A Correlation Between the Permeability Characteristics of a Series of Peptides Using an *in Vitro* Cell Culture Model (Caco-2) and Those Using an *in Situ* Perfused Rat Ileum Model of the Intestinal Mucosa

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In an attempt to establish an *in vitro/in situ* correlation of intestinal permeability data, the permeability coefficients (P_{app}) for a series of model peptides, which were determined using an *in situ* perfused rat ileum model, were compared to the permeability coefficients (P_{mono}) determined using an *in vitro* cell culture model (Caco-2). The model peptides, which were all blocked on the N-terminal (acetyl, Ac) and the C-terminal (amide, NH_2) ends, consisted of D-phenylalanine (F) residues (e.g., $AcFNH_2$, $AcFFNH_2$, $AcFFFNH_2$). To alter the degree of hydrogen bonding potential, the nitrogens of the amide bonds were sequentially methylated [e.g., $AcFF(Me)FNH_2$, $AcF(Me)F(Me)FNH_2$, $Ac(Me)F(Me)F(Me)FNH_2$, Ac-(Me)F(Me)F(Me)FNH(Me)]. These peptides were shown not to be metabolized in the *in situ* perfused rat ileum system. The results of the transport experiments showed that there were poor correlations between the apparent permeability coefficients (P_{app}) determined in an *in situ* perfused rat ileum model and the octanol–water partition coefficients ($r = 0.60$) or the hydrogen bonding numbers ($r = 0.63$) of these peptides. However, good correlations were observed between the *in situ* P_{app} values for these peptides and their partition coefficients in heptane–ethylene glycol ($r = 0.96$) and the differences in their partition coefficients between octanol–water and isooctane–water ($r = 0.86$). These results suggest that lipophilicity may not be the major factor in determining the intestinal permeability of these peptides and that hydrogen bonding potential may be a major contributing factor. A good correlation ($r = 0.94$) was also observed between the P_{app} values determined for these peptides in the *in situ* perfused ileum model and those P_{mono} values determined in the *in vitro* cell culture model (Caco-2) (Conradi *et al., Pharm. Res.* 8:1453–1460, 1991). These results suggest that the permeability values determined in the Caco-2 cell culture model may be a good predictor of the intestinal permeability of peptides.

KEY WORDS: permeability; peptides; *in vitro* cell culture; Caco-2; *in situ* perfusion; rat ileum; intestinal mucosa.

INTRODUCTION

Through rational drug design, medicinal chemists have synthesized many peptides with novel therapeutic potential (1). However, one of the major problems in developing these compounds as therapeutic agents is their low oral bioavailability (2–4). Low bioavailability can result from several factors, including low intestinal mucosal cell permeability, first-pass metabolism in the intestinal mucosa and the liver, rapid liver clearance (3), and rapid metabolism in the blood or peripheral tissues. The metabolic liability of peptides has been resolved in part by the incorporation of bioisosters of the peptide bond that are not susceptible to hydrolysis by peptidases (4). However, medicinal chemists have not achieved this same degree of success in designing peptides with enhanced cell membrane permeability or peptides with minimal potential to be cleared by the liver (5).

Recently, an *in vitro* cell culture model of the intestinal mucosa, consisting of human colon carcinoma cells (Caco-2) grown on microporous membranes, has been developed (6–9) and used to improve our understanding of structural features that influence peptide permeability (5,10–12) and to estimate the intestinal permeability of drugs (14,15). To date, investigators have attempted to correlate *in vitro* cell culture permeability data with percentage absorption after an oral dose (14,15). Since the bioavailability of a solute is dependent on a number of factors, with intestinal cell permeability being only one of these factors, this approach is problematic.

Therefore, in this study we have measured the permeability of a series of model peptides using an *in situ* perfused rat ileum model and attempted to correlate these data with the physicochemical characteristics (e.g., hydrogen bond numbers, partition coefficient in octanol–water, isooctane–water, and heptane–ethylene glycol) of the peptides. In addition, since the permeability characteristics of these model peptides were recently studied *in vitro* using the Caco-2 cell culture system (10,12), attempts were made to correlate these Caco-2 cell permeability data with the permeability data obtained using the *in situ* rat perfused ileum model.

