

## Regulation of Paracellular Absorption of Cimetidine and 5-Aminosalicylate in Rat Intestine

Simon Yuji Zhou,<sup>1</sup> Nusara Piyapolrunroj,<sup>2</sup> Li-heng Pao,<sup>3</sup> Cheng Li,<sup>4</sup> Guangyu Liu,<sup>5</sup> Ellen Zimmermann,<sup>6</sup> and David Fleisher<sup>5,7</sup>

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**Purpose.** Isolating the relative contributions of parallel transcellular and paracellular transport to the intestinal absorption of small hydrophilic molecules has proven experimentally challenging. In this report, luminal appearance of drug metabolite is utilized as a tool to assess the contribution of paracellular transport to the absorption of cimetidine and 5-aminosalicylate (5ASA) in rat small intestine.

**Methods.** Steady-state intestinal absorption and elimination of cimetidine and 5ASA were studied in single-pass intestinal perfusions in rats.

**Results.** Both drugs were metabolized in intestinal epithelia with subsequent metabolite secretion into the intestinal lumen. Jejunal cimetidine absorption decreased with increasing perfusion concentration while the ratio of luminal metabolite to luminal drug loss increased. Cimetidine uptake at perfusion concentrations above 0.4 mM resulted in over 80% drug elimination into the jejunal lumen. Inhibition of intracellular metabolism of cimetidine by methimazole did not alter epithelial uptake but totally abolished transepithelial cimetidine flux indicating an elevation of intracellular cimetidine. Similarly, co-perfusion of 5ASA with cimetidine and methimazole totally abolished 5ASA absorption but increased luminal levels of N-acetyl 5ASA indicating an increase in intracellular uptake of 5ASA.

**Conclusions.** Cimetidine and 5ASA absorption across rat jejunal epithelia are exclusively paracellular. Elevation of intracellular cimetidine, inferred from mass balance considerations, restricts paracellular transport of both drugs.

**KEY WORDS:** 5-aminosalicylate; cimetidine; paracellular transport; intestinal absorption; intestinal metabolism.

### INTRODUCTION

On the basis of a leaky epithelial structure, intestinal absorption of small hydrophilic drug molecules is projected to include parallel transcellular and paracellular transepithelial pathways (1). Access to the paracellular space is limited since

entry surface has been estimated to constitute only 1/10000 of the total surface area of the intestinal epithelia (2). Further restrictions to this absorption pathway are provided by tight junction control of solute entry (3,4). These limitations argue against a substantial paracellular absorption component even when drug partition coefficients are low (5,6). However, paracellular transport contributions to absorption have been projected for some drugs as a function of molecular size (7), charge (8) and shape (9).

Paracellular contributions to drug absorption are difficult to assess experimentally. The most common methods to evaluate paracellular drug transport involve correlation with small hydrophilic markers and measurement of transepithelial resistance subsequent to application of direct or alternating current. Mannitol is commonly employed as a marker to factor paracellular contributions to the absorption of other solutes (10). There have been some conflicting experimental results regarding the transport of mannitol and other monosaccharides (11,12). Changes in electrical resistance and impedance are presumed to reflect changes in the availability of the lateral intracellular space to transport of small inorganic ions. However, transcellular influences on electrical current can be substantial and may complicate the interpretation of experimentally measured changes in transepithelial resistance (13).

This study shows that two hydrophilic compounds are absorbed exclusively by paracellular transport in rat jejunum. It is demonstrated, using intestinal metabolite measurements and mass balance considerations, that intracellular drug uptake does not result in transcellular transport of cimetidine and 5ASA from *in situ* jejunal perfusion. Furthermore, intracellular drug levels are controlled by drug metabolism with subsequent metabolite secretion across the apical membrane. Intracellular drug does not appreciably exit the basolateral membrane and, hence, there is no transcellular contribution to the absorption of these drugs. Most importantly, metabolite inhibition studies suggest that intracellular cimetidine levels provide a sensing mechanism to restrict paracellular solute transport and drug absorption.

