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

Vaishali Pade[1] and Salomon Stavchansky[1,2]

Received March 10, 1997; accepted June 12, 1997

Purpose. The objective of this investigation was to determine, using the Caco-2 cell culture model, the extent to which the paracellular and transcellular routes contributed to the transport of passively absorbed drugs. An effort was also made to determine the controlling factors in this process.

Methods. We selected a heterologous series of drugs with varying physicochemical parameters for the investigation. Effective permeability coefficients of the model drugs (naproxen, phenytoin, salicylic acid, chlorothiazide, furosemide, propranolol, diltiazem, ephedrine, and cimetidine), at pH 7.2 and pH 5.4, were estimated using confluent monolayers of Caco-2 cells. The biophysical model approach, based on molecular size restricted diffusion within an electrostatic field of force, used by Adson *et al.* (1,2), was employed to estimate the permeability coefficients of the ionized and unionized forms of the drugs for the paracellular and transcellular route.

Results and Conclusions. The permeability coefficients of the acidic drugs was greater at pH 5.4, whereas that of the basic drugs was greater at pH 7.2 and the transcellular pathway was the favored pathway for most drugs, probably due to its larger accessible surface area. The paracellular permeability of the drugs was size and charge dependent. The permeability of the drugs through the tight junctions decreased with increasing molecular size. Further, the pathway also appeared to be cation-selective, with the positively charged cations of weak bases permeating the aqueous pores of the paracellular pathway at a faster rate than the negatively charged anions of weak acids. Thus, the extent to which the paracellular and transcellular routes are utilized in drug transport is influenced by the fraction of ionized and unionized species (which in turn depends upon the pKa of the drug and the pH of the solution), the intrinsic partition coefficient of the drug, the size of the molecule and its charge.

KEY WORDS: permeability; paracellular; transcellular; transport, Caco-2.

INTRODUCTION

Transport of solutes across the intestinal epithelium can occur transcellularly or paracellularly. The extent to which these pathways contribute to the overall flux of the drug depends upon the environment of the gastrointestinal tract, the physicochemical parameters of the solute, and the properties of the intestinal epithelium. Paracellular transport involves only passive diffusion, whereas transcellular transport can occur by passive, facilitated or active processes. Generally, hydrophilic, passively transported, polar solutes diffuse through the paracellular route, while the more lipophilic solutes use the transcellular route. This research article focuses on describing the effective permeability coefficients of diverse, passively absorbed solutes in terms of their paracellular and transcellular components. The Caco-2 cell culture model, an established model for studying intestinal absorption (3–6), was used for this purpose. The Caco-2 cell line, which is derived from a human colorectal adenocarcinoma, undergoes spontaneous enterocytic differentiation in culture, forming monolayers of polarized enterocytes that exhibit morphological and functional similarities to the small intestinal epithelium (7–9). The effective permeability coefficients measured across Caco-2 cell monolayers includes contributions from the aqueous boundary layer, the polycarbonate filter support and the cell monolayer. The permeability of the cell monolayer itself is a combination of the drug's permeability through the transcellular and the paracellular pathways. Further, for passively absorbed drugs, the relative contribution of each of these pathways to drug transport depends upon the pKa, partition coefficient, molecular radius and charge of the drug, the pH of luminal solution, and the area of the absorbing surface after accounting for the permeability through the aqueous boundary layer and the filter support. The effective permeability coefficients of model drugs: naproxen, phenytoin, salicylic acid, chlorothiazide, furosemide, propranolol, diltiazem, ephedrine, and cimetidine, were estimated using fifteen day old Caco-2 monolayers grown on microporous polycarbonate membranes. Transport studies were conducted at pH 7.2 and pH 5.4 (pHs which are representative of the human small intestine) to study the effect of pH, pKa, and partition coefficient on permeability. The effect of molecular size and charge on permeability was also investigated.

