A Model of Human Small Intestinal Absorptive Cells. 1. Transport Barrier

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Received December 7, 1989; accepted July 23, 1990

The Caco-2 cell culture model of human small intestinal absorptive cells was used to investigate transepithelial transport. Transport of permeability markers such as mannitol demonstrated that Caco-2 monolayers became less permeable with increasing age in culture. Cells were routinely used for transport studies between day 18 and day 32. A transport index was determined for each compound by calculating the ratio of transport of the molecules under investigation to transport of an internal standard such as the permeability marker mannitol. Comparison of transport rates at 4 and 37°C was a simple approach for differentiating primary transport mechanisms (passive paracellular, passive transcellular, or transporter-mediated) but must be coupled with additional experimental manipulations for definitive determination of transport pathways. Compounds predicted to undergo predominantly paracellular transport (mannitol, FITC, PEG-900, and PEG-4000), transporter-mediated transcellular transport (glucose, biotin, spermidine, or alanine), or lipophilic transcellular transport (alprenolol, propranolol, clonidine, or diazepam) showed differential effects of temperature on rates of transport as well as the transport index.

KEY WORDS: paracellular; transcellular; transport index; drug absorption; Caco-2; epithelial.

INTRODUCTION

Investigations of intestinal transport have often utilized *in situ* or *in vivo* animal models or *ex vivo* transport models with intestinal sheets, everted sacs, or brush border fractions. Animal absorption studies are time-consuming, require large amounts of sample, and can show great variability due to luminal contents, mucus layer, hepatic clearance, and animal-to-animal or species-to-species variation. *Ex vivo* models have been limited by rapid tissue degradation following removal from the body. For these reasons, a number of groups have utilized cell culture models of small intestinal absorptive cells with the human Caco-2 cell line (1–3). Advantages include small sample volumes, high throughput, use of human cells, and low variability between replicates. The Caco-2 transport model described herein makes it possible to elucidate factors regulating transport across the intestinal epithelium.

MATERIALS AND METHODS

Materials. Radioisotopes were from New England Nuclear. Tissue culture reagents were from Gibco. All other chemicals were from Sigma.

Maintenance of Cell Stock. Caco-2 cells were obtained from American Type Culture Collection (passages 28) or from Dr. D. Alpers, Washington University (passage 54). Cells were routinely maintained in T-75 tissue culture flasks which were not collagen-coated. These stock cells were passaged before reaching confluency to minimize spontaneous differentiation following confluency. The growth medium contained Dulbecco's MEM with high bicarbonate (44 m*M*), high glucose (25 m*M*), nonessential amino acids, 10% heat-inactivated fetal bovine serum, and no antibiotics. Cells were harvested with 0.25% trypsin plus 0.02% EDTA (2–5 min at 37°C), resuspended, and seeded into new growth vessels. Cells were plated at 10,000 cells/cm^2, maintained in 10% CO_2, and fed every 2 days. Cells reached confluency after 5–7 days in culture.

Growth of Cells on Microporous Filters. Caco-2 cells were grown on 12-mm Millicell CM filter inserts (Millipore) in 24-well cluster dishes. The filters were coated with 100 μl type 1 rat tail collagen diluted 1:4 with 60% ethanol and air-dried in a laminar flow hood overnight. Caco-2 cells growing on filter inserts were fed every 2 days with 250 μl medium in the apical chamber and 500 μl in the basal chamber.

Light and Electron Microscopy. Caco-2 cells growing on microporous filters were rinsed with phosphate-buffered saline and fixed in 2.5% glutaraldehyde, 0.1 *M* sodium cacodylate (pH 7.4), 2% sucrose, and 2 m*M* $CaCl_2$ for 2–3 hr at ambient temperature. Cells were then treated with osmium tetroxide, dehydrated, embedded in Epon, sectioned, and poststained with lead citrate and uranyl acetate.

Transport Protocol. Filter inserts were rinsed with Hank's balanced salts containing 5 m*M* glucose. Permeabilty markers such as ^{3}H-mannitol were included as internal standards with each sample. The labeled samples were added to the apical chamber (200 μl), and transport buffer without label was added to the basal chamber (400 μl). Transport rates were determined by replacing the medium in the basal chamber at 15-min intervals. This was accomplished by moving the insert itself to a new well containing fresh medium at each time point. Solutions were not stirred or agitated during transport experiments. Cumulative transport rates were then determined by adding data from the discrete 15-min intervals. The means and standard errors of replicates are shown.

