

## Intestinal Uptake of Cimetidine and Ranitidine in Rats

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The H<sub>2</sub>-receptor antagonists exhibit unusual absorption behavior in that double peaks often occur after oral administration. Moreover, administration with some high potency antacids decreases the extent of absorption. To date, no explanation that can completely account for these observations has been advanced. One problem is that there is a lack of consensus as to the mechanism of absorption of the H<sub>2</sub>-receptor antagonists from the gastrointestinal tract. In the studies reported here, the mechanism and regional dependence of intestinal uptake of two H<sub>2</sub>-receptor antagonists, cimetidine and ranitidine, were investigated in rats using the *in vitro* everted ring technique. The uptake rate of cimetidine from both jejunum and colon was linear with concentration (in the range of 0.0005-40 mM), and there was no significant competition for uptake in the presence of the structurally similar H<sub>2</sub>-receptor antagonists, famotidine and ranitidine. In the case of ranitidine too, the uptake rate from the jejunum and colon was linear with concentration (in the range of 0.0005-5 mM), and there was no competition for uptake by either famotidine or cimetidine. These data indicate that uptake of cimetidine and ranitidine in the rat jejunum and colon occurs by a predominantly passive process. Both cimetidine and ranitidine exhibited regional differences in uptake rate. Uptake tended to be greatest in the ileum, similar in duodenum and jejunum, and lowest in the colon. However, differences in uptake rates between locations in the small intestine appeared to be too modest to account for the double peak behavior of either compound.

**KEY WORDS:** cimetidine; ranitidine; H<sub>2</sub>-receptor antagonists; uptake; uptake rate; mechanism of transport; double peaks.

### INTRODUCTION

Cimetidine and ranitidine are H<sub>2</sub>-receptor antagonists (Figure 1) used in the treatment of duodenal ulcers and in the management of hypersecretory states. Cimetidine has a pK<sub>a</sub> of 7.1, an octanol/water partition coefficient of 2.5 at pH 9.2 and an aqueous solubility of 6 mg/ml. Ranitidine is freely water soluble (>1 g/ml) and has a pK<sub>a</sub> of 8.2. The rate and extent of absorption of cimetidine and ranitidine are found to be variable based on whether they are administered with food or with antacids. Following oral administration in the fasted state, two peaks have been observed in the plasma profiles of both H<sub>2</sub>-receptor antagonists but this behavior is circumvented by administering the drug with a meal (1,2). Coadministration of high potency antacids with cimetidine

or ranitidine results, in some cases, in a lower bioavailability (3,4). Although many explanations have been advanced for the effects of food and antacids on H<sub>2</sub>-receptor antagonist absorption (5) none have satisfactorily explained all the data.

One important aspect of the absorption process is the mechanism by which the drug is absorbed and how efficient this process is at various locations within the gastrointestinal tract. The mechanism(s) of absorption of H<sub>2</sub>-receptor antagonists have not been fully explored with some reports suggesting the possibility of active transport (6) while others indicate that paracellular uptake is important (7). Few studies have addressed the regional dependence of uptake within the small intestine, nor has the colonic transport of the H<sub>2</sub>-receptor antagonists been studied extensively. In view of the paucity of data and lack of consensus in the literature, the present study sought to further investigate the mechanisms and regional differences in intestinal transport of cimetidine and ranitidine.

### METHODS

**Chemicals:** {N-methyl-<sup>3</sup>H} Cimetidine (1.0 mCi/ml, 25 Ci/mmol) was obtained from Amersham Corp., IL. {N-methyl-<sup>3</sup>H} Ranitidine (20 μCi/ml, 6.5 Ci/mmol) was a gift from Glaxo Inc., NC. Cimetidine, ranitidine and famotidine were purchased from Sigma Chemical Co., MO. Scintigest® was obtained from Fisher Scientific, NJ and Cytoscient® was purchased from ICN Biomedical Inc., CA. All other analytical reagent grade chemicals were obtained commercially.

