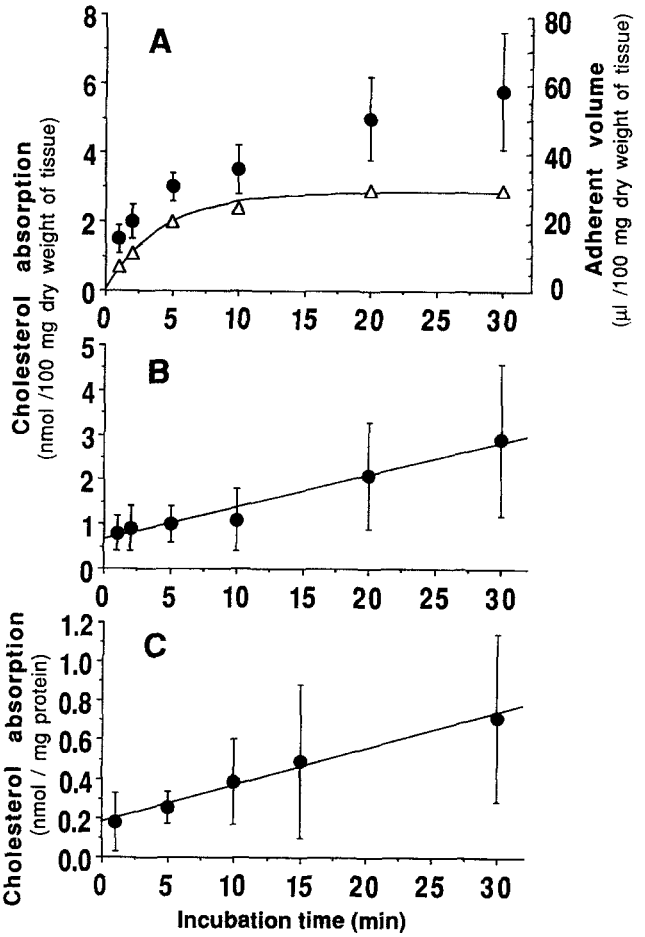

Fig. 9. Cholesterol absorption by segments of rabbit jejunum mounted in the modified Ussing chamber described in Fig. 8. A circular segment (~0.8 cm^2) of rabbit jejunum was incubated in the Ussing chamber at 23°C with 0.6 ml of mixed taurocholate micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol, 0.1 mM cholesterol, labeled with [^{3}H]cholesterol) dispersed in buffer A containing a trace amount of inulin-[^{14}C]carboxylic acid. The micellar dispersion was continuously gassed with pure oxygen and mixed as described in Methods. After various periods of time the incubaton was terminated by aspirating the micellar dispersion from the donor compartment and rinsing the tissue with buffer A. The intestinal segment was removed from the chamber, dissolved in tissue solubilizer, and the radioactivities of [^{3}H]cholesterol and inulin-[^{14}C]carboxylic acid were determined by liquid scintillation counting. (A) [^{3}H]cholesterol absorption (●) and adherent fluid (△) per 100 mg dry weight of tissue determined as described under Methods. The solid line represents a computer fit from which a half time of about 3 min was derived. The error bars represent the standard deviation of 2–4 measurements. (B) The effective [^{3}H]cholesterol absorption per 100 mg dry weight of tissue was calculated from the primary data (solid circles in Fig. 9A) by subtracting the amount of cholesterol present in the adherent fluid. The solid line represents a linear least-square fit to the experimental data: $y = 0.64 + 0.073 x$ ($r^2 = 0.972$). (C) [^{3}H]cholesterol absorption by small-intestinal mucosa scraped from segments of rabbit jejunum after incubation with mixed taurocholate micelles. After terminating the incubation some mucosa (0.5–1.5 mg protein) was immediately scraped from part of the intestinal segment; the scrapings were dissolved in 1 ml of 1% SDS and protein and radioactivities were determined. The [^{3}H]cholesterol uptake was corrected for adherent fluid. The solid line represents a linear least-square fit to the experimental data: $y = 0.18 + 0.019 x$ ($r^2 = 0.985$). The error bars represent the standard deviation of 3 measurements.

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BBMV which reduces cholesterol uptake to levels characteristic of passive cholesterol transfer.

The good agreement in cholesterol absorption between BBMV and enterocytes (cf. Fig. 4 and Table 4) indicates that both models handled with proper care are suitable for lipid absorption measurements. The agreement of the results obtained with these two BBM models under conditions of normalized sucrase activity can be taken as evidence that the protein(s) catalyzing sterol uptake resides in the BBM and not in the basolateral membrane. Sucrase-isomaltase is the most abundant protein of the BBM (21) and as such it is used as a marker enzyme of this membrane. As BBMV are ATP-depleted, cholesterol absorption must be an energy-independent process.