Analysis of Transported Products. Aliquots from each time point were counted in a Beckman LS 6800 liquid scintillation counter with Beckman Ready Gel scintillation fluid for aqueous samples. Integrity of radiolabeled compounds before and after transport across Caco-2 cells or blank filters was determined for all compounds tested with the exception of mannitol, fluorescein isothiocyanate (FITC), alanine, and methionine. Transport across blank filters had no effect on integrity of radiolabeled compounds, but incubation with Caco-2 cells resulted in the metabolism of some compounds. In cases where HPLC analyses were performed (glucose, mannitol, biotin, diazepam, alprenolol, clonidine, PEG-900, PEG-4000, propranolol, and spermidine), the transport val-

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0724-8741/91/0200-0210$06.50/0

ues reported have been corrected for percent intact compound.

Glucose and mannitol were analyzed on a Regis Amino Analytical column (4.6 mm × 25 cm; 5-μm bead diameter; 100-Å pore size) eluted using an isocratic system of acetonitrile (83%) in water with 0.1% NH_4OH at a flow rate of 1.3 ml/min. Biotin, diazepam, alprenolol, clonidine, PEG-900, and PEG-4000 were analyzed on a Beckman Ultrasphere ODS Analytical column (4.6 mm × 25 cm; 5-μm bead diameter; 80-Å pore size). Flow rates for biotin, diazepam, alprenolol, and clonidine were 1.3 ml/min, while 0.8 ml/min was used for PEG-900 and PEG-4000. Biotin was chromatographed using a 10-min acetonitrile gradient (15–35%) in water with 0.1% H_3PO_4. Alprenolol was eluted with an isocratic system of 70% acetonitrile in water plus 0.1% H_3PO_4, whereas clonidine was eluted with the same system containing 20% acetonitrile. PEG-900 and PEG-4000 were eluted with an isocratic system of water plus 30 or 40% acetonitrile, respectively. Propranolol and spermidine were analyzed on a Whatman SCX analytical column (4.6 mm × 12.5 cm; 0.8 ml/min). Propranolol was eluted using an isocratic system of 0.5 *M* ammonium phosphate in water with 20% acetonitrile, pH 3.5, whereas spermidine was eluted with the same system with 30% acetonitrile.

Data Calculation. The cumulative transport rates (μmol per cm^2 or % per cm^2) were calculated by correcting for volume, surface area of filters, specific activity, counting efficiency, and percentage integrity of labeled compounds.

Transport Index. The transport index was determined by dividing the percentage transport of the compound by the percentage transport of the internal standard (mannitol). Control experiments demonstrated that the percentage mannitol transport was not effected by mannitol concentration from 0.1 μ*M* to 10 m*M*, allowing utilization of tracer amounts of mannitol for determination of the transport index.

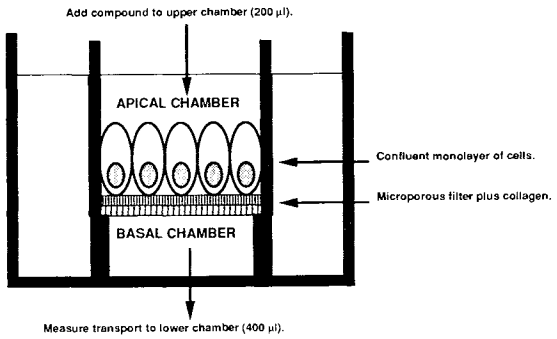
RESULTS

Tissue Culture Model of Human Small Intestinal Absorptive Cells and Transport Protocol

Caco-2 cells were grown on microporous filters as shown diagramatically in Fig. 1. Following growth of cells to a confluent monolayer, transport was determined by adding compounds to the upper (apical) chamber, equivalent to the luminal surface of intestines, and monitoring appearance in the lower (basal) chamber, equivalent to the serosal surface. Permeability markers such as mannitol were included as an internal standard with each sample to measure basal monolayer permeability. The internal standard allowed a straightforward determination of the validity of data generated by measuring background transport level in each monolayer insert.