MATERIALS AND METHODS

Peptides

The model peptides [$AcFNH_2$, **1**; $AcFFNH_2$, **2**; $AcFFFNH_2$, **3**; $AcFF(Me)FNH_2$, **4**; $AcF(Me)F(Me)FNH_2$, **5**; $Ac(Me)F(Me)F(Me)FNH_2$, **6**; and Ac(Me)F(Me)F(Me)-FNH(Me), **7**] were synthesized and radiolabeled with ^{14}C in the acetamide carbon position with specific activities of approximately 110 mCi/mmol using previously described procedures (10,12). The peptides were stored in a CH_3OH:$CHCl_3$ (50/50) solution at −70°C. All other reagents were analytical grade.

Rat Intestinal Perfusion Studies

Male Sprague–Dawley rats (300–350 g) were obtained from the University of Kansas Animal Care Unit. Cannulation of the jugular vein, the ileum, and the mesenteric vein of the rat was accomplished sequentially using a modification of a published method (16). Briefly, the rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (20 mg/rat). A PE-50 cannula (0.965-mm o.d. × 0.58-mm i.d.) was inserted into the jugular vein for the blood supply from donor rat. A longitudinal midline incision was made in the

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abdomen of the rat, and the distal site of the small intestine (ileum) at 20 cm above the beginning of the colon was cannulated with an L-shaped glass cannula (4-mm o.d. × 2-mm i.d. × 1 cm) attached to the intestine with silk thread. Prewarmed (37°C) perfusate buffer (Hanks' balanced salt solution, pH 7.4, containing 25 m*M* glucose and 10 m*M* HEPES) was perfused through the cannulated ileum using a positive displacement minipump (FMI Lab. Jr., Fluid Metering Instrument Co., Oyster Bay, NY). The mesenteric vein coming from the ligated intestinal loop was isolated by careful blunt dissection. A specially designed mesenteric vein cannula [1 cm of Silastic tubing (0.047-in. o.d. × 0.025-in. i.d.) attached to a polyethylene tubing (PE-50) filled with heparinized saline solution] was inserted into the mesenteric vein. The cannula was secured in the mesenteric vein with 2–3 drops of cyanoacrylate adhesive. The animal was heparinized by i.v. administration of 0.3 mL of heparin solution (1000 U/mL) into the femoral vein. Within 10 sec after the cannulation of the mesenteric vein, freshly drawn heparinized rat blood was infused into the jugular vein of the rat at a rate of 0.85 mL/min using a constant infusion pump (Harvard Apparatus, Inc., South Natick, MA) to replenish the blood lost through the mesenteric vein. The hematocrit values were not changed between the first and the last blood samples obtained from the mesenteric vein.

Transport Studies with Model Peptides in Perfused Rat Ileum

For drug transport studies, about 10 cm of the cannulated rat ileum was perfused with Hanks' balanced salt solution using a minipump until the outflow from the cannulated ileum became visually clear. Then a solution (150 μL) of a ^{14}C-labeled model peptides (2 μCi) was injected into the recirculating stream of the perfusate through an in-line tee containing an injection port with a Teflon-faced septum (Universal Septum Injector, Anspec Inc., Ann Arbor, MI). Samples of the perfusate were collected from the tubing located in front of the perfused ileum at 2, 4, 7, 10, 20, and 30 min while simultaneously collecting blood from the mesenteric vein cannula in separate containers. The perfusate flow rate was 2.0 mL/min. At the end of the experiment, the blood samples were weighed to determine the blood volume and centrifuged for 3 min at 3000 rpm to provide the plasma fraction. The length and diameter of perfused rat ileum were measured using a thread at the end of the experiment. The total volume of the perfusion system was 6.0–7.0 mL.

Analytical Methods

The perfusate samples were divided into two fractions. One fraction was used to determine levels of radioactivity by liquid scintillation counting (Beckman LS5801). Another fraction was analyzed by HPLC using procedures described previously (10). Briefly, the system consisted of a Beckman Model pump (112 Solvent Delivery Module) and a Perkin-Elmer LCI-100 integrator. The column was a Brownlee Sphere-5 RP-18 (5 μm, 4.6 × 100 mm) and RP-18 guard column (1.5 cm). The mobile phase consisted of 40% acetonitrile, 60% distilled water, 0.02% trifluoroacetic acid (TFA), and 0.02% dimethyloctylamine (DMOA). The flow rate was 1.0 mL/min at room temperature. A perfusate sample (50 μL) was injected into the C-18 column and the effluent was collected at 0.4-min time intervals. Three milliliters of scintillant (3a70B from RPI Corp., Mount Prospect, IL) was added to 400 μL of effluent and the level of radioactivity was determined by liquid scintillation counting. The level of radioactivity of model peptides in the plasma was determined by liquid scintillation counting.