**Incubation Media:** McIlvaine buffer (pH 6) containing 0.2 M sodium phosphate (626 ml/l) and 0.1 M citric acid (374 ml/l) was used for preparing the 'cold' drug solutions. Radiolabeled drug was then added and the solutions placed in beakers in a 37°C water bath (Precision® Dubnoff Metabolic Shaking Incubator, GCA).

**Tissue Preparation and Incubation:** Male Sprague-Dawley rats weighing 300-350 g were fasted for 15-18 hours prior to study, with water available *ad libitum*. The rat was anesthetized with an intraperitoneal injection of sodium pentobarbital at a dose of 6.5 mg/100 g body weight, more if deemed necessary by the pain withdrawal response. The first 15 cm of the small intestine from the pyloric junction (duodenum), the next 15 cm (jejunum) and the portions of the intestine 15 cm proximal to the cecum (ileum) and about 10 cm distal to the cecum (colon) were excised and then the rat was sacrificed. The intestinal segments were immediately placed in a pan containing 0.9% sodium chloride solution surrounded by ice. Each of the excised segments were tied at one end with a suture thread and then everted on a glass rod. Approximately 2-3 mm rings (15-30 mg) were cut from the intestinal segments using a razor blade and transferred to a petri dish containing oxygenated buffer. After a short preincubation in pH 6.0 McIlvaine buffer, the tissue rings were placed in wire baskets that were lowered into radiolabeled drug solutions at 37°C in the shaking water bath. The baskets were removed after the desired incubation interval, tissue rings immediately rinsed with ice-cold saline, gently blotted dry on Kimwipes® tissue paper (Kimberly-Clark Corp, GA) and then individually placed in pre-weighed scintillation vials. The vials were reweighed and the exact tissue weight

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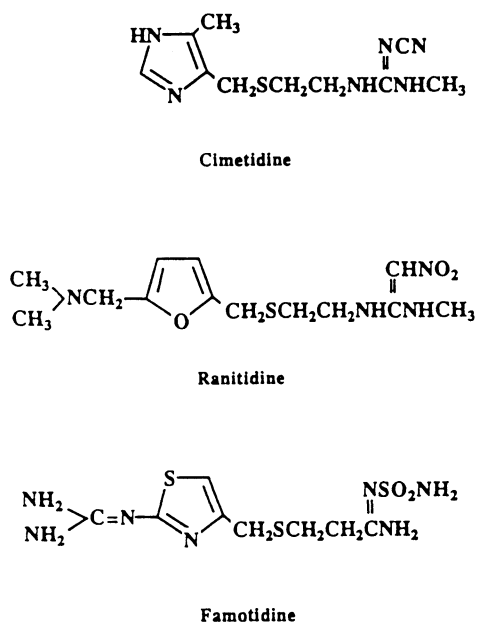


Figure 1. Chemical structure of H<sub>2</sub>-receptor antagonists.

obtained by difference. The tissue was solubilized for 5 hours at 60°C in Scintigest®. The samples were left overnight at ambient temperature after 10 ml of the scintillation cocktail (Cytoscint®) was added and radioactivity measured on a scintillation counter (Beckman® LS 9000, Beckman Instruments Inc., CA). The calibration standard was prepared by adding a known volume of the radioactive drug to 10 ml of scintillation cocktail.

**Preliminary Experiments:** It was necessary to decide on the appropriate experimental conditions of incubation time and shaking rate to be used in the subsequent site and concentration dependency studies for each compound. First, uptake was studied as a function of time and the linear region of uptake was determined. Extrapolation of the linear segment to the zero time point gave the extent of non-specific intestinal tissue binding. An incubation time period was then chosen from the linear region where tissue binding accounted for less than 25% of the uptake. Next, uptake was studied at three different shaking rates (40, 60 and 75 cycles/min) to determine the contribution, if any, of aqueous resistance to uptake and an appropriate shaking rate was chosen.

**Method Validation Experiments:** The everted ring technique was validated by characterizing the transport mechanisms of compounds that have been previously reported to be either passively or actively absorbed by other experimental methods. Passive transport was demonstrated for hydrocortisone (transcellular route) and mannitol (paracellular route) while active transport was observed for 3-O-methyl glucose. Polyethylene glycol 4000 provided a negative control for intestinal transport.