Light and Electron Microscopy of Caco-2 Cells

Thick sections (1 μm) showed that Caco-2 cells grew to a confluent monolayer on microporous filters (Fig. 2). As previously reported (2), the cells became taller and more uniform and developed longer microvilli with increasing age in culture. Transmission electron microscopy at day 22 showed well-differentiated cells with microvilli and tight junctions at the apical surface (Fig. 3). The paracellular channel has been outlined for emphasis in the upper micrograph.



Transport Protocol:

1. Rinse cells with Hank's balanced salts solution.
2. Add compound in Hank's to upper chamber; incubate at 4°C or 37°C.
3. Remove and replace medium in lower chamber at 15 min intervals.
4. Measure radiolabel, fluorescence, or activity in basal solutions and starting material.
5. Calculate cummulative % transport and molar transport per square cm.
6. Calculate transport rate relative to theoretical maximum across filters without cells.
7. Calculate ***transport index*** relative to internal standard.

Fig. 1. Tissue culture insert and transport protocol. Caco-2 cells were plated onto the microporous filters (apical chamber) and allowed to grow to confluency. Transport rates were determined by adding compounds to the upper (apical) chamber and monitoring appearance in the lower (basal) chamber.

Transport of Permeability Markers and Barrier Function of Cells

After cells had reached confluency, transport of permeability markers such as mannitol was used to measure the degree of monolayer permeability (Fig. 4). Mannitol transport across filters with and without cells (3.0 ± 1.5 and 90.6 ± 8.8%/cm^2/hr, respectively) showed that the Caco-2 cell layer was a substantial barrier to mannitol transport.

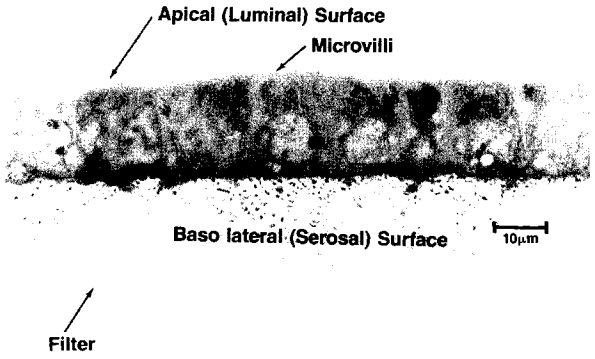


Fig. 2. Light micrograph of Caco-2 cells (day 22) on microporous filters. Sections (1 μm) were stained with Richardson's blue. Note uniform monolayer of cells, microvilli at apical surface, and filter. 1325×; reduced 50% for reproduction.