MATERIALS AND METHODS

Chemicals

^{14}C-mannitol (56.7 mCi/mmol), ^{14}C-salicylic acid (56.1 mCi/mmol), ^{14}C-phenytoin (47.2 mCi/mmol), ^{3}H-diltiazem (84.2 Ci/mmol), and ^{3}H-propranolol (21.2 Ci/mmol) were obtained from New England Nuclear Research Products (Boston, MA). ^{3}H-cimetidine (21 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). All labeled compounds used in the permeability experiments had radiochemical purity greater than 99%. Unlabeled furosemide, naproxen sodium, chlorothiazide, ephedrine, salicylic acid, phenytoin, propranolol, diltiazem, and cimetidine, were obtained from Sigma Chemical Company (St. Louis, MO). Solvents used in high performance liquid chromatographic procedures were of HPLC grade. All other reagents were analytical grade.

Cell Culture

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD). Cells from passage number 35 to 41 were used for the transport experiments. The details for the cell culture techniques have been previously described (3). Fifteen day old confluent monolayers of fully differentiated

[1] Pharmaceutics Division, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712.

[2] To whom correspondence should be addressed. (e-mail: stavchansky@mail.utexas.edu)

0724-8741/97/0900-1210$12.50/0

Caco-2 cells were grown in 162 cm^2 T-flasks, obtained from Corning Costar Corporation (Cambridge, MA). Cells for transport studies were grown in Transwell® cell culture chambers (diameter 24 mm, pore size 3.0 μ, growth area 4.71 cm^2), also obtained from Corning Costar. The integrity of the monolayers was checked by using mannitol as a paracellular marker, and by measuring the transepithelial electrical resistance.

Transport Studies

Transport of each drug from the apical to the basolateral chamber was studied at pH 7.2 and pH 5.4. The pH 7.2 transport buffer was comprised of phosphate buffer saline (PBS), 15 mM HEPES and 1 g/liter glucose, whereas the pH 5.4 transport buffer comprised of PBS containing 10mM sodium citrate and 10 mM citric acid, with 1 g/liter glucose. All transport experiments were conducted in triplicate at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity. The amount of drug transported to the receiver was normalized to account for loss in the apical concentration during each time interval. The effective permeability coefficients were calculated using equation 1.

$$P_e = \left(\frac{V_d}{A} \cdot \frac{\Delta\%}{\Delta t}\right) \quad (1)$$

where, P_e is the effective permeability coefficient measured in cm/sec, V_d is the volume (cm^3) in the donor compartment, A is the surface area of the monolayers, 4.71 cm^2, and $\Delta\%/\Delta t$ is the percent mass transported per time interval. Finally, a mass balance calculation was also performed to determine if accumulation or metabolism of the solute, or adsorption to the apparatus had occurred.

The average recovery from the apical to basolateral dircetion at pH 7.2 with and without the monolayer were found to be 91.1 ± 13.7, and 102 ± 6.14, respectively. From the basolateral to apical direction the recoveries were found to be 97.2 ± 9.81 and 103 ± 5.54, respectively. Finally, the recovery from the apical to basolateral direction with and without the monolayer were found to be 94.8 ± 11.7 and 103.9 ± 5.76, respectively.

Estimation of the Relative Contribution of the Paracellular and Transcellular Pathways to Drug Transport

The effective permeability coefficient, P_e, is composed of permeability coefficients of the various transport barriers in series, namely the aqueous boundary layer, the cell monolayer and the filter support. The total resistance to the transport of a solute can be expressed as the sum of the resistances offered by each barrier, as shown in equation 2 (2).

$$\frac{1}{P_e} = \frac{1}{P_{abl}} + \frac{1}{P_f} + \frac{1}{P_m} \quad (2)$$

/here, P_{abl} is the permeability coefficient of the aqueous boundry layer, P_f is the permeability coefficient of the polycarbonate lter support, and, P_m is the permeability coefficient of the cell onolayer. For the unstirred system, Burton and coworkers ersonal communication) have determined the P_{abl} to be 8×10^{-5} cm/sec. This value is in close agreement with the value orted by Adson of 5.4×10^{-5} cm/sec at 25 rpm. In our culations we use 8×10^{-5} cm/sec.