**Concentration Dependency Experiments:** To investigate the possibility that transport in the jejunum and colon may be saturable, uptake rate in the concentration range of 0.0005-40 mM and 0.0005-5 mM was studied for cimetidine and ranitidine respectively ( $n \geq 3$  rats at each concentration, 4 rings per rat).

**Competitor Experiments:** If the intestinal uptake mechanism of H<sub>2</sub>-receptor antagonists involves a carrier, then they may be expected to compete for the same carrier due to their structural similarity. This hypothesis was used to further explore the possibility of carrier mediated transport of cimetidine and ranitidine. Uptake of cimetidine was studied at a concentration of 3.0 mM (expected cimetidine concentration in the gut following ingestion of a 300 mg tablet) in the presence of either five-fold excess of famotidine or ten-fold excess ranitidine and the results compared to the uptake from a control solution containing cimetidine alone. Likewise, ranitidine uptake from a 0.1 mM solution in the presence of five-fold excess of either cimetidine or famotidine was compared to its uptake from a solution containing ranitidine only ( $n = 3$  rats for each potential inhibitor, 4 rings per rat).

**Regional Dependency Experiments:** The uptake rate of cimetidine and ranitidine from different intestinal sites was compared at pH 6.0 ( $n \geq 3$  rats for each location, 4 rings from each rat).

**Data Analysis:** Uptake values are expressed in terms of nmoles per gram of tissue while uptake rates are expressed as nmoles per gram of tissue per minute of the incubation time. Different treatments were compared by one-way ANOVA ( $\alpha = 0.05$ ). Linearity, significance of the slopes and 95% confidence intervals (C.I.) for the slopes were determined by simple regression analysis (Statview™ SE + Graphics, version 1.03, Abacus Concepts, Inc., Berkley, CA).

## RESULTS

**Preliminary Experiments:** The jejunal and colonic uptakes of cimetidine were linear for 90 seconds while ranitidine uptake was linear for 60 seconds in both regions. To ensure that binding was less than 25% of total uptake for each compound, subsequent incubations were carried out for 90 seconds for cimetidine and 60 seconds for ranitidine. No significant difference was observed in the uptake of either ranitidine or cimetidine at the three shaking rates. Hence, subsequent experiments were performed at a shaking rate of 75 cycles/min.

**Concentration Dependency Experiments:** Figures 2 and 3 represent the jejunal and colonic uptake rate of cimetidine and ranitidine as a function of concentration. Uptake rate of the two H<sub>2</sub>-receptor antagonists appeared to be linear with concentration from both sites over the wide concentration range studied. The relationship between uptake rate and concentration for cimetidine in the jejunum was  $UR_{\text{jej}} = -10 + 101[\text{CIM}]$  with  $R^2 = 0.99$ ,  $p = 0.0001$ , and 95% C.I. (slope) = 96-105; while in the colon the relationship was  $UR_{\text{col}} = -4 + 70[\text{CIM}]$  with  $R^2 = 0.998$ ,  $p = 0.0001$ , and 95% C.I. (slope) = 69-72. For ranitidine the equivalent expressions were  $UR_{\text{jej}} = -10 + 103[\text{RAN}]$  with  $R^2 = 0.99$ ,  $p = 0.0001$ , and 95% C.I. (slope) = 93-113; and  $UR_{\text{col}} = -7 + 71[\text{RAN}]$  with  $R^2 = 0.979$ ,  $p = 0.0002$ , and 95% C.I. (slope) = 57-86.