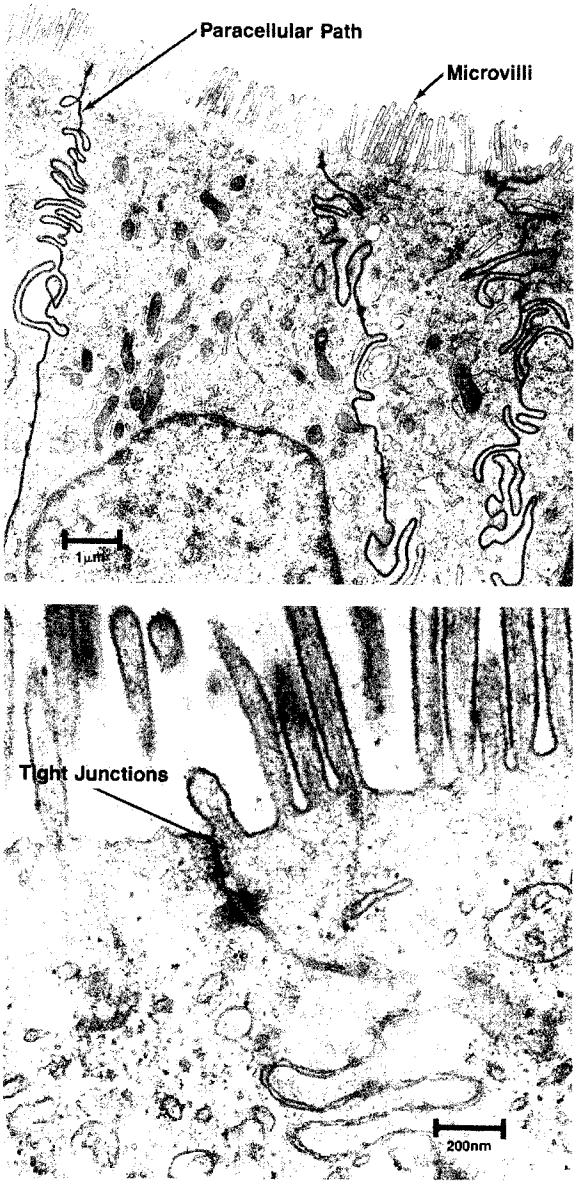


Fig. 3. Transmission electron micrographs of Caco-2 cells (day 22) on microporous filters. Note tight junctions, microvilli, and paracellular path. The paracellular path has been outlined in the top micrograph for emphasis. 16,500×, top; 96,000×, bottom; reduced 35% for reproduction.

Effect of Age on Permeability and Active Transport

Permeability of monolayers as measured by mannitol transport decreased continually with increasing days in culture (Fig. 5). Even though cells at day 7 appeared to be confluent in the microscope, the monolayers showed high levels of permeability to mannitol, presumably due to incomplete formation of tight junctions. Glucose transport, which involves both passive paracellular and carrier-mediated transcellular mechanisms in Caco-2 cells (4), was measured as an indicator of active transepithelial transport (not shown). Transport of glucose remained high throughout the timespan investigated, indicating that a viable active transport system existed in this intestinal transport model. Cell monolayers

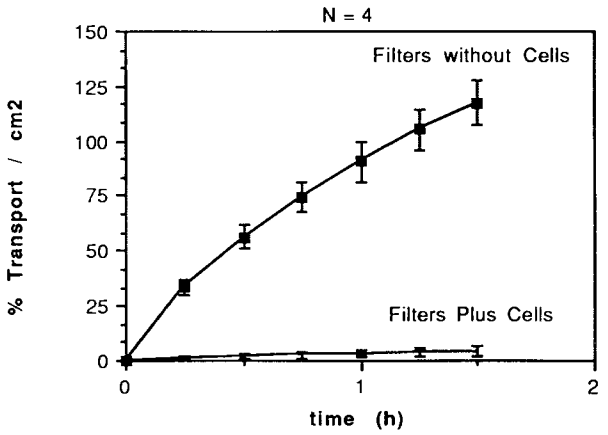


Fig. 4. Mannitol transport across filters with or without Caco-2 cell monolayers. Radiolabeled mannitol was added to the apical chamber and its rate of flux to the basal chamber was monitored. Caco-2 cells on filters caused about a 30-fold reduction in the rate of mannitol flux. $N = 4$.

were routinely used for transport studies between day 18 and day 32.

Effect of Concentration on Transport

Transport of mannitol was investigated across a concentration range from 50 μ*M* to 8 m*M*. The percentage of the total mannitol which was transported per hour was not concentration dependent; however, the amount transported (μmol per cm^2) increased with increasing mannitol concentration (Fig. 6A). This illustrates the importance of evaluating transport rates in terms of percentage of total as well as total amount transported. In contrast, the percentage transport of glucose, which involves saturable transporter-mediated mechanisms, was concentration dependent (Fig. 6B).

Effect of Temperature on Passive and Transporter-Mediated Transport

Transport of permeability markers such as mannitol is

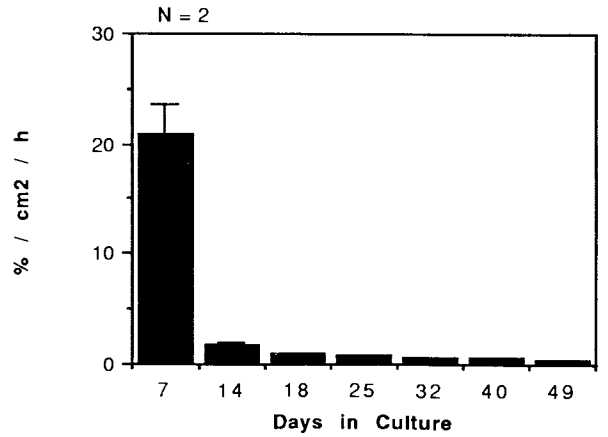


Fig. 5. Effect of days in culture on Caco-2 transport of mannitol. Cells at day 7 were confluent but showed substantial permeability as measured by mannitol flux. Cells became less permeable with increasing time in culture. Cells were used for transport studies between day 18 and day 32. $N = 2$.