### MATERIALS AND METHODS

#### Chemicals

5-aminosalicylic acid, cimetidine, 2(N-morpholino)ethanesulfonic acid (MES), and polyethylene glycol 4000 (PEG 4000) were purchased from Sigma Chemical Co., (St. Louis, MO). Acetic acid anhydride and <sup>14</sup>C-PEG 4000 were obtained from Aldrich Chemical Co, (St. Louis, MO) and New England Nuclear (Boston, MA) respectively. N-acetyl-5ASA was synthesized in house by reacting 5-ASA with acetic acid anhydride under catalysis of triethylamine in ethyl ester (14). Smith Kline Beecham Pharmaceuticals (King of Prussia, PA of Prussia) kindly provided cimetidine S-oxide. All the other chemicals were of reagent grade and HPLC grade.

#### Single-Pass Perfusion Experiments

Experimental procedures have been previously described (15) and approved by the University of Michigan Committee on Use and Care of Animals. Briefly, male Sprague-Dawley

<sup>1</sup> Candidate Synthesis Enhancement and Evaluation, Pfizer Central Research, Eastern Point Road, Groton, Connecticut 06340.

<sup>2</sup> Department of Pharmacy, Silpakorn University, Nakorn-pathom 73000, Thailand.

<sup>3</sup> School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, ROC.

<sup>4</sup> Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute, Kenilworth, New Jersey 07033-1300.

<sup>5</sup> College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48105-1065.

<sup>6</sup> Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan 48109.

<sup>7</sup> To whom correspondence should be addressed. (e-mail: fleisher@umich.edu)

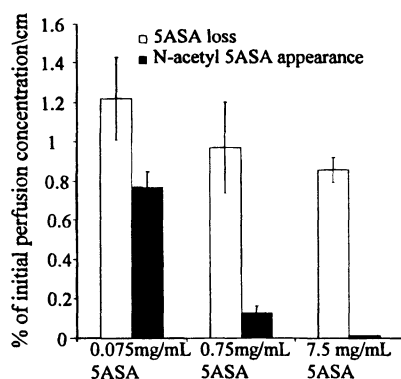
**ABBREVIATIONS:** 5ASA; 5-aminosalicylate.

rats weighing 250–300 g were fasted overnight for 16–22 hours with free access to water. Following IM anesthesia with 87 mg/kg ketamine and 13 mg/kg xylazine, the small intestine was surgically exposed and 10 cm of jejunum was ligated for perfusion. Perfusion flow rate was 0.12 mL/min and exiting perfusate samples were collected every 15 minutes over a period of 105 minutes after steady state absorption was achieved (40 minutes). Blood samples were collected during steady state perfusion. Scintillation counting of the radiolabeled nonabsorbable PEG 4000 marker in the outlet perfusate was measured and compared with the initial inlet counts to correct outlet solute concentrations for net water flux. Drug and metabolite concentrations in the outlet perfusate were measured by HPLC. The perfused jejunal length was measured after obtaining the final perfusate sample prior to animal sacrifice.

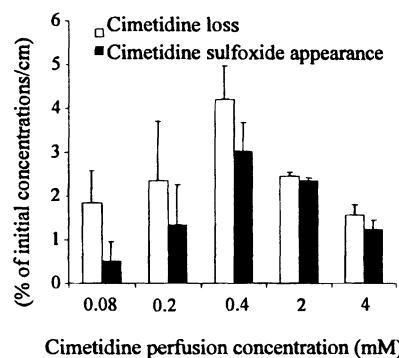
Both 5ASA and cimetidine solutions were freshly prepared and analyzed to insure stability prior to initiating perfusion. Solutions were prepared in MES buffer (10 mM MES, 5 mM KCl and 0.1% PEG 4000). Subsequent to addition of 5ASA and/or cimetidine, the final solutions were made isotonic ( $290 \pm 20$  mOsm/kg) at a pH of 6.5 by adjusting NaCl and 1N NaOH, respectively.