Permeability Coefficient of the Filter Support

The permeability coefficient for the filter was estimated using equation 3.

$$P_f = \frac{\varepsilon_f \cdot D \cdot F\left(\frac{r}{R_f}\right)}{\delta_f} \quad (3)$$

where, ε_f is the filter porosity (0.15), δ_f is the filter thickness (10 μ), and R_f is the radius of the pores of the filter (1.5 μ). $F(r/R_f)$ is the molecular sieving function which compares the radius of the molecule to the radius of the filter pore, and D is the aqueous diffusion coefficient of the molecule.

Permeability Coefficient of the Cell Monolayer

In order to determine the relative contribution of the transcellular and paracellular routes on the diffusion of the ionized and the unionized species, the following approach, developed by Adson *et al.* was applied (1,2). It was assumed that only the unionized species of the drug could partition into the cell membrane, and diffuse across the cell (transcellular transport), whereas, both unionized and ionized species could diffuse across the tight junctions (paracellular transport). Based on this assumption, the permeability of the monolayer is expressed by equation 4.

$$P_m = f_u(P^o_{trans} + P^o_{para}) + (1 - f_u)P^{\pm}_{para} \quad (4)$$

where, f_u is the fraction of unionized species, P^o_{trans} is the permeability of the unionized species for the transcellular route, P^o_{para} is the permeability coefficient of the unionized species for the paracellular route, and $P^{\pm}_{para}$ is the permeability coefficient of the ionized species (cationic or anionic) for the paracellular route.

Adson *et al.* (1) have derived a systematic and quantitative approach to passive transport by the paracellular route across cultured cell monolayers, using several hydrophilic solutes of varying molecular size and charge. The approach utilizes the theory of molecular size-restricted diffusion of cations and anions within a negative electrostatic field of force (10). The permeabilities of cations and anions across the aqueous pores of the paracellular pathway of the cell monolayer are expressed by equation 5 and 6.

$$P^+_{para} = \frac{\varepsilon \cdot D \cdot F\left(\frac{r}{R}\right)}{\delta}\left(\frac{\kappa}{1 - e^{-\kappa}}\right) \quad (5)$$

$$P^-_{para} = \frac{\varepsilon \cdot D \cdot F\left(\frac{r}{R}\right)}{\delta}\left(\frac{\kappa}{e^{\kappa} - 1}\right) \quad (6)$$

where, P^+_{para} and P^-_{para} represent the paracellular permeabilities of the cations and anions, respectively, ε is the porosity, D is the aqueous diffusion coefficient of the ion, r is the radius of the molecule, R is the radius of the pore, F(r/R) is the molecular sieving factor, and κ is the dimensionless electrochemical energy function across the pore of length, δ. The electrochemical energy function is estimated using equation 7.

$$\kappa = \frac{ez|\Delta\Psi|}{k_B T} \tag{7}$$

where, e is the unit charge of an ion, 4.8×10^{-10} esu, z is the valence of the ion (sign included), $|\Delta\Psi|$ is the potential drop across the barrier, k_B is the Boltzmann constant, 1.38×10^{-23} Joules/Kelvin, and T is the temperature in degrees Kelvin.

The permeability of the neutral molecule, P^o_{para}, is given by equation 8.

$$P^o_{para} = \frac{\varepsilon \cdot D \cdot F\left(\frac{r}{R}\right)}{\delta} \tag{8}$$

The molecular radii of the drugs were estimated from their molecular volumes using equation 9, which is the equation for the volume of a sphere.

$$V = \frac{4}{3}\pi r^3 \tag{9}$$

where, V is the molecular volume and r is the molecular radius. The molecular weights of the model drugs used in this investigation were in the range of 100–450 g/mole, and therefore, these molecules were assumed to be spherical molecules.

The program, SAVOL (11), was used to calculate the molecular volumes of the model drugs. The aqueous diffusion coefficients of the model drugs at 37°C were, then, estimated using the Stokes-Einstein equation. Equation 10 represents the Stokes-Einstein equation for small, spherical molecules (12).

$$D = \frac{k_B T}{6\pi\eta r} \tag{10}$$

where, D is the diffusion coefficient in cm^2/sec, k_B is the Boltzmann constant, T is the temperature in degrees Kelvin, η is the viscosity of water (0.006915 poise at 37°C), and r is the radius of the molecule in cm.