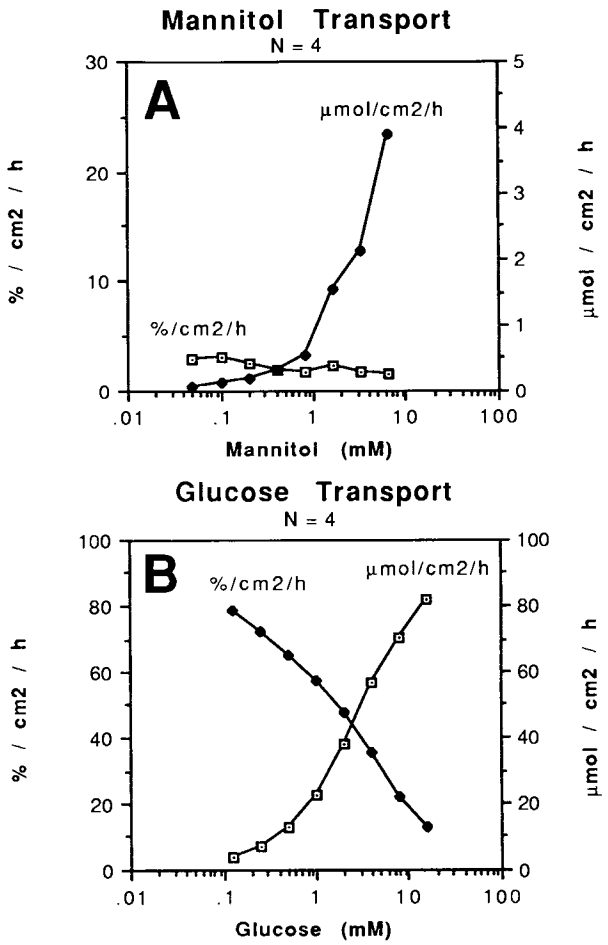


Fig. 6. Effect of concentration on passive and transporter-mediated transport. Percentage transport of mannitol (passive) was not concentration dependent (A), whereas percentage transport of glucose (transporter mediated) was concentration dependent (B). The total amount transported (μmol per cm^2 per hr) was concentration dependent in both cases. $N = 4$.

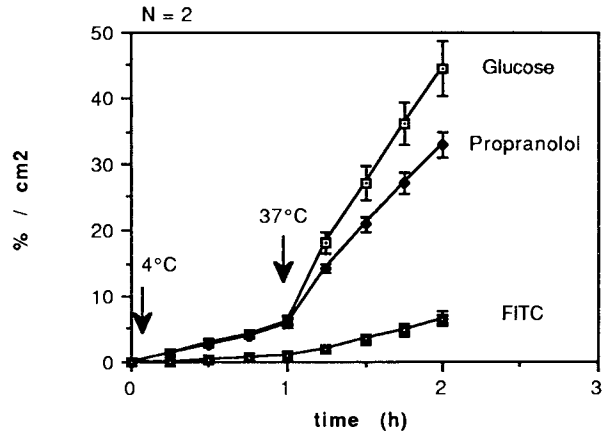


Fig. 7. Effect of temperature on passive hydrophilic, passive lipophilic, and transporter-mediated transport. Transport of 50 μ*M* FITC (passive hydrophilic), 75 μ*M* propranolol (passive lipophilic), and 5 m*M* glucose (transporter mediated) was measured at 4 and at 37°C. Averages of duplicates with standard errors are shown. All three compounds were applied to the cells in the same incubation mixture. FITC transport was low at both temperatures and showed a small increase when the temperature was raised. Glucose and propranolol both showed large increases in transport at the higher temperature. $N = 2$.

believed to involve passive mechanisms and to occur predominantly through the aqueous paracellular channel (5). Comparison of transport at low temperature and physiological temperature was used to elucidate transport mechanisms, since processes requiring energy generation or membrane flow (e.g., endosomal uptake) should be greatly reduced at low temperature. Transport of compounds was first measured for 1 hr at 4°C, followed by a shift to 37°C for 1 hr (Fig. 7). Compounds representative of three types of transport mechanisms were investigated in the same incubation mixture. Hydrophilic fluorescent compounds such as fluorescein isothiocyanate (FITC) have previously been used to measure passive transport through the paracellular pathway (2). Lipophilic compounds such as propranolol have been shown to be transported through cells by passive transcellular mechanisms (6). Glucose is an example of transporter-mediated transport mechanism. The glucose transporter has been demonstrated in Caco-2 cells (4).