### Analytical Methods

Chemical analysis of 5ASA and cimetidine and their respective metabolites in perfusion solutions were determined by HPLC. Perfusate and plasma-extracted 5ASA (5-aminosalicylate) and N-acetyl 5ASA were separated by reverse phase HPLC with fluorescence detection as previously described (14). Cimetidine and cimetidine S-oxide were analyzed by cationic exchange chromatography with UV detection (16). The HPLC system consisted of a Waters 501 HPLC pump, Waters 770 autosampler, Shimadzu CR 501 chromatopac integrator, Anspec linear strip recorder, either LiChrosorb RP-C18 column ( $250 \times 4.6$  mm) and ABS 980 fluorescence detector (317 nm excitation, 417nm emission cut off) for 5ASA analysis or Nucleosil SA cation exchange column ( $250 \times 4.6$  mm) and Waters AM 486 Tunable UV absorbance detector for cimetidine analysis. Both methods had been previously cross validated to show no interference with each other.



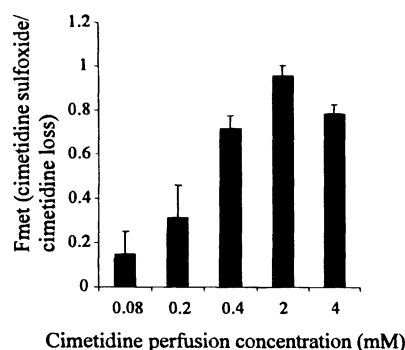
**Fig. 1.** Mean  $\pm$  SEM luminal drug (5ASA) loss and metabolite (N-acetyl 5ASA) appearance in rat jejunal lumen as a function of perfusion drug concentration normalized by the length of the perfused segment. Jejunal absorption is assessed as the difference between luminal drug lost and metabolite appearance.



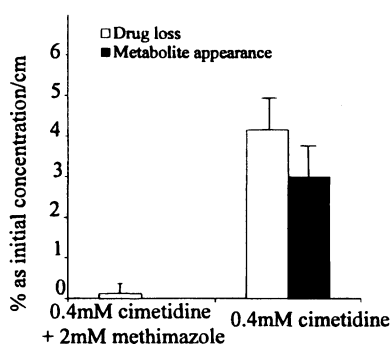
**Fig. 2.** Mean  $\pm$  SEM luminal drug (cimetidine) loss and metabolite (cimetidine S-oxide) appearance in rat jejunal lumen as a function of perfusion drug concentration normalized by the length of the perfused segment. Jejunal absorption is assessed as the difference between luminal drug lost and metabolite appearance.

### RESULTS

Jejunal absorption of 5ASA and cimetidine was assessed from perfusate data as the percent of drug lost from the intestinal lumen minus the percent appearing in the lumen as metabolite. Jejunal 5ASA metabolism significantly reduced drug absorption at low 5ASA perfusion concentration but had no significant impact on absorption at high 5ASA concentration (Fig. 1). On the contrary, cimetidine metabolism did not affect drug absorption at lower cimetidine perfusion concentrations (from 0.08 to 0.4 mM) but significantly decreased drug absorption at high cimetidine concentrations (2 and 4 mM) (Figs. 2 and 3). Inhibition of cimetidine intracellular metabolism by methimazole reduced absorption of cimetidine from 0.4mM perfusions to near zero (Fig. 4). On a mass balance basis, co-perfusion of 5ASA and cimetidine with methimazole totally abolished transepithelial transport of 5ASA at 0.075 (Fig. 5a) and 0.75 mg/mL (Fig. 5b). Furthermore, the percent of 5ASA metabolite appearing in the jejunal lumen is greater at the higher drug perfusion concentration under these same conditions. Pre-treating the intestine by infusing cimetidine and methimazole prior to 5ASA co-perfusion with cimetidine and methimazole reduced the systemic levels of 5ASA and N-acetyl-5ASA from 3 and 25  $\mu$ g/mL to zero and 5  $\mu$ g/mL, respectively (Fig. 6).