Partition Coefficients of Model Peptides

The octanol–water, isooctane–water, and heptane–ethylene glycol partition coefficients for model peptides 1–7 were taken from Refs. 10 and 12.

Kinetic Analysis of the Time Profiles of Disappearance from the Perfusate and Appearance in the Mesenteric Vein of Model Peptides

To assess the permeability of model peptides in the perfused rat ileum, a compartment model was designed to describe the perfusion system. The rate of appearance of model peptide (dX/dt) in the mesenteric vein plasma is proportional to the concentration of model peptides in the perfusate (C_p) based on the assumptions made in the compartment model (see details in the legend to Fig. 1):

$$dX/dt = CL_{app}C_p \quad (1)$$

where CL_{app} is the apparent transport clearance of model peptide across the perfused ileum. By integrating both sides of Eq. (1) from time 0 to time t, when the perfusion was terminated, we obtain

$$CL_{app} = X_{(0-t)}/AUC_{(0-t)} \quad (2)$$

where $X_{(0-t)}$ is the accumulated amount of model peptides from time 0 to time t, and $AUC_{(0-t)}$ is the area under the concentration–time profile of model peptides in the perfusate. $AUC_{(0-t)}$ was calculated by a trapezoidal method (17). To compare the membrane permeability of model peptides between the perfused rat ileum and the Caco-2 cell systems, the CL_{app} values determined in the perfused rat ileum were transformed into the apparent permeability coefficient (P_{app}) using the following equation:

$$P_{app} = CL_{app}/SA \quad (3)$$

where SA is the effective surface area of the perfused rat ileum through which the transport of model peptides occur. The surface area was calculated from the radius and the length of the perfused rat ileum (18).

RESULTS AND DISCUSSION

For these studies, a series of model peptides consisting of D-phenylalanine (F) residues (e.g., $AcFNH_2$, **1**; $AcFFNH_2$, **2**; $AcFFFNH_2$, **3**), which were blocked on the N-terminal (acetyl, Ac) and the C-terminal (amide, NH_2) ends, was employed. To alter the degree of hydrogen bonding potential, the nitrogens of the amide bonds were sequentially methylated [e.g., $AcFF(Me)FNH_2$, **4**; $AcF(Me)F(Me)FNH_2$, **5**; $Ac(Me)F(Me)F(Me)FNH_2$, **6**; Ac(Me)F(Me)F(Me)FNH(Me), **7**]. The permeability coefficients (P_{mono}) of these peptides determined using an *in vitro* cell culture model (Caco-2) of the intestinal mucosa have been reported

