Effect of Polyethylene Glycol 400 on the Intestinal Permeability of Carbamazepine in the Rabbit

Lillian E. Riad[1] and Ronald J. Sawchuk[1,2]

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Because of the limited solubility of carbamazepine, aqueous solutions are usually prepared using glycols as cosolvents. This research focuses on the effect of varying the composition of polyethylene glycol 400 (PEG-400) in aqueous solutions on rabbit intestinal permeability of carbamazepine in the duodenojejunum and the ascending colon using an *in situ* perfusion technique. In both segments the intestinal permeability varied inversely with the percentage of PEG-400, when the concentration of carbamazepine in the perfusing solution was maintained constant. The decreased permeability may be explained by a reduction in the thermodynamic activity of carbamazepine with increased concentrations of PEG-400, as well as by reverse solvent drag because of the hyperosmolarity of the perfusing solutions.

KEY WORDS: intestinal permeability; *in situ* perfusion; partition coefficient; thermodynamic activity.

INTRODUCTION

Aqueous solubility and lipid solubility are major determinants of intestinal drug absorption. The antiepileptic drug (1), carbamazepine (CBZ; 5-carbamyl-5H-dibenzo[b,f] azepine; Tegretol), which has an octanol/phosphate buffer partition coefficient of 58, is a neutral lipophilic compound (2). Because of its limited water solubility (0.072 g/liter), aqueous solutions of CBZ using cosolvents such as propylene glycol (3–6) and polyethylene glycol 400 (PEG-400) (7–10) have been prepared to examine its pharmacokinetics in both humans and animals.

The objective of this research was to examine the effect of altering the PEG-400 content of aqueous solutions of CBZ on its intestinal permeability in different intestinal regions, using an *in situ* perfusion technique (11,12) in the rabbit (13). Perfusate solutions containing the same concentration of CBZ but different PEG-400 composition in normal saline were studied, allowing an investigation of intestinal permeability of CBZ in the duodenojejunum and the ascending colon.

MATERIALS AND METHODS

Segment Preparation

Male New Zealand White rabbits (Birchwood Farm Rabbitry, Grantsburg, WI) weighing 2.8–3.6 kg were used for this procedure. Prior to surgery, animals were fasted for 16–18 hr (14), and water was allowed *ad libitum*. Anesthesia was achieved by intramuscular injection of 45 mg/kg ketamine (Parke-Davis, Morris Plains, NJ; 100 mg/ml) and 2 mg/kg acepromazine (Butler, Columbus, OH; 10 mg/ml) for muscle relaxation. A second dose of 25 mg/kg of ketamine and 2 mg/kg of acepromazine was given 15 min later. After the abdominal area was shaved a longitudinal midline incision was made. The intestinal segment of interest was isolated and exposed.

The proximal end of the duodenojejunal segment was tied off below the bile duct and 3 cm from the pyloric sphincter, using surgical silk. A Color-Course four-way stopcock and its extension tubing (Travenol Laboratories, Inc., Morton Grove, IL) were used as a cannula. The desired length of the intestinal segment was then measured, and the distal end was cannulated using an L-shaped glass cannula.

For the colon, the proximal end was tied off immediately after the *ampulla coli*, the desired length measured, and the distal end tied off. Incisions were made at each end, and solid fecal debris was expelled by gentle manipulation. The rest of the fecal debris was forced out by infusing 37°C normal saline through the proximal end. Finally, both the proximal and the distal ends were cannulated as described earlier.

To cleanse the intestinal segment of interest additionally, the proximal cannula was connected to a 60-ml syringe (Beckton Dickinson & Co., Rutherford, NJ). Using an infusion pump (Harvard Apparatus, Millis, MA), 37°C normal saline was infused at a rate of 1.1 ml/min until the effluent was clear. The remaining saline in the segment was expelled by carefully infusing air so as not to cause excessive intraluminal pressure. The intestinal segment under study was arranged in a uniform S to multi-S pattern, depending upon the length, to avoid kinks and ensure uniformity in fluid flow. The isolated segment was kept warm and moist by frequent application of 37°C normal saline to a gauze covering.

Preparation of the Perfusing Solutions

To prepare the perfusing solution, a 500-μl aliquot of a (1 μg/μl) CBZ solution in methanol was transferred to a 100-ml volumetric flask, and the methanol was evaporated on an N-EVAP evaporator (Organomation Associates Inc., South Berlin, MA) at 45°C under nitrogen. The solid residue was dissolved in normal saline; 10%, v/v, PEG-400 (Sigma Chemical Co., St. Louis, MO) in normal saline; or 50%, v/v, PEG-400 in normal saline. One microcurie of [^{14}C]polyethylene glycol 4000 (PEG-4000) (Amersham Corp., Arlington Heights, IL; sp act, 60 mCi/mmol) was added as a nonabsorbable marker to monitor water transport across the intestinal segment.