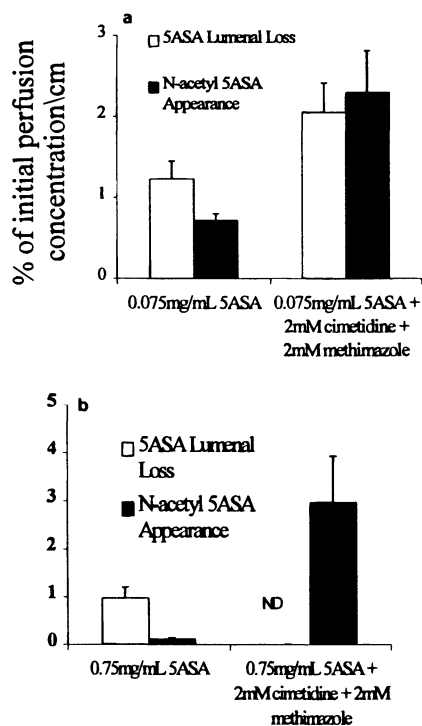
**Fig. 3.** Mean  $\pm$  SEM fraction of cimetidine lost from the jejunal lumen appearing as cimetidine S-oxide ( $F_{met}$ ) as a function of perfusion drug concentration normalized by the length of the perfused segment.



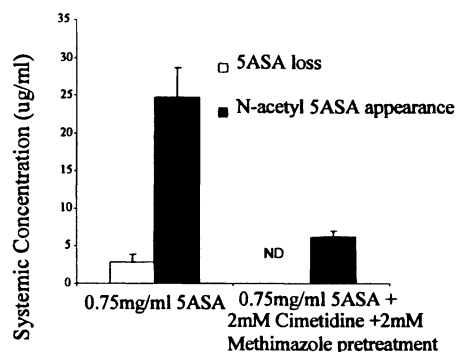
**Fig. 4.** Mean  $\pm$  SEM luminal drug (cimetidine) loss and metabolite (cimetidine S-oxide) appearance in rat jejunal lumen from 0.4 mM cimetidine perfusion solutions in the presence and absence of 2 mM methimazole (sulfoxidation inhibitor) normalized by the length of the perfused segment.

## DISCUSSION

As a zwitterion with a low and pH-independent partition coefficient ( $P_{\text{octanol}/\text{H}_2\text{O}} = 0.03$ ), 5ASA absorption via lipid partitioning and permeation is not favorable. Recent studies suggested that 5ASA is taken up into intestinal cells by a low-capacity carrier-mediated transport process (14). In rat jejunal



**Fig. 5.** (a) Mean  $\pm$  SEM luminal drug (5ASA) loss and metabolite (N-acetyl 5ASA) appearance in rat jejunal lumen from 0.075 mg/mL 5ASA perfusion solutions in the presence and absence of 2 mM cimetidine and 2 mM methimazole normalized by the length of the perfused segment. (b): Mean  $\pm$  SEM luminal drug (5ASA) loss and metabolite (N-acetyl 5ASA) appearance in rat jejunal lumen from 0.75 mg/mL 5ASA perfusion solutions in the presence and absence of 2 mM cimetidine and 2 mM methimazole normalized by the length of the perfused segment.



**Fig. 6.** Mean  $\pm$  SEM plasma drug (5ASA) and metabolite (N-acetyl 5ASA) concentrations from 0.75 mg/mL 5ASA perfusion solutions and 0.75 mg/mL 5ASA co-perfusion with 2 mM cimetidine and 2 mM methimazole following a half hour pre-treatment of 2 mM cimetidine and 2 mM methimazole perfusion. Plasma samples were obtained just prior to stopping the jejunal perfusion process. ND = not detected.

perfusions, 5ASA absorption is significantly countered by intestinal drug elimination at low drug concentrations where 5ASA N-acetylation capacity is not saturated (Fig. 1). This is also observed to a lesser extent in ileal perfusions. With increasing 5ASA perfusion concentrations, carrier transport is dominated by a passive transport process. Based on these results, 5ASA absorption was projected to include intracellular uptake with subsequent transcellular transport in parallel with passive absorption via the paracellular pathway (14). However, on a mass balance basis, the data in this report indicates that intracellular drug does not exit the basolateral membrane so that significant transcellular absorption does not occur.