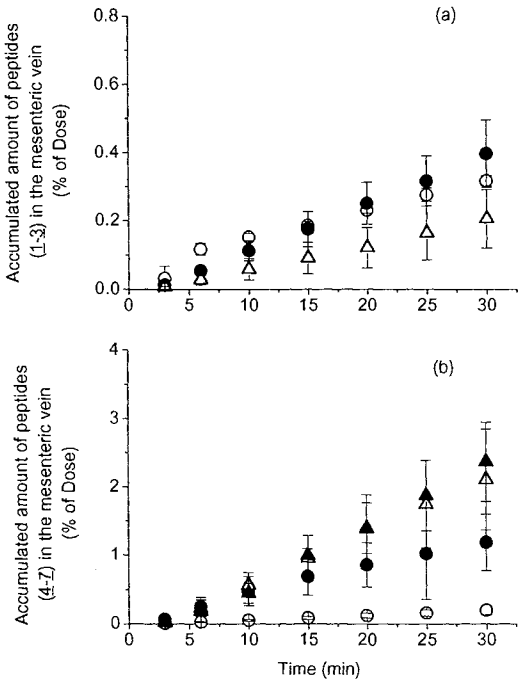

Fig. 1. Time profiles of appearance in the mesenteric vein of model peptides: (a) 1 (○), 2 (●), and 3 (△); (b) 4 (○), 5 (●), 6 (△), and 7 (▲). After a bolus administration of 150 μL (2 μCi) of the model peptides into a stream of the perfusate, the rat ileum was perfused for 30 min in a recirculation mode. The perfusate samples were withdrawn at the designated times and the blood outflowing from the mesenteric vein was collected at various sampling time intervals. The levels of radioactivity in the perfusate and mesenteric vein plasm were determined as described under Materials and Methods. To assess the permeability of model peptides across the perfused rat ileal membrane, the following assumptions were made: (a) the transport of model peptides across the intestinal membrane follows first-order kinetics; (b) efflux of the model peptides or metabolites back into the ileum compartment does not occur; and (c) the extraction of model peptides during a single pass through the perfused ileum is low enough to approximate the concentration of model peptides in the ileum compartment to the concentration in the reservoir compartment. These assumptions, used in the derivation of Eq. (1), were supported by the following observations. The mesenteric vein blood was continuously withdrawn during the perfusion experiment; thus, sink conditions in the mesenteric vein compartment were maintained throughout the perfusion experiment. Therefore, only unidirectional transport (from ileum to mesenteric vein) of the model peptides should occur under these conditions. No metabolites derived from model peptides were identified when the perfusate samples were analyzed by HPLC. Finally, the single-pass extraction of model peptides through the perfused rat ileum was very small (<3%).

previously (10,12). Using the published *in vitro* permeability data and the data generated in this study using the *in situ* model, it was possible to establish a correlation between the *in vitro* and the *in situ* permeability characteristics of this series of peptides.

Before we undertook the determination of the kinetics of transport of model peptides 1–7 in the perfused rat ileum model, studies were conducted to determine if these peptides were susceptible to metabolism. Samples of the intestinal perfusate obtained after a 30-min recirculatory perfusion of model peptides 1–7 at 37°C were analyzed by HPLC. No metabolites of model peptides 1–7 were identified in the perfusate samples. The retention times of each of the model peptides in the perfusate samples were identical to those of the standard peptides (i.e., **1**, 3.6 min; **2**, 4.8 min; **3**, 6.4 min; **4**, 8.8 min; **5**, 7.6 min; **6**, 13.6 min; **7**, 14.8 min). These results suggested that the model peptides were not degraded during the perfusion experiments. Our results are consistent with data reported by Karls *et al.* (13), who administered this same series of model peptides into the duodenum of rats. Analysis of the contents of the duodenum and the blood obtained from the mesenteric vein using HPLC revealed no apparent metabolism of these model peptides.

During the recirculatory perfusion of the ^{14}C-labeled model peptides 1–7, both the perfusate and the mesenteric vein plasma were analyzed. The levels of radioactivity in the perfusate decreased slightly with time (data not shown). The decreases in the concentration of the model peptides in the perfusate were comparable to that of [^{3}H]PEG (4000) (data not shown), which is an impermeable volume marker. However, the levels of accumulated radioactivity in the mesenteric vein plasma increased with time of perfusion (Figs. 1a and b). The amounts of radioactivity appearing in the mesenteric vein plasma varied depending on the model peptide (Figs. 1a and b). To identify the physicochemical factors responsible for the different membrane permeabilities of the model peptides, we quantitated the flux of model peptides across the perfused rat ileum. A simple compartment model was used to calculate the transport clearance (CL_{app}) values of the model peptides, which were then transformed into the permeability coefficients (P_{app}). The P_{app} values generated using this *in situ* rat ileum model and the physicochemical properties of the model peptides are summarized in Table I.

When the *in situ* permeability coefficients (P_{app}) of the model peptides were plotted as a function of lipophilicity, as measured by partition coefficients in octanol–water, a poor correlation (r = 0.60) was observed (Fig. 2a), suggesting that the lipophilicity is not the major determining factor in the transport of the model peptides. A poor correlation (r = 0.63) was also observed when the *in situ* P_{app} values were plotted as a function of the hydrogen bonding number for the peptide (Fig. 2b). However, when these *in situ* P_{app} values were plotted as a function of the differences in their partition coefficients in octanol–water and in isooctane–water (Fig. 2c) or their partition coefficients determined in heptane–ethylene glycol (Fig. 2d), excellent correlation coefficients (r = 0.86 and 0.96, respectively) were observed.