Perfusions

A 5 μg/ml solution of CBZ in 0, 10, or 50%, v/v, PEG-400 in normal saline was perfused at a constant rate of 0.27 ml/min through either a 50-cm dudenojejunal segment or a 30-cm colon segment. The lag time, an estimate of the mean transit time of perfusing solution, was recorded. Effluent perfusate samples were collected every 10 min in 5- or 10-ml

[1] Department of Pharmaceutics, College of Pharmacy, 308 Harvard Street S.E., Minneapolis, Minnesota 55455.
[2] To whom correspondence should be addressed.

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glass Vacutainers (Beckton Dickinson & Co., Rutherford, NJ) for 160–170 min. For CBZ solutions prepared in normal saline, volumes of the effluent samples were determined gravimetrically, assuming a density of unity; otherwise, they were measured volumetrically.

Determination of [^{14}C]PEG-4000 Purity

The radiochemical purity of PEG-4000 was examined by eluting 0.5 μCi PEG-4000 through a Sephadex G-15 (Sigma Chemical Co., St. Louis, MO). The column used was a 0.9 × 55-cm glass column (Biorad, Richmond, CA). Ten grams of Sephadex G-15 was hydrated and packed in the column to a bed height of 38.5 cm. Phosphate buffer (0.025 mol/liter, pH 7) was used in preparing the gel and in eluting the applied sample. Aliquots were collected every 2 min for 90 min at a flow rate of 0.5 ml/min using a fraction collector (Retriever II, Isco, Lincoln, NE).

Determination of the Partition Coefficient of CBZ

A 5-ml aliquot of the aqueous phase (normal saline; 10%, v/v, PEG-400 in normal saline; or 50%, v/v, PEG-400 in normal saline) was transferred to 35-ml centrifuge tubes. A 20-μl aliquot of a standard methanolic solution containing 1 μg/μl of CBZ was used to spike the aqueous phase. This was followed by the addition of 5 ml of octanol. Each PEG-400 composition was studied in triplicate. Tubes were stoppered and shaken at 180 cycles/min on a mechanical shaker (Eberbach Corp., Ann Arbor, MI) at 37°C. After equilibrium was attained, tubes were centrifuged at 750 *g* for 5 min, and both phases were separated. The concentrations of CBZ in both phases were determined. In this study, the concentration of the drug and volumes of the immiscible phases were held constant, and the composition of PEG-400 in the aqueous phase was varied.

Analytical Methods

Perfusion Studies. When perfusate solutions were prepared in normal saline, effluent perfusate samples were extracted and analyzed for CBZ using a microbore HPLC method developed in our laboratory (15) with the following modifications. Ten microliters of cyheptamide, the internal standard, was used. Aliquots of the effluent perfusate samples (0.5 ml) were used. The volumes of the 0.2 mol/liter phosphate buffer (pH 11.2) and the organic solvent (5% t-butyl alcohol in chloroform) were proportionally scaled to 1 and 10 ml, respectively. A high-pressure liquid chromatograph (Hewlett-Packard, 1090L) was used, employing an oven temperature of 36°C and a flow rate of 0.5 ml/min. Column effluent was monitored at 212 nm using a variable-wave length UV detector (Shimadzu, Model SPD-6A), and peak heights were determined electronically.

When perfusate solutions were prepared in 10 or 50% PEG-400 in normal saline, effluent samples were analyzed as follows. Tubes used for both the standard curve and effluent samples were spiked with 10 μl of 0.03 μg/μl cyheptamide. The standard curve tubes were further spiked with standard solutions of CBZ to provide concentrations ranging from 0.02 to 4.0 μg/ml. The methanol in all tubes was evaporated at 45°C, and the solid residue was reconstituted in 100 μl of the mobile phase, 45:55, v/v, methanol:distilled water, by vortex mixing. For the standard-curve tubes, an additional 100 μl of drug-free 10 or 50%, v/v, PEG-400 in normal saline was added, but for the sample tubes, 100-μl aliquots of the effluent perfusate samples were added, and tubes were vortexed. Samples were transferred to microvials, and 10 μl was injected using the same system described earlier.

The original solution, the effluent perfusate samples, and the samples resulting from the gel permeation experiment were also analyzed for [^{14}C]PEG-4000 using liquid scintillation counting. A 200-μl sample aliquot was mixed with 10 ml of scintillation fluid, Ecolume (ICN Biomedicals, Inc., Irvine, CA). Samples were counted using a scintillation counter (Beckman LS-3801, Beckman Instruments, Inc., Fullerton, CA) with automatic quench correction. To measure the concentration of [^{14}C]PEG-4000 in the original solution, duplicate samples were collected from the inlet tubing at the beginning and end of the experiment.