With a  $pK_a$  of 7.1, cimetidine exists as both neutral and cationic species at normal intestinal pH. Although cimetidine's intrinsic partition coefficient ( $P_{\text{octanol}/\text{H}_2\text{O}} = 2$ ) is not as low as that of 5ASA, passive absorption via lipid partitioning is not consistent with its pH- and concentration-dependent absorption profile. Concentration-dependent permeability was observed in both rat jejunal and ileal perfusion studies (17). Jejunal cimetidine absorption was countered by secretion of a cimetidine metabolite into the jejunal lumen (Figs. 2 and 3) while luminal metabolite is not detectable from ileal perfusions (17). As depicted in Figs. 2 and 3, jejunal secretion of cimetidine S-oxide increases with increasing cimetidine concentration up to 0.4 mM in jejunal perfusions. At concentrations above 0.4mM, the fraction of cimetidine secreted as metabolite ( $F_{\text{met}}$ ) levels off above 0.8 (Fig. 3). The fact that the ratio of metabolite appearance to drug loss is close to 1 at high luminal cimetidine concentrations suggests that most of the drug entering jejunal epithelial cells is not absorbed systemically but secreted back across the apical membrane as the S-oxide metabolite. In parallel with this process, systemic cimetidine absorption defined as the difference between luminal drug loss and metabolite appearance is proportional to cimetidine perfusion concentration up to 0.4 mM.

The relationship between cimetidine transmucosal transport and jejunal elimination was further explored in metabolism inhibition studies. Methimazole, a potent flavin monooxygenase S-oxidation inhibitor (16), which does not effect cimetidine uptake in intestinal brush border membrane vesicles (18), totally abolishes the appearance of cimetidine S-oxide in the lumen

and reduces jejunal cimetidine absorption to near zero. Since methimazole inhibits cimetidine microsomal metabolism but not intracellular drug uptake, higher cimetidine levels in jejunal cells are projected than would be the case from cimetidine perfusions without the inhibitor. Reduced absorption (to near zero in the presence of methimazole, Fig. 4) at higher cellular cimetidine levels indicates that transcellular transport does not contribute to cimetidine absorption. Rather, the cellular level of cimetidine serves as an indicator to down-regulate cimetidine absorption by the paracellular pathway. It is inferred from mass balance that decreased cimetidine absorption with increasing drug concentration above 0.4 mM results from an intracellular drug effect on the paracellular pathway in rat jejunal perfusion studies (Fig. 2).

Similar to cimetidine, 5ASA intracellular uptake in rat jejunum was shown not to contribute to 5ASA absorption when the drug is co-perfused with cimetidine and methimazole. As shown in Figs. 5a, 5b and 6, this drug combination not only reduces cimetidine absorption but also abolishes 5ASA absorption. While systemic 5ASA absorption is eliminated under these conditions, the observation of higher luminal levels of N-acetyl 5ASA indicates that intracellular uptake of 5ASA is increased versus controls (Fig. 5a and 5b). The increased intracellular 5ASA uptake does not promote its absorption since the drug is eliminated as metabolite into the intestinal lumen. Previous observations showing that basolateral-to-apical flux is greater than flux in the other direction provides evidence for the existence of basolateral carriers for these drugs (14). The data documenting minimal basolateral exit of intracellular drug in this study suggests that the basolateral transporters for 5ASA and cimetidine are unidirectional serving to support intestinal secretion rather than transcellular absorption. For both 5ASA and cimetidine, intracellular uptake by either carrier mediated transport or lipid partitioning does not facilitate drug transcellular transport across the basolateral membrane. Thus, the only significant route for systemic absorption of 5ASA and cimetidine from perfused rat jejunum is via the paracellular pathway.