In contrast to the kinetic curves obtained with BBMV and intact enterocytes, linear kinetics and significantly lower rates of cholesterol absorption are observed with intestinal segments mounted in the Ussing chamber. From the equilibration curve of inulin [^{14}C]carboxylic acid with the intestinal segments (cf. triangles in Fig. 9A) a half time of ~3 min was derived. As the rate of diffusion of bile salt micelles to the BBM is expected to be similar or slower than that of inulin, this finding means that the diffusion of the bile salt micelles to the BBM is actually slower than the absorption of cholesterol by the BBM. We conclude that with intestinal segments the diffusion of the donor particles to the site of absorption is the rate-limiting step and very likely responsible for the linear kinetics observed (Figs. 9B and C). The mucus layer at the surface of the BBM produced by the Goblet cells may provide a diffusion barrier slowing down the diffusion of bile salt micelles. However, in the in vitro situation, the mucus layer is probably artificially enhanced in as much as cell debris accumulate at the surface of intestinal segments due to surface adsorption.

Evidence presented in Figs. 4 and 6 rules out that whole bile salt micelles are taken up or endocytosed by the BBM. This result is in agreement with the generally accepted view that endocytosis of lipid particles at the BBM does not contribute significantly to lipid absorption (4, 5, 39). The percentage of taurocholate taken up by the BBMV or the plasma membrane of enterocytes (cf. inset of Fig. 4, Fig. 6) is distinctly different from that of cholesterol taken up by these membranes. The increase in taurocholate uptake observed by enterocytes over 4 h (Fig. 2) is interpreted to be due to cell lysis so that the bile salt will equilibrate with organelles and cytosolic components.

Cholesteryl ester absorption

Figure 7 shows that in the first kinetic phase cholesterol and cholesteryl oleyl ether molecules are taken up by the BBM with similar rate constants. This finding points to the lipid uptake mechanism of BBM being lipid-unspecific. The results of Fig. 7 do not necessarily imply that the uptake of cholesteryl esters is a quantitatively significant process in small intestine because the solubility of cholesteryl esters in bile salt micelles is limited. Nevertheless, cholesteryl esters present in the lumen of the small intestine will have a finite probability of being solubilized in bile salt micelles and taken up by the BBM; this follows from a consideration of the ratio of the concentration of cholesterol (~2.4 mM) and cholesteryl esters (~0.2 mM) in the lumen of the small intestine (5) which is quite similar to the ratio of the maximal solubility of cholesterol (~0.2 mM) and cholesteryl oleate (~0.01 mM) in mixed bile salt micelles (4.8 mM sodium taurocholate, 0.3 mM 1-monooleoyl-*sn*-glycerol).

With all three BBM models it is apparent that compared to cholesterol, only about half of cholesteryl oleyl ether is taken up. This difference is due to the second kinetic phase of cholesteryl oleyl ether uptake which probably reflects differences in the flip-flop movement of free and esterified cholesterol. This movement is probably significantly slower for esterified cholesterol (2) than for free cholesterol which was shown to be quite efficient in red blood cell membranes (40). Under physiological conditions cholesteryl esters taken up in the outer monolayer of small-intestinal BBM probably undergo hydrolysis prior to their transverse motion across the BBM. This process might be catalyzed by pancreatic cholesterol esterase which was shown to bind specifically to small-intestinal BBM (41).

Concluding remarks

There is no single reliable model of the BBM, therefore the combined use of different BBM models is compulsory in lipid absorption studies. BBMV are a simple model, relatively easy in handling and interpreting the results, and therefore suitable for routine work. Due to the limitations of this model, the results obtained with BBMV should be verified in more elaborate models such as intact enterocytes, intestinal segments, CaCo-2 cells, and in vivo systems.

APPENDIX

Equation 1 is valid for biphasic exchange reactions (9):

$$x = x_\infty + [x_0 - x_{I\infty}]\, e^{-k_I[(a_I+b_I)/a_I]t} + [x_{I\infty} - x_\infty]\, e^{-k'_I[(a_2+b_2)/a_2]t} \qquad \textit{Eq. 1)}$$

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where x_0, x, $x_{1\infty}$, and x_∞ represent the fractions or percentages of the labeled lipid in the donor at times 0, t, at an intermediate state, and at the final equilibrium, respectively; k_1 and k_1' are the pseudo-first-order rate constants of the first and the second kinetic phases, respectively; a_1 and b_1 represent the lipid pools of acceptor and donor, respectively, available during the first kinetic phase; a_2 and b_2 are the lipid pools available during the second kinetic phase. Half time values for the second kinetic phase were calculated from

$$t_{1/2} = \frac{\ln 2}{k_1' (a_2 + b_2)/a_2}$$

The term in the denominator was derived by curve fitting.

Intermediate state $x_{1\infty}$ and final equilibrium x_∞ are related to the kinetic lipid pools as follows:

$$x_{1\infty} = b_2 + [b_1/(a_1 + b_1)] \qquad Eq.\ 2)$$

Assuming that all sterol molecules of small mixed bile salt micelles are readily accessible to lipid absorption equation 2 may be simplified:

$$x_{1\infty} = b/(a_1 + b) \qquad Eq.\ 3)$$

$$x_\infty = b/(a + b) \qquad Eq.\ 4)$$

where a and b represent the total kinetic lipid pools of acceptor and donor, respectively. The lipid pools of the acceptor membrane a_1 and a_2 being available during the first and the second kinetic phases of sterol absorption, respectively, are given by

$$a_1 = a\,\frac{x_\infty\,(1 - x_{1\infty})}{x_{1\infty}\,(1 - x_\infty)} \qquad Eq.\ 5)$$

$$a_2 = a - a_1 \qquad Eq.\ 6)$$

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