**Competitor Experiments:** Famotidine and ranitidine did not appear to cause any significant reduction in cimetidine uptake rate from a 3 mM solution (Table 1). Similarly, there was no significant reduction in ranitidine uptake rate from a

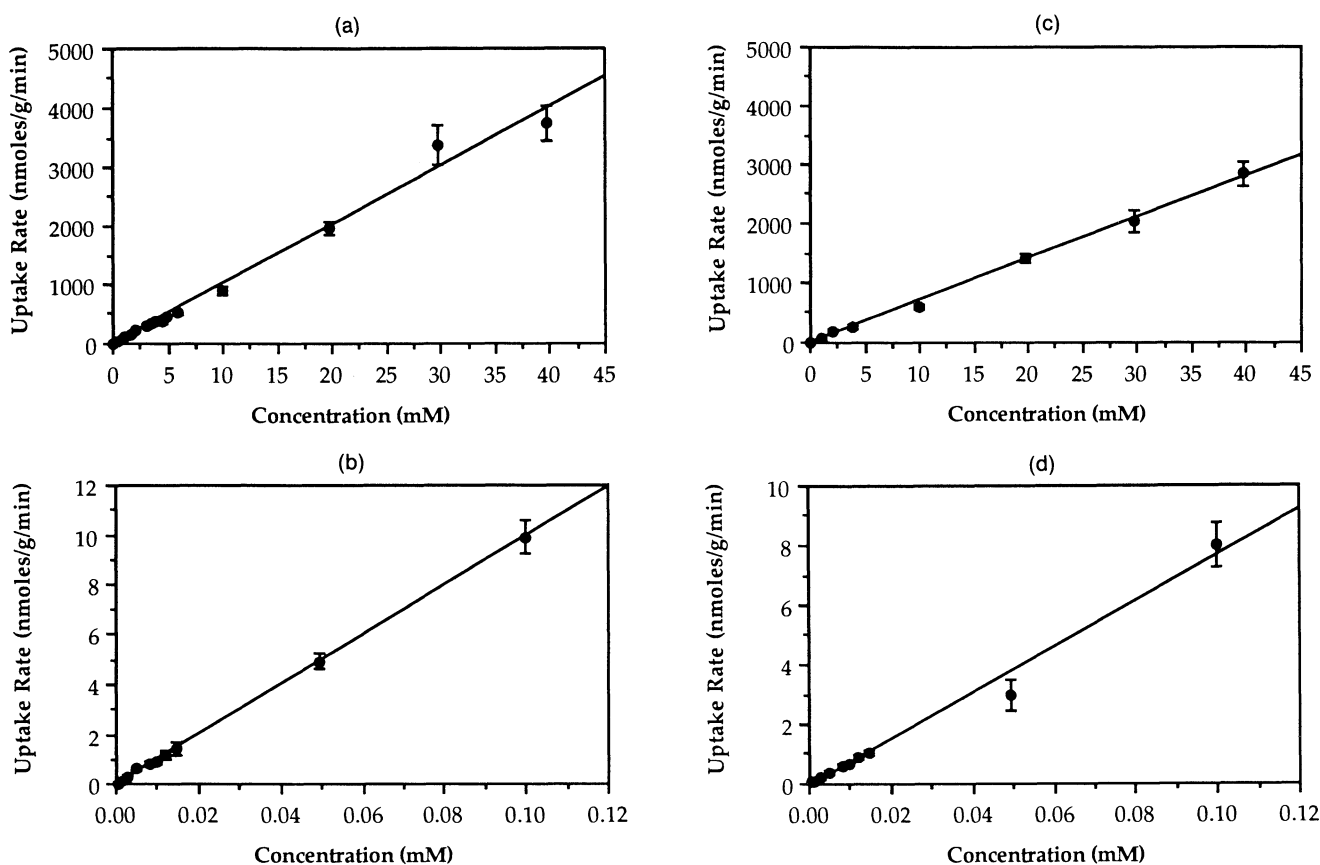


Figure 2. Uptake of cimetidine as a function of concentration (a) 0 - 40 mM, jejunum (b) 0 - 0.1 mM, jejunum (c) 0 - 40 mM, colon and (d) 0 - 0.1 mM, colon. Each point represents the mean  $\pm$  standard error of four rings per rate for three rats.

0.1 mM solution in the presence of excess of either cimetidine or famotidine (Table 1).