Partition Coefficient Determination. For the aqueous phase, 100-μl sample aliquots were diluted (1:2) and analyzed as described above, with the exception that 20 μl was injected. For the octanol phase, a 50-μl sample aliquot was diluted (1:3), and 10 μl was injected.

Data Analysis

Perfusion Experiments. To examine the intestinal permeability of CBZ, the following equation (11) describing the fraction of drug remaining to be absorbed at steady state was used:

$$\{C(l)/C(0)\}_{ss} = \exp(-2\pi rlP_e/Q) = \exp(-A \cdot P_e/Q) \quad (1)$$

where $C(0)$ is the concentration of drug entering the intestinal segment, $C(l)$ is the concentration leaving the intestinal segment, r is the radius of the intestinal lumen in cm, l is the length of the intestinal segment (cm), A is the surface area available for absorption (cm^2), P_e is the effective permeability coefficient (cm/min), and Q is the rate at which perfusate is introduced into the intestinal segment (ml/min).

Since there is transmucosal fluid movement that is dependent upon both the intestinal site (16) and the nature of the perfusing solution (17), the $C(l)/C(0)_{ss}$ should be corrected for net water flux. In these experiments, two approaches (volume monitoring and the use of a nonabsorbable marker) were examined to correct for net water movement. The fraction absorbed from an intestinal segment is a function of the ratio of $R(e)$, the exit rate of drug in the perfusate, to the input rate, $R(i)$. Thus,

$$F_a = 1 - \{R(e)/R(i)\}_{ss} \quad (2)$$

where $R(e) = C(l) \cdot Q_{out}$ and $R(i) = C(0) \cdot Q_{in}$ and

$$\{R(e)/R(i)\}_{ss} = \exp(-A \cdot P_e/Q) \quad (3)$$

$\{R(e)/R(i)\}_{ss}$ is a time-averaged term calculated as follows:

$$\{R(e)/R(i)\}_{ss} = AUC_{\{R(e)/R(i)\}}/(t_2 - t_1) \quad (4)$$

where the numerator is the area under the $R(e)/R(i)$–time curve calculated over the period $(t_2 - t_1)$, during which steady state is maintained.

Equation (3) is solved for the area–permeability product, or absorptive clearance of CBZ:

$$A \cdot P_e = -Q \cdot \ln\{R(e)/R(i)\}_{ss} \quad (5)$$

To compare between regions, area–permeability products may be normalized to segment length.

RESULTS

[^{14}C]PEG-4000 as a Nonabsorbable Marker

Water flux was monitored by determining both the volume of effluent samples and the concentration of the assumedly nonabsorbable marker, [^{14}C]PEG-4000, in effluent samples. When drug concentrations were corrected using both methods, the resulting ratios, $R(e)/R(i)$, did not agree. The fraction of marker disappearing at steady state was plotted as a function of the volume ratio, efflux volume/influx volume [$V(eff)/V(inf)$], for different percentages of PEG-400 in normal saline (Figs. 1A and B). In some experiments up to 45% of the [^{14}C]PEG-4000 disappeared during a collection interval. The negative values observed in the fraction absorbed may have been due to carry over from previous intervals, variable peristaltic activity, or reversible binding of the marker.

Of the total radioactivity applied on the Sephadex column, 97.3% was collected in fractions 7 through 9, followed by 2.7% as low molecular weight fractions, which agreed with the data provided by the manufacturer.

These data suggest that PEG-4000 was absorbed during the perfusion, with the proportion disappearing dependent on water flux. However, F_a of the marker was not time dependent (Figs. 2A and B), suggesting no decrease in tissue viability during the experiment. Incomplete recovery of the marker accounts for the disagreement between the correction techniques used.

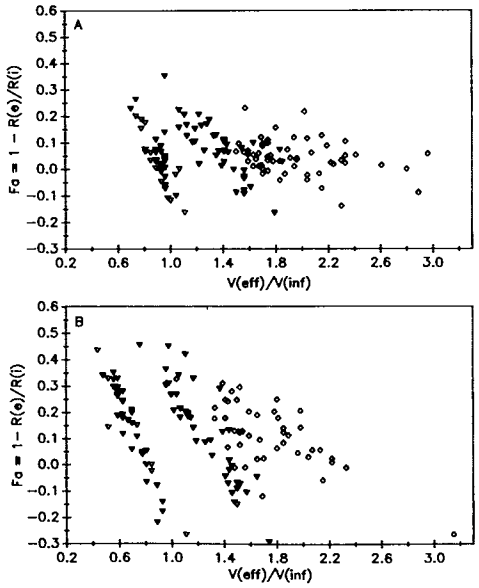

Fig. 1. Relationship between the fraction of [^{14}C]PEG-4000 absorbed and the volume ratio in (A) duodenojejunum and (B) colon. (▽) 0% PEG-400, (▼) 10% PEG-400, and (◇) 50% PEG-400.