The dimensionless molecular sieving function, F(r/R), compares the molecular radius, r, with the pore radius, R, and, $0 < F(r/R) < 1$. The molecular sieving function for cylindrical channels is calculated using the Renkin equation (13,14), and is expressed by equation 11.

$$F\left(\frac{r}{R}\right) = \left[1 - \left(\frac{r}{R}\right)\right]^2\left[1 - 2.104\left(\frac{r}{R}\right) + 2.09\left(\frac{r}{R}\right)^3 - 0.95\left(\frac{r}{R}\right)^5\right] \tag{11}$$

RESULTS AND DISCUSSION

Model drugs selected for the investigation included both acidic drugs: naproxen, phenytoin, salicylic acid, chlorothiazide, and furosemide, and basic drugs: propranolol, diltiazem, ephedrine and cimetidine. The drugs belong to different structural classes, and have a wide range of partition coefficients. The physicochemical parameters of the model dugs along with the molecular radii (estimated using equation 9) and the aqueous diffusion coefficients at 37°C (estimated using equation 10) are presented in Table I.

Transport Studies

The apical to basolateral transport of the model drugs at pH 7.2 and pH 5.4 was linear over the time periods studied and is depicted in Figures 1 and 2. The effective permeability coefficients of the model drugs across Caco-2 monolayers at pH 7.2 and pH 5.4 are presented in Table II. For the acidic drugs, naproxen, phenytoin, salicylic acid, chlorothiazide, and furosemide, the fraction of unionized species is greater at pH 5.4 compared to pH 7.2. Consequently, it was observed that the permeability at pH 5.4 was higher than the permeability at pH 7.2. Similarly, in the case of the basic drugs, propranolol, diltiazem, ephedrine, and cimetidine, the fraction of unionized species is greater at pH 7.2 than at pH 5.4. Therefore, the transport of the basic drugs was observed to be faster at pH

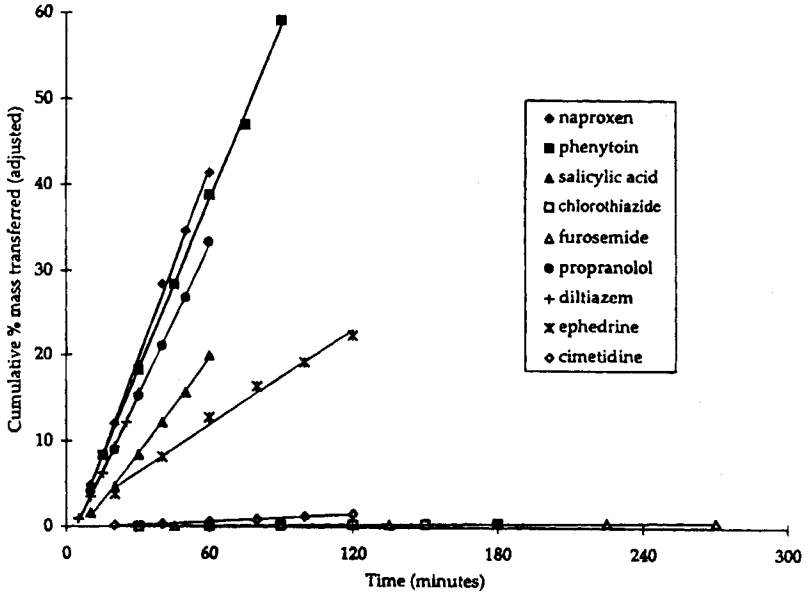

Fig. 1. Apical to basolateral transport of the model drugs across Caco-2 cell monolayers at pH 7.2.