**Regional Dependency Experiments:** Although ileal uptake rate of cimetidine had a tendency to be higher than the jejunal or duodenal uptake rate (Figure 4), one way ANOVA showed no significant difference at any of the three concentrations (0.02, 4 and 20 mM) studied. Ileal uptake rate of ranitidine was significantly higher than either jejunal or duodenal uptake rate (Figure 4). Colonic uptake rate of the  $H_2$ -receptor antagonists, though substantial, was significantly lower than their jejunal uptake rate (Figure 5).

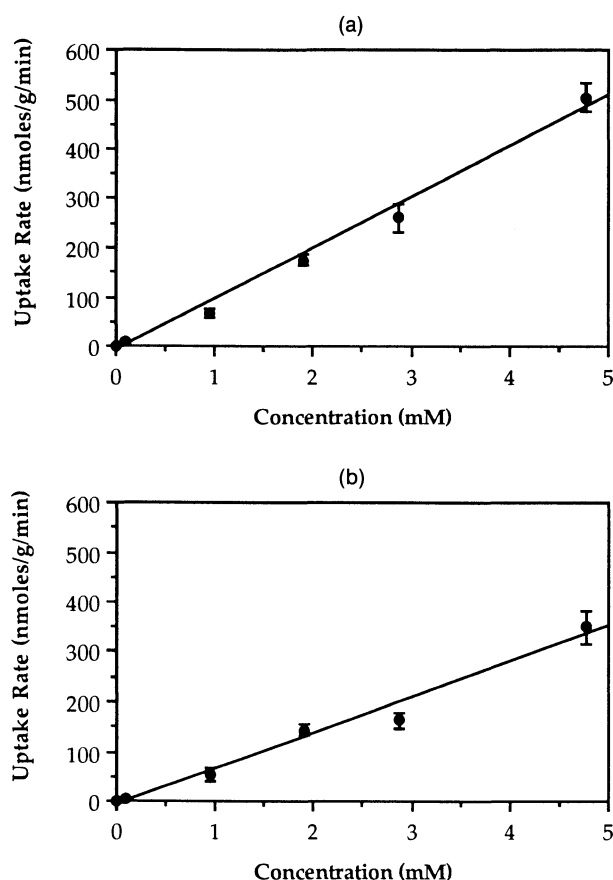
The jejunal uptake rate of cimetidine was significantly higher than the jejunal uptake rate of ranitidine (Figure 5).

## DISCUSSION

**Passive versus Active Transport:** In the present work, concentration dependency experiments indicated a predominantly passive transport of cimetidine and ranitidine in the rat jejunum and colon. In a previous report, inhibition of cimetidine transport across the rat intestinal everted sacs by 2,4-dinitrophenol (DNP) was noted at concentrations below 2 mM at pH 7.2 (6). The authors interpreted these results in terms of an active transport process for cimetidine that is observable at low substrate concentrations but is masked by a diffusion process at higher concentrations. Results of the Barber study cannot be considered conclusive, however,

since the everted sacs were randomized with respect to position along the intestine and therefore any differences in uptake with intestinal sites were not accounted for. Moreover, the drug solution was incubated for one hour in the sacs and the sulfoxide metabolite (amount unspecified by the authors) was detected. Both deterioration of tissue viability and metabolism may, therefore, have influenced the interpretation of the results. Chen observed some concentration dependency of jejunal uptake at pH 6.0 over the concentration range of 0.004 - 40 mM during *in situ* perfusions in rats, also suggesting the possibility of carrier mediated transport of cimetidine (8). However, neither 2,4-DNP nor histidine had a significantly inhibitory effect on the jejunal permeability of cimetidine in the Chen study. There was no evidence for saturable uptake over the wide concentration range investigated in our study. Also, no competition for intestinal uptake by structurally similar  $H_2$ -receptor antagonists was observed. Ranitidine but not famotidine has been found to competitively inhibit the kidney tubular transport of cimetidine (9) although ranitidine did not inhibit cimetidine transport in isolated brush border membrane vesicles from bovine choroid plexus despite evidence of saturable transport at that location (10).