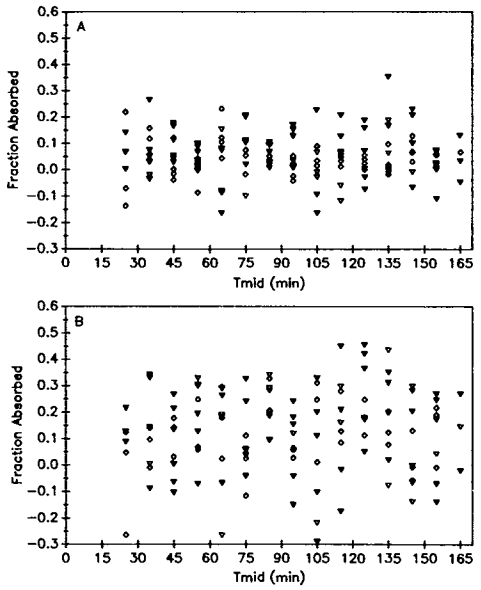

Fig. 2. Relationship between the fraction of [^{14}C]PEG-4000 absorbed and time in (A) duodenojejunum and (b) colon. (▽) 0% PEG-400, (▼) 10% PEG-400, and (◇) 50% PEG-400.

Effect of the Composition of PEG-400 on the Intestinal Permeability of CBZ

Because of the apparent absorptive loss of PEG-4000 in this study, correction for water transport utilized volumetric measurements. $R(e)/R(i)$ plotted versus the midpoint time of the collection interval (t_{mid}) is shown in Figs. 3 and 4 for the duodenojejunum and ascending colon, respectively. Steady states were attained in 30–40 min of perfusion. For both segments, the fraction of CBZ absorbed at steady state decreased with increasing percentage of PEG-400 in normal saline. A summary of the data is given in Table I. Calculated area–permeability products, normalized to length, show that the overall effective permeability decreases with increasing percentage of PEG-400 in the perfusing solution in both segment sites (Table I).

Correlation Between *in Vitro* and *in Situ* Data

To explain the effect of PEG-400 on the intestinal per-

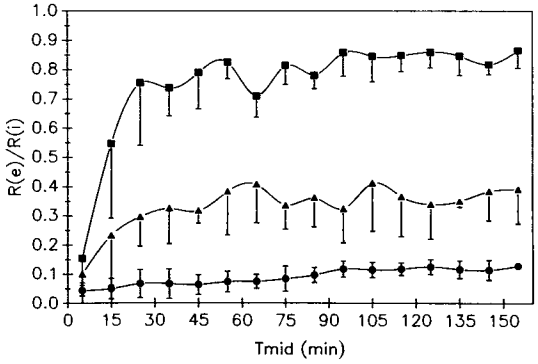

Fig. 3. Effect of the composition of PEG-400 on $R(e)/R(i)$ during duodenojejunal perfusion of CBZ. (●——●) 0% PEG-400, (▲——▲) 10% PEG-400, and (■——■) 50% PEG-400.

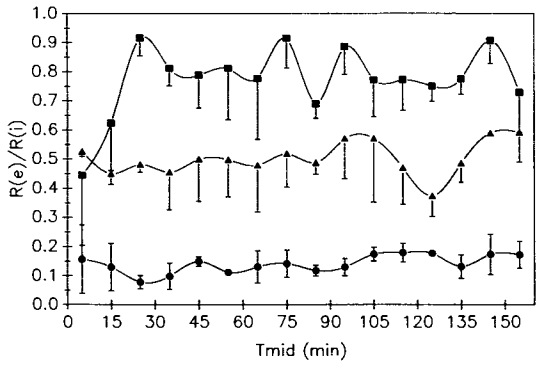

Fig. 4. Effect of the composition of PEG-400 on $R(e)/R(i)$ during colon perfusion of CBZ. (●——●) 0% PEG-400, (▲——▲) 10% PEG-400, and (■——■) 50% PEG-400.