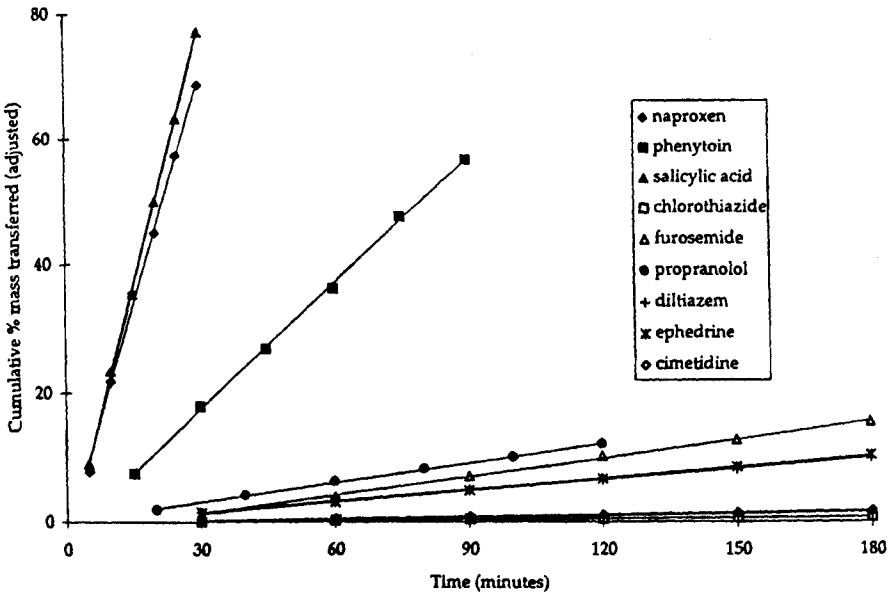

Fig. 2. Apical to basolateral transport of the model drugs across Caco-2 cell monolayers at pH 5.4.

7.2 as compared to pH 5.4. These observations are in agreement with the pH-partition theory which states that a drug is absorbed from the gastrointestinal tract mainly in its unionized form (15).

Permeability Coefficient of the Filter Support

The molecular restriction factor, $F(r/R_f)$, comparing the radius of the molecule with the radius of the filter, was calculated for each model drug. The pore radius of the polycarbonate membrane support was sufficiently large so that molecular size-restricted diffusion was negligible, and $F(r/R_f) \approx 1$.

Permeability Coefficient of the Cell Monolayer

The permeability coefficient for the cell monolayer was then simply calculated by subtraction of $1/P_f$ and $1/P_{abl}$ from $1/P_e$. The next step was to determine the extent to which the paracellular and transcellular pathways contributed towards the passage of the drug across the cell monolayer. In order to calculate the paracellular permeabilities of the unionized and ionized species using equation 5, 6, and 8, it was necessary to get an estimate of the effective pore radius of the cell monolayer. It has been reported that the pore radius of human colonic T84 cells is between 3.6 and 11.6 Å (16), and that of jejunal epithelia is between 6.7 to 8.8 Å (17). Based on these reports, the effective pore radius of the Caco-2 cell monolayer was assumed to be 7.75 Å, which is the average of 6.7 Å and 8.8 Å. Regardless of weather the jejunal or the T84 cell data is chosen the difference between the averages of the T84 cells and jejunal epithelial pore radiuses is slight and it is not expected to influence the final analysis of the data or the conclusions arrived from the data.

Mannitol, a neutral molecule, is absorbed exclusively by passive diffusion through the paracellular route. Therefore, P_m of mannitol was utilized in the estimation of ε/δ, using equation 8. The effective permeability coefficients of mannitol ranged from 0.14×10^{-6} to 0.26×10^{-6}, ($0.2 \times 10^{-6} \pm 0.04 \times 10^{-6}$, n = 12).

$$\frac{\varepsilon}{\delta} = \frac{P_m^{\text{mannitol}}}{D \cdot F\left(\frac{r}{R}\right)} = 0.267 \text{ cm}^{-1}$$

It was assumed that the ratio remained constant through the duration of the transport experiments.