**Paracellular versus Transcellular Transport:** The paracellular pathway would appear to be accessible to the  $H_2$ -receptor antagonists based on the fact that their molecular weights are under 350 and a major fraction of these com-



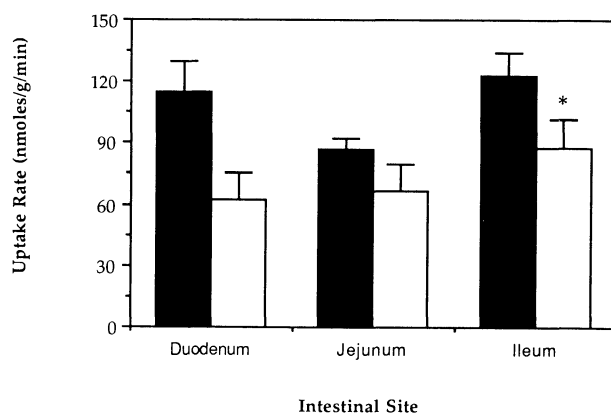
**Figure 3.** Uptake of ranitidine as a function of concentration (a) jejunum and (b) colon. Each point represents the mean  $\pm$  standard error of four rings per rat for three rats.

pounds would be in the positively charged form in the intestinal pH range. The transport of cations is generally favored over nonionic species or anions through the tight junctions (11). Gan *et al.* have reported a possible paracellular passive diffusion pathway for the absorption of ranitidine based on their results using the CACO-2 cell culture system (7). Hu has shown that cimetidine membrane permeability at pH 7.5 increased in the presence of 3-O-methyl glucose, a nonmetabolizable sugar (12). This increase due to the monosaccha-

**Table I.** Jejunal Uptake Rate of Cimetidine and Ranitidine in the Presence of other H<sub>2</sub>-Receptor Antagonists. Data Represent the Mean  $\pm$  Standard Deviation of Four Rings Per Rat for Three Rats

Compound	Inhibitor	[Inhibitor]	Uptake Rate (nmol/g/imin/mM) <sup>a</sup>
Cimetidine, 3 mM	None	—	197 $\pm$ 47
	Ranitidine	24.0 mM	149 $\pm$ 36
	Famotidine	22.0 mM	165 $\pm$ 30
Ranitidine, 0.1 mM	None	—	82 $\pm$ 20
	Cimetidine	6.0 mM	94 $\pm$ 26
	Famotidine	4.5 mM	85 $\pm$ 14

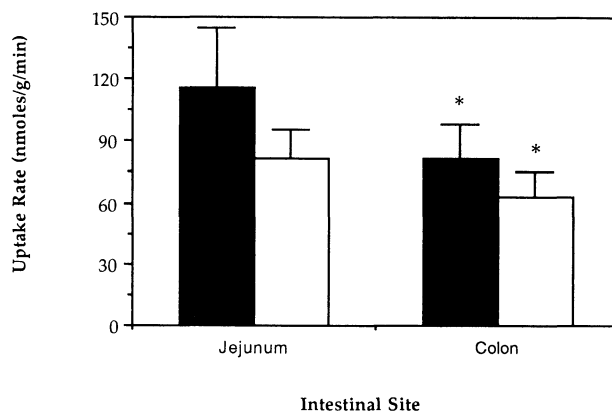
<sup>a</sup> Differences in cimetidine and ranitidine uptake rate between treatments are not statistically significant ( $p > 0.05$ ).



**Figure 4.** Regional dependence of uptake of cimetidine (solid columns) and ranitidine (open columns) from the small intestine. Data represent the mean and standard deviation of four rings per rat for three rats. \*Differences between ileal vs. jejunal or duodenal uptake rate of ranitidine are statistically significant ( $p < 0.05$ ).

ride generated water drag suggests a significant paracellular component in the transport of cimetidine. If the paracellular pathway is assumed to be the only route of transport, the higher uptake rate of cimetidine can be explained by its molecular size being smaller than that of ranitidine, since the rat intestine is reported to be more permeable to smaller organic cations (13).