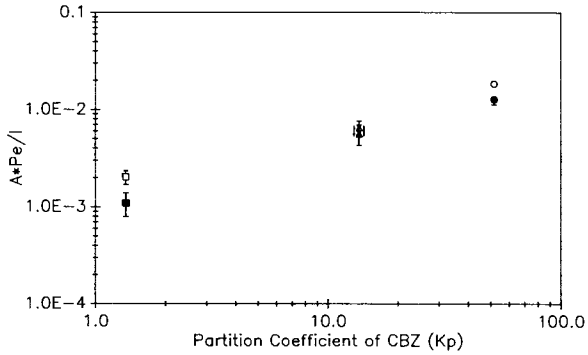

Fig. 5. Effect of the composition of PEG-400 on the intestinal permeability–lipophilicity of CBZ. Duodenojejunum—(●), (▲), and (■); colon—(○), (△), and (□); for 0, 10, and 50% PEG-400, respectively.

meability, the partition coefficients (K_p) of CBZ in octanol and aqueous systems containing different percentages of PEG-400 were determined. The mean ± SD of three determinants at 0, 10, and 50% PEG-400 in normal saline were 51.8 ± 1.97, 13.6 ± 0.663, and 1.35 ± 0.0436, respectively. Figure 5 shows that for all three solvent systems, the log of the intestinal permeability of CBZ in both segments is linearly related to the corresponding log of the octanol/aqueous partition coefficient.

DISCUSSION

This study examines the effect of varying the composition of aqueous PEG-400 solutions on the intestinal permeability of CBZ, a drug practically insoluble in water. This is of interest because PEG is frequently employed as a solubilizing agent for CBZ for experimental purposes, and it has been used as a probe to examine gastric, jejunal, ileal, and colonic permeabilities under different conditions (18,19).

Effect of PEG-400 on Intestinal Permeability of CBZ

Since PEG-400 has been used to increase the solubility of CBZ, the effect of the solvent system on intestinal permeability was examined independent of drug concentration. Water flux was monitored both volumetrically and by using an assumedly nonabsorbable marker, [^{14}C]PEG-4000. As

Table I. *In Situ* Perfusion Data for Carbamazepine in the Duodenojejunum and Ascending Colon with Varying PEG-400 Composition

	0% PEG-400 (mean ± SD)	10% PEG-400 (mean ± SD)	50% PEG-400 (mean ± SD)
	Duodenojejunum		
Rabbits (n)	3	3	4
Body weight (kg)	2.96 ± 0.125	3.06 ± 0.101	3.07 ± 0.0946
$C(0)$ (μg/ml)	4.64 ± 0.403	5.02 ± 0.189	4.93 ± 0.214
Segment length (cm)	49.3 ± 1.53	50.7 ± 1.15	50.8 ± 4.53
Lag time (min)	14.7 ± 1.1	13.2 ± 7.62	11.2 ± 6.15
$\{R(e)/R(i)\}_{ss}$	0.100 ± 0.0251	0.357 ± 0.0998	0.814 ± 0.0572
$A \cdot P_e$ (ml/min)	0.572 ± 0.0787	0.284 ± 0.0718	0.0562 ± 0.0191
$A \cdot P_e/l$ (ml/min · cm)	0.0127 ± 0.00145*	0.00560 ± 0.00134**	0.00109 ± 0.000301***
	Ascending colon		
Rabbits (n)	3	3	3
Body weight (kg)	3.04 ± 0.085	2.90 ± 0.0351	3.39 ± 0.3
$C(0)$ (μg/ml)	4.72 ± 0.225	4.97 ± 0.0833	4.64 ± 0.161
Segment length (cm)	28.0 ± 1.32	29.3 ± 1.53	29.0 ± (0)
Lag time (min)	27.5 ± 0.986	23.8 ± 2.56	10.9 ± 1.75
$\{R(e)/R(i)\}_{ss}$	0.148 ± 0.018	0.502 ± 0.0624	0.806 ± 0.028
$A \cdot P_e$ (ml/min)	0.516 ± 0.0325	0.187 ± 0.0352	0.0584 ± 0.0094
$A \cdot P_e/l$ (ml/min · cm)	0.0184 ± 0.000404*	0.00640 ± 0.00123**	0.00202 ± 0.000325***

* Significantly different, Student t test, $P = 0.003$.
** Nonsignificantly different, Student t test, $P = 0.487$.
*** Significantly different, Student t test, $P = 0.011$.

shown in Figs. 1A and B and in earlier studies (20,21), PEG-4000 appeared to be absorbed, causing an overestimate in the $\{R(e)/R(i)\}_{ss}$ ratio and, therefore, an underestimate in the extent of absorption of CBZ.

The effect of varying the composition of PEG-400 in perfusate solutions was examined by comparing the area–permeability products normalized to length for CBZ in both the duodenojejunal and the colon segments. A major factor in the reduction of the fraction absorbed with increasing PEG-400 concentration appears to be a decrease in thermodynamic activity of CBZ in solution when increasing PEG-400 concentrations are utilized in the perfusate. This is reflected by a corresponding alteration in the *in vitro* octanol/aqueous partition coefficient (K_p). The *in vitro* data show decreasing K_p values with increasing PEG-400 concentrations in the aqueous phase. The relationship between normalized absorptive clearance of CBZ and K_p is depicted in Fig. 5.