As shown in Fig. 7, transport of all three compounds showed varying degrees of temperature dependency. FITC transport was 1.1% per cm^2 per hr at 4°C and increased to 5.4% per cm^2 per hr at 37°C. The change in transport rates from 4 to 37°C can be represented as either fold increase (37°C ÷ 4°C) or absolute difference (37°C − 4°C). FITC transport increased 4.3% per cm^2 per hr when the temperature was raised to 37°C, which represented a 4.8-fold increase. Propranolol transport at 4°C was 5.7% per cm^2 per hr, which increased to 32.9% per cm^2 per hr at 37°C. This was an increase of 27.2% per cm^2 per hr, or 5.7-fold increase. Glucose transport was 6.2% per cm^2 per hr at 4°C and went up to 44.6% per cm^2 per hr at 37°C. This was an increase of 38.4% per cm^2 per hr, or 7.2-fold increase.

The data in Fig. 7 show that the compound undergoing passive hydrophilic transport (FITC) exhibited the smallest effect of temperature, the compound undergoing transporter-mediated transport (glucose) showed the greatest effect of temperature, and the compound undergoing passive lipophilic transport (propranolol) was intermediate. The effect of temperature on transport of a more extended series of compounds is shown in Table I. Unlike the data presented in Fig. 7, separate wells were incubated at the two temperatures in order to avoid possible effects of low temperature incubation on cell physiology. Caco-2 cells used in Table I were at days 29–32, whereas cells in Fig. 7 were at day 18. Note that cells at day 18 routinely exhibited about two-fold higher mannitol transport than cells at day 32, so that data in Fig. 7 and Table I may not be directly comparable. Compounds in Table I were selected based on their predicted mechanisms of transport. Mannitol, PEG-900, and PEG-4000 have frequently been used as indicators of passive hydrophilic transport through the paracellular pathway (5,7). Five compounds were selected which have been reported to undergo transporter-mediated transport across intestinal epithelium (glucose, alanine, methionine, biotin, and spermidine) (8–10). The glucose transporter, however, is the only one of these transporters which has been demonstrated in

Table I. Effect of Temperature on Different Mechanisms of Transport

Sample	Conc. (μM)	N	% transport/cm²/hr		Change from 4 to 37°C	
			4°C	37°C	-Fold increase (37°C/4°C)	Difference (37°C − 4°C)
Hydrophilic permeability markers						
Mannitol[a]	0.26	3	0.33 ± 0.02	0.72 ± 0.05	2.18	0.39
PEG-900	741.00	3	0.11 ± 0.01	0.74 ± 0.07	6.73	0.63
PEG-4000	2500.00	3	0.18 ± 0.00	0.46 ± 0.24	2.56	0.28
Potential transporter-mediated compounds						
Glucose	5000.00	3	3.15 ± 0.10	30.14 ± 3.12	9.57	26.99
Alanine[a]	5.95	3	0.77 ± 0.05	4.12 ± 0.43	5.35	3.35
Methionine[a]	0.001	3	0.68 ± 0.03	3.90 ± 0.47	5.73	3.22
Biotin	0.10	3	0.14 ± 0.01	0.52 ± 0.04	3.71	0.38
Spermidine	0.14	3	0.18 ± 0.00	0.49 ± 0.42	2.72	0.31
Lipophilic compounds						
Alprenolol	0.05	3	1.26 ± 0.00	5.87 ± 0.13	4.66	4.61
Propranolol	0.18	3	2.16 ± 0.00	9.90 ± 0.65	4.58	7.74
Clonidine	0.12	3	5.80 ± 0.00	44.88 ± 5.45	7.74	38.08
Diazepam	0.06	3	11.97 ± 0.00	39.49 ± 1.72	3.30	27.52

[a] HPLC analysis not performed.

Caco-2 cells (4). Lipophilic compounds such as small organic molecules generally exhibit relatively high transport by passive transcellular mechanisms (6).