Earlier, Conradi *et al.* (10,12) and Burton *et al.* (11) showed that good correlations exist between the Caco-2 cell permeability (P_{mono}) of these model peptides and their hydrogen bond numbers, their partition coefficients in heptane–ethylene glycol, and the differences in their partition coefficients between octanol–water and isooctane–water. These solvent systems are measures of the hydrogen bonding potential of the peptides (11). These Caco-2 cell permeability data could not be correlated with the lipophilicity of the peptides as measured by octanol–water partition coefficients (10–12). These observations are consistent with the hypothesis presented by Stein (19); that is, if the rate-limiting step in the transport of a polar solute across a cell membrane is the desolvation, there should be a correlation between the

Table I. Physicochemical and Permeability Properties of Model Peptides 1–7

Peptide	No. of hydrogen bonds	logPC[a]			P_{mono} Caco-2[b]	P_{app} *in situ* intestinal perfusion[c]
		Octanol–water	Isooctane–water	Heptane–ethylene glycol		
$AcFNH_2$ (1)	5	0.05	−4.92	−5.46	8.83	1.52
$AcFFNH_2$ (2)	7	1.19	−5.29	−6.52	2.26	1.40
$AcFFFNH_2$ (3)	9	2.30	−5.02	−7.10	0.60	0.333
$AcFF(Me)FNH_2$ (4)	8	2.63	−4.20	−6.28	2.88	0.569
$AcF(Me)F(Me)FNH_2$ (5)	7	2.53	−3.10	−5.14	6.11	5.12
$Ac(Me)F(Me)F(Me)FNH_2$ (6)	6	2.92	−1.67	−4.20	16.7	9.42
Ac(Me)F(Me)F(Me)FNH(Me) (7)	5	3.24	−0.69	−2.86	33.9	13.3

[a] Partition coefficient values (PC) were taken from Refs. 10 and 12.
[b] Permeability coefficient values (P_{app}) (10^{-6} cm/sec) determined in Caco-2 cell monolayer were taken from Refs. 10 and 17.
[c] Permeability coefficient values (P_{app}) (10^{-6} cm/sec) in the *in situ* perfused rat ileum model were determined as described under Materials and Methods.

total number of hydrogen bonds the solute can make with water and its permeability. In other words, for a polar solute to cross a cell membrane, the hydrogen bonds formed between a polar solute and water need to be broken. Thus, the energy required for breaking the hydrogen bonds between the polar solute and water may be a significant barrier in the transport of a polar solute across a lipophilic cell membrane.

The data presented in this manuscript suggest that the physicochemical factors (e.g., hydrogen bonding potential) that influence the permeability of a peptide across a monolayer of Caco-2 cells are the same physicochemical factors that affect its permeability across the intestinal mucosa as measured using the *in situ* perfused ileum model. If similar physicochemical factors influence the permeability of a peptide *in vitro* and *in situ*, then a correlation should exist between the permeability coefficients determined using these different models of the intestinal mucosa.

When the P_{app} values for the model peptides determined in the *in situ* perfused ileum model (Table I) were plotted as a function of the P_{mono} values determined in the *in vitro* Caco-2 cell model (Table I), an excellent correlation (r = 0.94; Fig. 3) between these experimental models was observed. These results are somewhat different from those reported earlier on the duodenal absorption of these peptides in the rat. In that study (13), a good correlation was observed for peptides 3–7, but peptides 1 and 2 had significantly greater absorption in the rat than predicted form the Caco-2 cell permeability. Karls *et al.* (13) argued that this lack of correlation for the entire series may represent a more significant contribution from paracellular flux for peptides 1 and 2 *in vivo*. This apparent discrepancy with the present work