Similar results were found in a study performed to examine the effect of varying the percentage of Tween 80 on the intestinal permeability of progesterone (12). In addition PEG-400 may affect the degree to which solvent drag contributes to CBZ absorption. Positive net water flux (water absorption from the lumen) can be viewed as entrainment of solute molecules in a paracellular stream of the solvent (22,23). This supplementary flux of solute caused by solvent drag is convective and is added to the diffusive flux. Reverse solvent drag (water flux into the lumen) may attenuate this convective route of transport. The magnitude of the contribution of solvent drag to transport depends on the lipophilicity of the molecule and the nature (tonicity) of the perfusing solution (17,24,25).

Since carbamazepine is a neutral lipophilic compound, its permeability through membranes is the primary determinant of its overall flux, which is therefore relatively insensitive to solvent drag. However, solvent drag has been shown to affect membrane permeability of lipid-soluble drugs, perhaps through an interaction of such molecules with water within the lipid part of the membrane (26).

Effect of Transmucosal Water Flux on the Fraction Absorbed

During the course of the experiments, volume ratios [V(eff)/V(inf)] ranging from 0.4 to 3.0 were observed. The effect of water flux on the fraction absorbed may be examined in the following equation:

$$F_a = 1 - \{R(e)/R(i)\}_{ss} = 1 - \exp(-A \cdot P_e/Q^*) \quad (6)$$

where Q^* is the average flow rate (ml/min) within a collection interval and is equal to

$$Q^* = Q \cdot V(\text{eff})/V(\text{inf}) \quad (7)$$

At high $A \cdot P_e/Q^*$, F_a approaches 1; at very small $A \cdot P_e/Q^*$

$$F_a \cong 2\pi rlP_e/Q^* = A \cdot P_e/Q^* = A \cdot P_e/\{Q \cdot V(\text{eff})/V(\text{inf})\} \quad (8)$$

Theoretical curves were generated to examine the fraction absorbed (F_a) as a function of V(eff)/V(inf) for drugs exhibiting differing area–permeability products. These

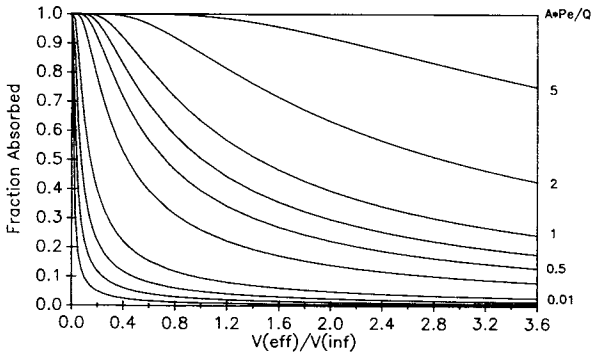

Fig. 6. Simulation of the fraction absorbed at steady state and the volume ratio for different permeability values.

curves, shown in Fig. 6, simulate the decrease in F_a with an increase in the volume ratio.

To examine the effect of transmucosal water movement, the fraction of CBZ absorbed at steady state was plotted versus the volume ratio. For each composition of PEG-400, results were pooled and examined as one data set. Equation (6) was fitted to the data using PCNONLIN, and the average permeability term corresponding to each composition of PEG-400 was obtained from the fit. A summary of the data is given in Table II.

Figures 7 and 8 show the line of best fit for F_a at each composition of PEG-400 for the duodenojejunum and colon, respectively. However, the system is more complex than that represented here, as these simulations assume that CBZ activity remains constant. For solutions containing PEG-400, reverse solvent drag resulting from the high osmotic pressure in the lumen caused a continuous dilution of the perfusing solvent during transit. Therefore, dilution of PEG-400 in solution increases the thermodynamic activity of CBZ, thus increasing F_a. This is observed by data tending to deviate toward higher F_a at high V(eff)/V(inf) values. Estimates of area–permeability products as a function of the changing composition of PEG-400 in the perfusing solutions are difficult to obtain since PEG-400 itself is absorbed from various intestinal regions (19).

Effect of Hyperosmolarity on the Intestinal Mucosa

Although no histological examination of the perfused segment was conducted, gross changes such as mucosal

Table II. Effect of Volume Ratio on the Fraction of Carbamazepine Absorbed

Duodenojejunum	
0% PEG-400	2.12 (0.0528)[a]
10% PEG-400	1.41 (0.0590)
50% PEG-400	0.370 (0.0287)
Ascending colon	
0% PEG-400	1.33 (0.0291)
10% PEG-400	0.858 (0.0326)
50% PEG-400	0.370 (0.0335)

[a] The parameter, $A \cdot P_e/Q$, and the standard error around it, produced from fitting Eq. (6).