As shown in Table I, hydrophilic compounds such as mannitol, PEG-900, or PEG-4000 which have routinely been used as permeability markers showed low transport at both 4 and 37°C (≤1%/cm²/hr). The difference in the rates at the two temperatures was small (≤1%/cm²/hr), even though the -fold increase was substantial in some cases (up to sixfold). Diazepam and other lipophilic compounds exhibited as much as 100-fold higher transport than the hydrophilic permeability markers at both 4 and 37°C. The difference between the two temperatures was large (up to 38%/cm²/hr), as was the -fold increase (≤8-fold). Transport of glucose, reported to occur by transporter-mediated mechanisms in Caco-2 cells (4), was greatly reduced at 4°C relative to 37°C. The difference as well as the -fold increase from 4 to 37°C was large (27%/cm²/hr and 10-fold, respectively).

These findings thus suggest that the magnitude of transport at 4 and 37°C, as well as the difference between the two

Table II. Correction Based on Transport across Filters Without Cells

Sample	Conc. (μM)	N	% transport/cm²/hr at 37°C		
			Filter alone	Filter + cells	Filter + cells ÷ filter alone
Hydrophilic permeability markers					
Mannitol[a]	0.26	3	75.63 ± 13.90	0.72 ± 0.05	0.95
PEG-900	741.00	3	72.73 ± 6.62	0.74 ± 0.07	1.02
PEG-4000	2500.00	3	49.73 ± 2.87	0.46 ± 0.24	0.92
Potential transporter-mediated compounds					
Glucose	5000.00	3	87.57 ± 6.40	30.00 ± 3.12	34.25
Alanine[a]	5.95	3	96.58 ± 6.23	4.12 ± 0.43	4.26
Methionine[a]	0.001	3	87.52 ± 8.73	3.90 ± 0.38	4.46
Biotin	0.10	3	82.88 ± 10.67	0.52 ± 0.04	0.63
Spermidine	0.14	3	69.78 ± 1.62	0.49 ± 0.42	0.70
Lipophilic compounds					
Alprenolol	0.05	3	39.63 ± 9.62	5.87 ± 0.13	14.81
Propranolol	0.18	3	42.53 ± 2.57	9.90 ± 0.65	23.28
Clonidine	0.12	3	78.43 ± 8.28	44.88 ± 5.45	57.22
Diazepam	0.06	3	66.08 ± 7.65	39.49 ± 1.72	59.76

[a] HPLC analysis not performed.

temperatures, may be indicative of the primary transport mechanisms. Further studies would be necessary, however, for definitive determination of transport pathways.

Correction for Transport Across Filters Without Cells

Since the microporous filters on which Caco-2 cells are grown may represent a significant barrier to transport, it is important to measure transport across collagen-coated blank filters (Table II). This correction can dramatically alter the apparent rate of transport of some compounds, particularly lipophilic compounds. For example, alprenolol transport across blank filters was approximately half the rate of mannitol transport. This system may not be appropriate for compounds having very low levels of transport across blank filters. Correction for transport across blank filters is one approach for normalization of transport of all compounds relative to each other. Note that the results reported herein have not been corrected for transport across blank filters, with the exception of the data in Table II.

Transport Index (TI)

Barrier integrity of Caco-2 cell monolayers was determined by measuring transport of the permeability marker mannitol which was included as an internal standard with each compound of interest. Rates of transport of compounds predicted to undergo passive paracellular transport (mannitol and PEG-4000), transporter-mediated transcellular transport (alanine, biotin, and spermidine), or lipophilic transcellular transport (alprenolol, clonidine, and diazepam) were compared at 4 and 37°C (Table III). A transport index was calculated by dividing the percentage transport of each compound by the percentage mannitol transport. A similar approach has been used by Cho *et al.* (11) to normalize transport of compounds across MDCK cells to transport of radiolabeled sucrose. Hydrophilic molecules undergoing passive transport had a low transport index relative to mannitol at 4 and 37°C (≤ 1). Lipophilic molecules had a high transport index at both 4 and 37°C (~10–30). Alanine, which is transported by the neutral amino acid transporter in intestines (8) and should undergo transporter-mediated transport if this transporter is expressed in Caco-2 cells, had a high transport index at 37°C (~4–8) and a slightly lower index at 4°C (~5–7). The high level of alanine transport relative to mannitol transport suggests that the alanine transporter may be expressed in Caco-2 cells; however, further experiments would be necessary to definitively answer this question. Two other compounds that have been reported to undergo transporter-mediated transport *in vivo,* biotin and spermidine (9,10), showed low levels of transport at both 4 and 37°C, suggesting that these transporters were not expressed in Caco-2 cells.