Table I. Physiochemical Parameters of the Model Drugs

Drug	Molecular weight (g/mole)	pKa	Intrinsic log P	Molecular radius × 10^8 (cm)	D_{aq} at 37°C × 10^6 (cm^2/sec)
Naproxen	230.3	4.2	2.8	3.69	8.88
Phenytoin	252.3	8.3	2.5	3.73	8.79
Salicylic acid	138.1	3.0	3.3	3.03	10.8
Chlorothiazide	295.7	6.7	−0.1	3.61	9.08
Furosemide	330.4	4.7	−0.8	3.91	8.39
Propranolol	259.3	9.5	3.6	3.84	8.54
Diltiazem	414.5	7.7	2.7	4.62	7.11
Ephedrine	165.2	9.6	0.9	3.43	9.58
Cimetidine	252.3	7.1	0.4	3.81	8.62

Table II. Mean Effective Permeability Coefficients of the Model Drugs Across Caco-2 Monolayers, at pH 7.2 and pH 5.4

Drug	P_e(Ap → Bl) × 10^6 cm/sec at pH 7.2	P_e(Ap → Bl) × 10^6 cm/sec at pH 5.4
Naproxen	39.5 ± 0.27	131.0 ± 1.19
Phenytoin	34.3 ± 1.75	35.11 ± 0.96
Salicylic acid	18.6 ± 0.86	143.8 ± 5.52
Chlorothiazide	0.15 ± 0.01	0.23 ± 0.01
Furosemide	0.12 ± 0.01	5.06 ± 0.21
Propranolol	30.1 ± 1.23	5.45 ± 0.08
Diltiazem	29.8 ± 0.22	2.98 ± 0.07
Ephedrine	10.2 ± 0.58	3.12 ± 0.19
Cimetidine	0.74 ± 0.09	0.50 ± 0.02

Adson *et al.* (1) have reported that κ, and the potential drop across the barrier, $|\Delta\Psi|$, are essentially identical among the singly charged, cationic and anionic permeants studied. Solutes used in their study included both weak acids and bases whose molecular radii ranged from 2.65 to 5.99 Å. The electrochemical energy function estimated by them for each permeant ranged from 0.62 to 0.78, and their estimate of $|\Delta\Psi|$ was 17.7 mV (range 15.9 mV to 20 mV). The potential drop across the barrier was, therefore, assumed to be 17.7 mV in our calculations, and the dimensionless electrochemical energy function, κ, was then estimated using equation 7.

$$k = \frac{ez|\Delta\Psi|}{kT} = 0.662$$

The plot of paracellular permeability (P_{para}(total)) versus molecular radius presented in Figure 3 shows the influence of molecular size and electrical charge on paracellular permeability. The intrinsic paracellular permeability of the drugs appeared to decrease with increasing size. It was also observed that the intrinsic paracellular permeability of the positively charged cations of weak bases was greater than that of the anions of weak acids, indicating that the paracellular pathway is cation-selective. This transport behavior of permeants conforms with the hypothesis that the paracellular route may be negatively charged (19). These results are similar to results of transport studies with hydrophilic extracellular permeants (neutral, cationic, anionic, zwitterionic) reported by Adson *et al.* (1), and to transport kinetics of ionic permeants across water-filled, negatively charged, porous collagen matrix of the cuticle of the roundworm, *Ascaris suum* (18).

The fraction of unionized and ionized species were, then, calculated, and from these, the permeabilities of the various molecular species across the parallel transcellular and paracellular pathways of the monolayer were obtained. The permeability of the unionized species for the transcellular pathway was obtained from equation 4, once the permeabilities of the ionized and unionized species for the paracellular route were known. The relative contribution of these two routes towards the transport of each drug at pH 7.2, and pH 5.4, is presented in Table III. It was observed that the drugs naproxen, phenytoin, salicylic acid, propranolol, diltiazem, and ephedrine, are transported mainly by the transcellular route in spite of the fraction of unionized species being very small at pH 7.2 and pH 5.4. This is probably due to the fact that the paracellular route represents a relatively small fraction of accessible area of the cell monolayer. It has been reported that the cell membrane occupies a surface area that is a thousand times greater than the area occupied by the paracellular spaces. Besides, it was also observed that the intrinsic paracellular permeability is influenced by molecular size and charge. Thus, the smaller accessible area, and its size and charge based discrimination make the paracellular route the less favorable route for drug transport. Further, it should also be noted that naproxen, phenytoin, salicylic acid, propranolol and diltiazem, have a fairly high intrinsic partition coefficient, making them lipophilic enough to take advantage of the large surface area for transcellular permeation. On the other hand, the drugs, furosemide and cimetidine, are intrinsically more hydrophilic. Also, furosemide exists mostly