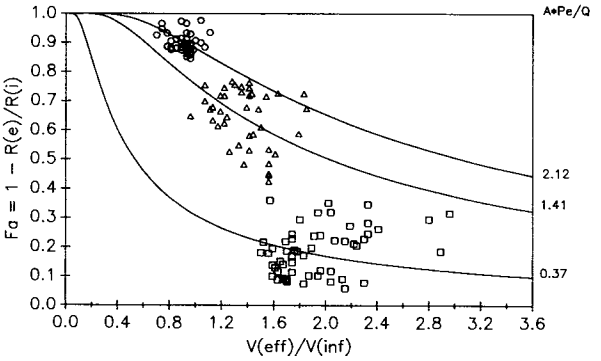

Fig. 7. Relationship between the fraction of CBZ absorbed at steady state and the volume ratio during duodenojejunal perfusions. (○) 0% PEG-400, (△) 10% PEG-400, and (□) 50% PEG-400.

sloughing were monitored. This was occasionally observed only when 50% PEG-400 in normal saline was employed. Viability of the segment was judged by the pink color and pulsating arcades of blood vessels perfusing the segment during the 3-hr studies. The disappearance of [^{14}C]PEG-4000 was also monitored, and although F_a was dependent on water flux, it was not a function of time (Figs. 2A and B). The variability observed in the data results from the contribution of solvent drag to the fraction absorbed.

The effect of PEG solutions on the histology of the intestinal mucosa has been examined using *in situ* rat intestinal loop segments (27,28). When 55%, w/v, of PEG-1000 in normal saline was used (27), osmolarity measured by freezing point (2000–4000 mosm/liter) was far more than its concentration would predict (860 mosm/liter). Extreme deviations between measured and calculated osmolarity were also obtained when PEG-2000 was used at varying concentrations, 10–30%, w/v (28). This anomolous osmotic behavior of PEG solutions induces considerable fluid loss from the mucosa to the lumen.

In summary, the effect of PEG-400 on the intestinal permeability of CBZ was examined in an *in situ* rabbit model. The results suggest that the observed decrease in intestinal permeability with increasing concentrations of PEG-400 may be attributed to a reduction in thermodynamic activity of CBZ in solution and to reverse solvent drag resulting from the hyperosmolarity of the perfusate solutions.

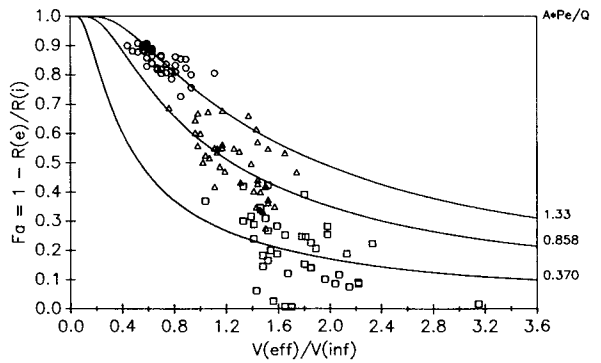

Fig. 8. Relationship between the fraction of CBZ absorbed at steady state and the volume ratio during colon perfusions. (○) 0% PEG-400, (△) 10% PEG-400, and (□) 50% PEG-400.