These data demonstrate that an evaluation of the effect of temperature on transport rates may provide an indication of transport mechanisms but must be coupled with other experimental manipulations for definitive determination of transport pathways.

DISCUSSION

Ideally, this cell culture model would have utilized cells derived from normal human small intestinal tissue. However, conditions have not yet been established for maintaining differentiated primary intestinal epithelial cells in culture. Caco-2 cells, which were originally derived from a human colon carcinoma by Fogh *et al.* (1), differentiate in culture into cells with many properties of small intestinal absorptive cells. These cells can be passaged many times and spontaneously undergo differentiation after reaching confluency. Properties of Caco-2 cells which are similar to small intestinal absorptive cells include ion transport (12–15), brush border hydrolases (15–18), vitamin uptake (19) and gut-neuropeptide hormone response (12). General morphological characteristics of small intestinal cells have also been demonstrated (16). Even though Caco-2 cells have been used extensively to investigate intestinal differentiation, very few papers have been published on their transport prop-

Table III. Caco-2 Transport Index Based on Mannitol as Permeability Marker

Compound	Concentration (m*M*)	Transport index[a] 4°C	37°C
	Hydrophilic permeability markers		
Mannitol[b]	0.01	(1.0)	(1.0)
	0.10	(1.0)	(1.0)
	1.00	(1.0)	(1.0)
	10.00	(1.0)	(1.0)
PEG-4000	0.01	0.08	0.11
	0.10	0.10	0.11
	1.00	0.11	0.11
	10.00	0.14	0.13
	Lipophilic compounds		
Alprenolol	0.01	13.33	11.02
	0.10	11.12	8.99
	1.00	ND[c]	10.50
Clonidine	0.01	22.64	18.99
	0.10	26.83	22.00
	1.00	19.25	22.44
	10.00	ND	23.50
Diazepam	0.004	29.58	22.45
	0.008	31.44	25.31
	0.016	25.43	19.29
	0.033	27.27	28.43
	Potential transporter-mediated compounds		
Alanine[b]	0.01	7.09	3.99
	0.10	5.65	7.52
	1.00	5.14	7.56
Biotin	0.01	0.30	0.27
	0.10	0.33	0.28
	1.00	0.31	0.25
	10.00	0.33	0.23
Spermidine	0.01	0.57	0.49
	0.10	0.66	0.48
	1.00	0.74	0.54
	10.00	ND	0.86

[a] (Compound % transport/hr) ÷ (mannitol % transport/hr).
[b] HPLC analysis not performed.
[c] Not determined.

erties. A recent paper by Hidalgo *et al.* (2) describes a similar cell culture model with Caco-2 cells growing on microporous filters. Our results on Caco-2 permeability as measured by flux of permeability markers were in agreement with their findings. In addition, Hidalgo *et al.* (2) measured resistance of Caco-2 monolayers and showed electrical resistance levels which were equivalent to those of small intestinal tissue (250–300 Ω/cm^2), a finding we also confirm (data not shown).

The transport index (TI) described herein is similar to that of Cho *et al.* (11). The TI provides a means of comparing transport rates of different compounds by calculation of a ratio based on percentage transport to percentage transport of an internal standard for measuring monolayer permeability. The internal standard makes it possible to monitor effects of compounds themselves on transport of permeability markers. The data obtained thus far suggest that TI determination at different temperatures may be indicative of the primary transport pathway, but further studies would be required for definitive determination of transport pathways.

The Caco-2 transport model has given us a means of isolating the intestinal epithelial cell barrier, and thus elucidating its role in oral bioavailability. Further studies are now under way to determine whether the Caco-2 model is representative of the human intestinal epithelial barrier and whether it can be used to predict *in vivo* intestinal absorption.

ACKNOWLEDGMENTS

Electron microscopy was done by Gabriele Nieses, and HPLC analysis was done by Sheldon Verrett. We would like to express our appreciation for their contributions to this project.

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