Though the everted ring technique cannot distinguish between paracellular and transcellular passive diffusion, the comparison of uptake between different sites gives some indication of the route of transport. The epithelial junctions become progressively tighter from the small intestine to the colon which should lead to decreased permeability of polar compounds. If the H<sub>2</sub>-receptor antagonists are only absorbed by paracellular mechanism, their uptake rate from the colon would be expected to be much smaller than was observed in this study. Since the colonic uptake of these compounds was found to be about 70-80% of the jejunal



**Figure 5.** Small versus large intestinal uptake of cimetidine (solid columns) and ranitidine (open columns). Data represent the mean and standard deviation of uptake rates from 37 experiments (cimetidine) or 12 experiments (ranitidine). Each experiment is the average of four rings per rat for three rats. \*Difference between colonic and jejunal uptake is statistically significant for both drugs ( $p < 0.05$ ).

uptake rate, the results suggest that the transcellular pathway may also be an important route of absorption.

The pH-dependent absorption of cimetidine observed in rats (14,15) and in dogs (16) further suggests that there is a transcellular component to cimetidine transport. The observation that the jejunal uptake rate of cimetidine is higher than that of ranitidine may be a reflection of the higher lipophilicity of cimetidine. Ranitidine is freely soluble in water and partitioning into organic phases is difficult to detect, while cimetidine has a log P of 0.4.

*Regional Dependence of Intestinal Uptake:* The H<sub>2</sub>-receptor antagonists appeared to be absorbed throughout the *small intestine* of the rat, with duodenal uptake rate being similar to jejunal uptake rate at pH 6. The difference between uptake rate from the ileum and the other regions was only significant for ranitidine.

Although the results from this study are qualitatively similar to those from previous studies (14,17), we observed much more modest regional differences in the uptake rate of cimetidine. In 1977, Griffiths *et al.* showed that the absorption of cimetidine from an unbuffered solution of [<sup>14</sup>C] cimetidine in normal saline into intestinal loops isolated *in situ* in rats was higher in the ileum than in the jejunum, but that absorption from the ileum was comparable to absorption from the duodenum (17). However, only two rats were used in this study. Kaneniwa *et al.* observed large regional differences in absorption after administration of a solution of cimetidine in 0.17% hydrochloric acid into intestinal loops ligated *in situ* in rats (14). The authors attributed the higher ileal absorption to the high ileal pH (pH 7.52). Even though the pH of the duodenum (pH 6.79) was close to the pH of the jejunum (pH 6.77 to 6.85), the duodenal absorption rate was about three times higher than the jejunal rate indicating that pH alone could not explain the intestinal site dependent differences in absorption. One problem in interpreting the results from the Kaneniwa study is that the administration of an unbuffered acidic cimetidine solution (pH~1.12) directly into the intestinal segments may have resulted in some tissue damage with resultant changes in permeability.

Funaki *et al.* proposed a discontinuous absorption model to explain the occurrence of double peaks based on the Kaneniwa study (18). According to this model, when cimetidine is administered in the solid form, most of it is absorbed from the duodenum, but the fraction that is not dissolved in the duodenum dissolves during its transit through the jejunum and is then absorbed from the ileum. However, the high aqueous solubility of cimetidine (6 mg/ml) suggests that dissolution would not be rate limiting to absorption. In view of the literature data with respect to pH effects and results presented here, it appears that regional differences in pH and uptake cannot completely account for the double peak phenomenon.

Comparing the *small intestine* and *colon*, cimetidine was absorbed to a significantly lesser but substantial extent in the colon than in the jejunum. Colonic uptake of ranitidine was also significantly lower than jejunal uptake. An earlier study in human subjects suggested that ranitidine is poorly absorbed following cecal dosing (19). On the other hand, ranitidine has been found to be absorbed to a significant extent following administration into the rectum in man (20). Since the rat results did not fully concur with the limited

human data available, it appears that further work needs to be done to determine whether rat colon is a good model for absorption of the H<sub>2</sub>-receptor antagonists in the human colon.

In conclusion, cimetidine and ranitidine appear to be absorbed by predominantly passive processes throughout the small intestine and colon. Uptake rates are slightly faster in the ileum and significantly slower in the colon when compared to duodenum and jejunum, with differences too modest to completely account for the double peak behavior.

#### ACKNOWLEDGMENTS

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