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REFERENCES

1. L. Bertilsson and T. Tomson. Clinical pharmacokinetics and pharmacological effects of carbamazepine and carbamazepine-10,11-epoxide. *Clin. Pharmacokinet.* 11:177–198 (1986).
2. J. W. Faigle, S. Brechbuhler, K. F. Feldmann, and W. J. Richter. The biotransformation of carbamazepine. In W. Birkmayer (ed.), *Epileptic Seizures-Behaviour-Pain,* Hans Huber, Bern, 1976, pp. 127–140.
3. M. Sumi, N. Watari, O. Umezawa, and N.Kaneniwa. Pharmacokinetic study of carbamazepine and its epoxide metabolite in humans. *J. Pharmaco-bio-Dyn.* 10:652–661 (1987).
4. R. H. Levy, W. H. Pitlick, A. S. Troupin, J. R. Green, and J. M. Neal. Pharmacokinetics of carbamazepine in normal man. *Clin. Pharmacol. Ther.* 17:657–668 (1975).
5. R. H. Levy, J. S. Lockard, J. R. Green, P. Friel, and L. Maris. Pharmacokinetics of carbamazepine in monkeys following intravenous and oral administration. *J. Pharm. Sci.* 64:302–307 (1975).
6. M. Sumi, N. Watari, H. Naito, O. Umezawa, and N. Kaneniwa. Influence of phenobarbital on pharmacokinetics of carbamazepine and its epoxide in the rabbit. *Yakugaku Zasshi* 107:984–991 (1987).
7. P. J. Wedlund and R. H. Levy. Time-dependent kinetics. VII. Effect of diurnal oscillations on the time course of carbamazepine autoinduction in the rhesus monkey. *J. Pharm. Sci.* 72:905–909 (1983).
8. W. H. Pitlick and R. H. Levy. Time-dependent kinetics. I. Exponential autoinduction of carbamazepine in monkeys. *J. Pharm. Sci.* 66:647–649 (1977).
9. I. H. Patel and R. H. Levy. Intramuscular absorption of carbamazepine in rhesus monkeys. *Epilepsia* 21:103–109 (1980).
10. I. H. Patel, P. J. Wedlund, and R. H. Levy. Induction effect of phenobarbital on the carbamazepine to the carbamazepine-10,11-epoxide pathway in rhesus monkeys. *J. Pharmacol. Exp. Ther.* 217:555–558 (1981).
11. N. F. H. Ho and W. I. Higuchi. Theoretical model studies of intestinal drug absorption. IV. Bile acid transport at perimicellar concentrations across diffusion layer-membrane barrier. *J. Pharm. Sci.* 63:686–690 (1974).
12. N. F. H. Ho, J. Y. Park, P. F. Ni, and W. I. Higuchi. Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. In W. Crouthamel and A. C. Sarapu (eds.), *Animal Models for Oral Drug Delivery in Man,* APhA, Washington, D.C., 1983, pp. 27–106.
13. R. J. Sawchuk and W. M. Awni. Absorption of cyclosporine from rabbit small intestine *in situ*. *J. Pharm. Sci.* 75:1151–1156 (1986).
14. J. T. Doluisio, G. H. Tan, N. F. Billups, and L. Diamond. Drug absorption. II. Effect of fasting on intestinal drug absorption. *J. Pharm. Sci.* 58:1200–1202 (1969).
15. L. E. Riad and R. J. Sawchuk. Simultaneous determination of carbamazepine and its epoxide and *trans*diol metabolites in plasma by microbore liquid chromatography. *Clin. Chem.* 33:1863–1866 (1988).
16. I. Johno, K. Kawakatsu, H. Kuwata, and S. Kitazawa. Segmental differences in transmucosal fluid movement and its effect on gastrointestinal drug absorption in rabbits. *Int. J. Pharm.* 25:255–263 (1985).
17. S. Kitazawa, H. Ito, and H. Sezaki. Transmucosal fluid movement and its effect on drug absorption. *Chem. Pharm. Bull.* 23:1856–1865 (1975).

18. V. S. Chadwick, S. F. Phillips, and A. F. Hoffman. Measurement of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). I. Chemical analysis and biological properties. *Gastroenterology* 73:241–246 (1977).
19. V. S. Chadwick, S. F. Phillips, and A. F. Hoffman. Measurement of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man. *Gastroenterology* 73:247–251 (1977).
20. J. R. Pappenheimer and K. E. Zich. Absorption of hydrophilic solutes from the rat small intestine. *J. Physiol. (London)* 371:138P (1985).
21. J. R. Pappenheimer and K. Z. Reiss. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membrane Biol.* 100:123–136 (1987).
22. N. Lifson and A. A. Hakim. Simple diffusive-convective model for intestinal absorption of a nonelectrolyte (urea). *Am. J. Physiol.* 211:1137–1146 (1966).
23. N. Lifson, L. M. Gruman, and D. G. Levitt. Diffusive-convective models for intestinal absorption of D_2O. *Am. J. Physiol.* 215:444–454 (1968).
24. A. Karino, M. Hayashi, T. Horie, S. Awazu, H. Minami, and M. Hanano. Solvent drag effect in drug intestinal absorption. I. Studies on drug and D_2O absorption clearances. *J. Pharm. Dyn.* 5:410–417 (1982).
25. A. Karino, M. Hayashi, S. Awazu, and M. Hanano. Solvent drag effect in drug intestinal absorption. II. Studies on drug absorption clearance and water influx. *J. Pharm. Dyn.* 5:670–677 (1982).
26. O. Kedem and A. Katchalsky. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* 45:143–179 (1961).
27. H. Kameda, T. Abei, S. Nasrallah, and F. L. Iber. Functional and histological injury to intestinal mucosa produced by hypertonicity. *Am. J. Physiol.* 214:1090–1095 (1968).
28. A. J. Bryan, R. Kaur, G. Robinson, N. W. Thomas, and C. G. Wilson. Histological and physiological studies on the intestine of the rat exposed to solutions of Myrij 52 and PEG 2000. *Int. J. Pharm.* 7:145–156 (1980).