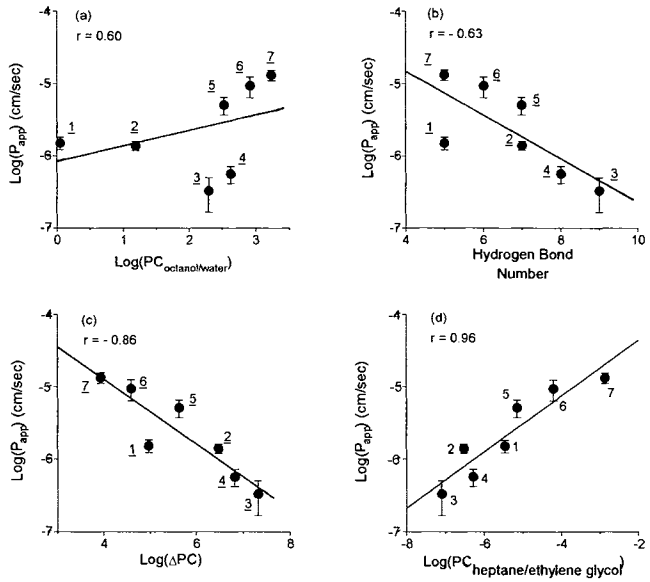

Fig. 2. Relationship between logP_{app} determined in the intestinal perfusion system and the $logPC_{octanol/water}$ (a), the hydrogen bond numbers (b), the ΔlogPC (c), and the $logPC_{heptane-ethylene\ glycol}$ (d). The values of hydrogen bond number, $PC_{octanol/water}$, ΔlogPC, and $PC_{heptane-ethylene\ glycol}$ are given in Table I. The values of P_{app} were determined as described under Materials and Methods and in the legend to Fig. 1. Values are averages of triplicate determinations.

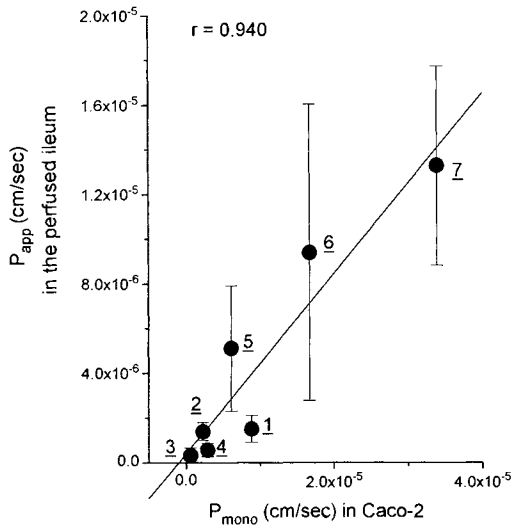

Fig. 3. Relationship between the P_{app} values determined in the *in situ* perfused rat ileum model and the P_{mono} values determined in the Caco-2 cell culture model. The P_{app} and P_{mono} values are given in Table I. Values are averages of triplicate determinations.

may reflect a difference in the density of the "aqueous pores" (paracellular route) in the duodenum compared to the ileum of the rat. Alternatively, it is possible that some vehicle effects were present in the earlier study which resulted in a solvent drag contribution to the transport of peptides 1 and 2.

The results reported in this manuscript indicate that the data generated from Caco-2 cell monolayer experiments (10–12) would not have estimated the "absolute" permeability values observed in the perfused rat ileum model. However, the Caco-2 permeability values would have accurately predicted the rank order of the permeabilities of these model peptides in this *in situ* model. This type of information about a series of drug candidates, when considered in light of the propensity of these compounds to undergo first-pass metabolism and/or liver clearance, will allow pharmaceutical scientists to make more intelligent decisions about which compounds to move into animal studies. In addition, when *in vitro–in situ* and *in vitro–in vivo* correlations can be demonstrated for a series of compounds, then the results of Caco-2 cell experiments will be helpful to medicinal chemists as they attempt to make structural modifications so as to optimize the oral bioavailability of peptides and peptide mimetics.

It should be noted that Burton *et al.* (20) recently described the existence of a saturable, apically polarized transport system in Caco-2 cells which reduces the apical-to-basolateral transport of some peptides. An analogous system has also been observed in the intestine of rabbit (N. F. H. Ho, J. S. Day, and P. S. Burton, unpublished data). Since this type of transport system has not yet been fully characterized in these intestinal preparations, the transport kinetics for the model peptides used in this study were not corrected for the effects of this apically polarized transport system.