Cellular uptake and intracellular metabolism determine intracellular concentrations of cimetidine with subsequent metabolite elimination into the intestinal lumen. At low cimetidine perfusion concentrations, enterocyte metabolism results in low intracellular cimetidine levels, and unhindered paracellular transport increases linearly with perfusion concentration (Fig. 1). At cimetidine concentrations sufficient to saturate metabolism (above 2 mM perfusion concentration) or at lower perfusion concentration in the presence of metabolic inhibitors, intracellular cimetidine levels are elevated. Given that there is minimal basolateral drug exit, the data indicate that elevated intracellular cimetidine concentrations pharmacologically restrict paracellular transport to reduce jejunal drug absorption (Fig. 7). Cell biology studies have established that epithelial tight junctions are under the influence of intracellular modulators of cytoskeleton activity (4,19). It is projected that cimetidine, and possibly other aminothiols, mimic an endogenous modulator of tight junctions to restrict paracellular transport. As indicated in the methimazole inhibition studies, increasing cellular cimetidine concentrations beyond a certain level totally restricts paracellular transport of both cimetidine and 5ASA.

While a number of experimental manipulations have been reported to expand the paracellular pathway, the experiments

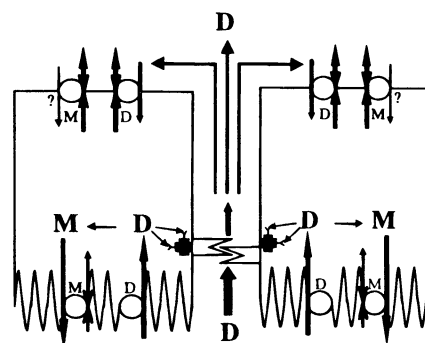


Fig. 7. Proposed mechanism for altered 5ASA intestinal absorption in presence of cimetidine and methimazole. D: cimetidine or 5ASA; M: cimetidine S-oxide or N-acetyl 5ASA; ?: unknown transporter operating counter to metabolite exit from the basolateral membrane.

detailed here document restrictive control of paracellular transport *in vivo* by cimetidine. Two other H<sub>2</sub>-antagonists, ranitidine and famotidine, have been shown to increase TEER across Caco-2 cells and the *in vitro* permeability of these two drugs decreased with increasing drug concentration. Caco-2 monolayers are tighter epithelia than small intestine and moderate restriction was demonstrated (20). The current *in situ* data shows complete restriction of the paracellular pathway to cimetidine and 5ASA in rat jejunum as mediated by intracellular cimetidine. These perfusion conditions provide an experimental tool to evaluate the relative contributions of transcellular and paracellular transport to the jejunal absorption of other small hydrophilic drugs.

This mechanism by which cellular drug uptake and metabolism regulates paracellular drug transport may be unique to jejunal epithelia which are leakier than neighboring duodenal and ileal epithelia. Given the absorptive function of the jejunum, a greater capacity for paracellular control might be expected. Clinical ramifications of this control are observed in the absorption of small hydrophilic molecules with potential paracellular transport. Cimetidine absorption is characterized by a second maximum in its plasma level-versus-time-profile that is not observed with food ingestion (21). In addition, cimetidine oral bioavailability has been reported to be highly variable (from 40% to 120%) and variability increases with increasing dose (22,23). These observations may be a function of cimetidine's capacity to regulate its paracellular absorption in the jejunum. Under fasted conditions, gastric emptying of cimetidine is rapid resulting in high drug levels in the jejunum supporting substantial self-inhibition via paracellular restriction and drug elimination. The resulting depression of drug absorption with high jejunal concentrations would result in plasma level double peaks from regionally dependent absorption. The mechanism is also consistent with the fact that decreases in cimetidine bioavailability with increasing dose are more profound with liquid than solid formulations (24). When cimetidine is administered at low oral dose, with food or in a sustained release formulation, the rate of presentation to jejunal epithelia and resultant self-inhibition of paracellular absorption is diminished.