In summary, a good correlation between membrane permeability and the hydrogen bonding potential for the model peptides (1–7) was demonstrated in an *in situ* perfused rat ileum. In addition, a good correlation between permeability values determined in an *in situ* perfused rat ileum and in an *in vitro* Caco-2 cell model was also observed, suggesting that the Caco-2 cell monolayer is a good experimental system for studying passive transport of peptides across the intestinal cell membrane.

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REFERENCES

1. C. Oliyai, C. Schoneich, G.-S. Wilson, and R. T. Borchardt. Chemical and physical stability of protein pharmaceuticals. In D. J. A. Crommelin and K. K. Midha (eds.), *Topics in Pharmaceutical Sciences 1991*, Med. Pharm. Scientific, Stuttgart, 1992, pp. 23–46.
2. V. H. L. Lee (ed.). *Peptide and Protein Delivery*, Marcel Dekker, New York, 1991.
3. V. H. L. Lee and A. Yamamoto. Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug. Deliv. Rev.* 4:171–207 (1990).
4. H. Nellans. Paracellular intestinal transport: Modulator of absorption. *Adv. Drug. Deliv. Rev.* 7:339–364 (1991).
5. P. S. Burton, R. A. Conradi, and A. R. Hilgers. Transcellular mechanism of peptide and protein absorption: Passive aspects. *Adv. Drug. Deliv. Rev.* 7:365–386 (1991).
6. I. J. Hidalgo, T. J. Raub, and R. T. Borchardt. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* 96:736–749 (1989).
7. G. Wilson, J. F. Hassam, C. J. Dix, I. Williamson, R. Shah, M. Mackay, and P. Artursson. Transport and permeability properties of human Caco-2 cells: An *in vitro* model of the intestinal epithelial cell barrier. *J. Control. Release* 11:25–40 (1990).
8. A. R. Hilgers, R. A. Conradi, and P. S. Burton. Caco-2 cell monolayer as a model for drug transport across the intestinal mucosa. *Pharm. Res.* 7:902–910 (1990).
9. P. Artursson. Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.* 79:476–482 (1990).
10. R. A. Conradi, A. R. Hilgers, N. F. H. Ho, and P. S. Burton. The influence of peptide structure on transport across Caco-2 cells. *Pharm. Res.* 8:1453–1460 (1991).
11. P. S. Burton, R. A. Conradi, A. R. Hilgers, N. F. H. Ho, and L. L. Maggiora. The relationship between peptide structure and transport across epithelial cell monolayers. *J. Control. Release* 9:87–98 (1992).
12. R. A. Conradi, A. R. Hilgers, N. F. H. Ho, and P. S. Burton. The influence of peptide structure on transport across Caco-2 cell. II. Peptide bond modification which results in improved permeability. *Pharm. Res.* 9:435–439 (1992).
13. M. S. Karls, B. D. Rush, K. F. Wilkinson, T. J. Vidmar, P. S. Burton, and M. J. Ruwart. Desolvation energy: A major determinant of absorption, but not clearance, of peptides in rats. *Pharm. Res.* 8:1477–1481 (1991).
14. W. Rubas, N. Jezyk, and G. M. Grass. Comparison of the permeability of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey and dog intestine and human drug absorption. *Pharm. Res.* 10:113–118 (1993).
15. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* 175:880–885 (1991).
16. K. R. Buttleworth and D. Pelley. Mesenteric venous blood sampling *in vivo* in the rat. *J. Physiol.* 232:60P–61P (1973).
17. K. C. Yeh and K. C. Kwan. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximations. *J. Pharmacokinet. Biopharm.* 6:79–98 (1978).
18. N. F. H. Ho, J. S. Day, C. L. Barsuhn, P. S. Burton, and T. J. Raub. Biophysical model approaches to mechanistic transepithelial studies of peptides. *J. Control. Release* 11:3–24 (1990).
19. W. D. Stein. The molecular basis of diffusion across cell membranes. In *The Movement of Molecules Across Cell Membranes*, Academic Press, New York, 1967, pp. 65–125.
20. P. S. Burton, R. A. Conradi, A. R. Hilgers, and N. F. H. Ho. Evidence for a polarized efflux system for peptides in the apical membrane of Caco-2 cells. *Biochem. Biophys. Res. Commun.* 190:760–766 (1993).