Cimetidine has also been found to promote a number of drug interactions primarily mediated by changes in hepatic metabolism and renal secretion (25). These interactions typically result in greater systemic exposure via slower drug elimination (26). While cimetidine reduces antipyrine (27) and

triamterene (28) clearance by 24.3% and 28% respectively, total drug recovery in the urine and area under the drug plasma level versus time curves (AUC) show that systemic drug exposure is actually *decreased*. This result indicates that cimetidine provides an additional interaction which reduces drug absorption. Similar examples of decreased drug absorption have been reported for tetracycline (29) and zinc (29) with oral co-administration of cimetidine. Ranitidine also has been reported to decrease the absorption of triamterene (30). Such interactions may result from the influence of intracellular H<sub>2</sub>-antagonists to reduce the paracellular transport of other drugs.

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#### REFERENCES

1. K. R. Spring. Routes and mechanism of fluid transport by epithelia. *Ann. Rev. Physiol.* **60**:105–119 (1998).
2. K. H. Soergel. Showdown at the tight junction. *Gastroenterology* **105**:1247–1250 (1993).
3. J. R. Turner, B. K. Rill, S. L. Carlson, D. C. Arnes, R. Kerner, R. J. Mrsny, and J. L. Madara. Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *Am. J. Physiol.* **273**:C1378–1385 (1997).
4. L. L. Mitic and J. M. Anderson. Molecular architecture of tight junctions. *Ann. Rev. Physiol.* **60**:121–42 (1998).
5. V. Pade and S. Stavchansky. Estimation of the relative contribution of the transcellular and paracellular pathway to the transport of passively absorbed drugs in the Caco-2 cell culture model. *Pharm. Res.* **14**:1210–1215 (1997).
6. H. Lennernas. Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation? *Pharm. Res.* **12**:1573–1582 (1995).
7. J. M. Mullin, C. W. Marano, K. V. Laughlin, M. Nuciglio, B. R. Stevenson, and P. Soler. Different size limitations for increased transepithelial paracellular solute flux across phorbol ester and tumor necrosis factor-treated epithelial cell sheets. *J. Cell. Physiol.* **171**:226–33 (1997).
8. G. M. Pauletti, F. W. Okumu, and R. T. Borchardt. Effect of size and charge on the passive diffusion of peptides across Caco-2 cell monolayers via the paracellular pathway. *Pharm. Res.* **14**:164–168 (1997).
9. H. Ghandehari, P. L. Smith, H. Ellens, P. Y. Yeh, and J. Kopecek. Size-dependent permeability of hydrophilic probes across rabbit colonic epithelium. *J. Pharmacol. Exp. Ther.* **280**:747–753 (1997).
10. R. M. van Elburg, J. J. Uil, F. T. Kokke, A. M. Mulder, W. G. van de Broek, C. J. Mulder and H. S. Heymans. Repeatability of the sugar-absorption test, using lactulose and mannitol, for measuring intestinal permeability for sugars. *J. Ped. Gastro. Nutr.* **20**:184–888 (1995).
11. L. Lazorova, J. Grasjo, P. Artursson, M. Bergstrom, F. Wu, E. Petterman-Bergstrom, M. Ogren, and B. Langstrom. Quantification and imaging of mannitol transport through Caco-2 cell monolayers using a positron-emitting tracer. *Pharm. Res.* **15**:1441–1444 (1998).
12. R. M. Schwartz, J. K. Furne, and M. D. Levitt. Paracellular intestinal transport of six-carbon sugars is negligible in the rat. *Gastroenterology* **109**:1206–1213 (1995).
13. J. L. Madara. Regulation of the movement of solutes across tight junctions. *Ann. Rev. Physiol.* **60**:143–159 (1998).
14. S. Y. Zhou, D. Fleisher, L. H. Pao, C. Li, B. Winward, and E. M. Zimmermann. Intestinal metabolism and elimination of 5-aminosalicylic acid. *Drug Metab. Dispos.* **27**:479–485 (1999).
15. Y. F. Hui, J. Kolars, Z. Hu, and D. Fleisher. Intestinal clearance of H<sub>2</sub>-antagonists. *Biochem. Pharmacol.* **48**:229–231 (1994).
16. X. Lu, C. Li, and D. Fleisher. Cimetidine sulfoxidation in small intestinal microsomes. *Drug Metab. Dispos.* **26**:940–942 (1998).
17. N. Piyapolrunroj, Y. S. Zhou, C. Li, G. Liu, E. Zimmermann, and D. Fleisher. Cimetidine intestinal absorption and elimination in rat small intestine. revision submitted to *Drug Metab. Dispos.* (1999).
18. N. Piyapolrunroj, C. Li, R. L. Pisoni, and D. Fleisher. Cimetidine transport in brush border membrane vesicles from rat small intestine. *J. Pharmacol. Exp. Ther.* **289**:346–353 (1999).
19. M. Perez, A. Barber, and F. Ponz. Modulation of intestinal paracellular permeability by intracellular mediators and cytoskeleton. *Can. J. Physiol. Pharmacol.* **75**:287–92 (1997).
20. L. S. Gan, S. Yanni, and D. R. Thakker. Modulation of the tight junctions of the Caco-2 cell monolayers by H<sub>2</sub>-antagonists. *Pharm. Res.* **15**:53–57 (1998).
21. P. V. Pedersen and R. Miller. Pharmacokinetics and bioavailability of cimetidine in humans. *J. Pharm. Sci.* **69**:394–348 (1980).
22. A. Grahnén, C. von Bahr, B. Lindstrom, and A. Rosen. Bioavailability and pharmacokinetics of cimetidine. *Eur. J. Clin. Pharmacol.* **16**:335–340 (1979).
23. A. Somogyi and R. Gugler. Clinical pharmacokinetics of cimetidine. *Clin. Pharmacokin.* **8**:463–495 (1983).
24. S. S. Walkenstein, J. W. Dubb, W. C. Randolph, W. J. Westlake, R. M. Stote, and A. P. Intoccia. Bioavailability of cimetidine in man. *Gastroenterology* **74**:360–365 (1978).
25. A. Somogyi and M. Muirhead. Pharmacokinetic interactions of cimetidine. *Clin. Pharmacokin.* **12**:321–366 (1987).
26. F. P. Guengerich. Role of cytochrome P450 enzymes in drug-drug interactions. *Adv. Pharmacol.* **43**:7–35 (1997).
27. L. B. Slusher and E. S. Vesell. Cimetidine-induced reduction in gastrointestinal absorption of antipyrine and rate constants for formation of its metabolites. *Clin. Pharmacol. Ther.* **35**:568–75 (1984).
28. M. R. Muirhead, A. A. Somogyi, P. E. Rolan, and F. Bochner. Effect of cimetidine on renal and hepatic drug elimination: studies with triamterene. *Clin. Pharmacol. Ther.* **40**:400–407 (1986).
29. G. C. Sturniolo, M. C. Montino, L. Rossetto, A. Martin, R. D'Inca, A. D'Odorico, and R. Naccarato. Inhibition of gastric acid secretion reduces zinc absorption in man. *J. Amer. Coll. Nutr.* **10**:372–375 (1991).
30. M. Muirhead, F. Bochner, and A. Somogyi. Pharmacokinetic drug interactions between triamterene and ranitidine in humans: alterations in renal and hepatic clearances and gastrointestinal absorption. *J. Pharmacol. Exp. Ther.* **244**:734–739 (1988).