Table III. Relative Contribution of the Transcellular and Paracellular Pathways to the Transport of Model Drugs Across Caco-2 Monolayers, at pH 7.2 and pH 5.4

	pH 7.2		pH 5.4	
Drug	Paracellular transport (%)	Transcellular transport (%)	Paracellular transport (%)	Transcellular transport (%)
Naproxen	0.11	99.89	0	100
Phenytoin	0.20	99.80	0.19	99.81
Salicylic acid	0.91	99.09	0	100
Chlorothiazide	74.34	25.66	69.3	30.7
Furosemide	55.46	44.54	1.36	98.64
Propranolol	0.29	99.71	2.50	97.50
Diltiazem	0.10	99.90	1.65	98.34
Ephedrine	2.18	97.82	7.89	92.11
Cimetidine	14.79	85.21	30.7	69.3

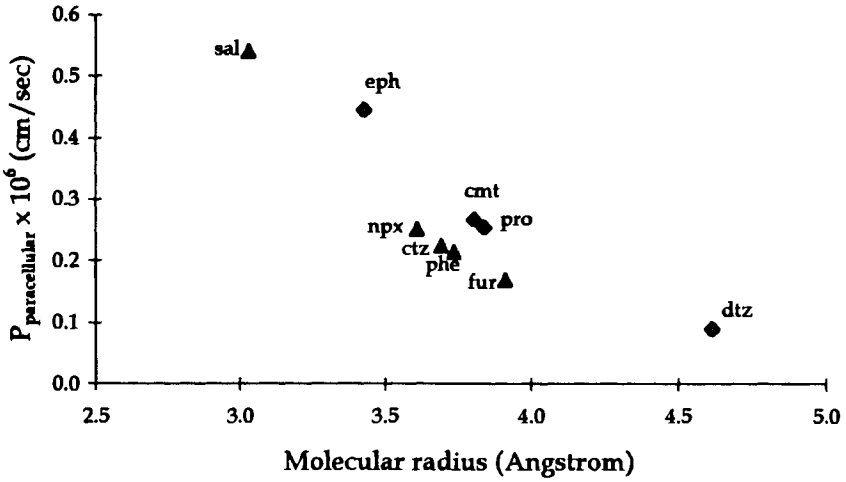

Fig. 3. Plot of paracellular permeability of the model drugs versus their molecular radii, illustrating the influence of molecular size and charge on paracellular permeability. (♦) cations, (▲) anions.

in the ionized form at pH 7.2, and cimetidine exists mostly in the ionized form at pH 5.4. Consequently, these drugs are absorbed to a greater extent by the paracellular pathway at these pHs. Chlorothiazide, however, is absorbed mainly by the paracellular pathway at both pHs, probably because of its extremely poor lipophilicity and solubility. Thus, the extent to which the various routes are utilized is influenced by the fraction of species, which in turn is determined by the pKa of the drug and the pH of the solution.

In summary, the extent to which the paracellular and transcellular routes are utilized is influenced by the fraction of ionized and unionized species (which in turn depends upon the pKa of the drug and the pH of the solution), the intrinsic partition coefficient of the drug, the size of the molecule and its charge. This research complements the work of Adson *et al.* (1,2), in attempting to understand the various factors controlling the absorption of passively transported drugs.

ACKNOWLEDGMENTS

The authors would like to acknowledge the help, and support extended by Dr. Philip S. Burton, Mr. A. Hilgers, Mr. R. Conradi, Dr. S. Valvani of The Upjohn Company, Kalamazoo, MI. In addition, we are grateful to Dr. Burton for his comments and discussion of this manuscript, and to Dr. R. S. Pearlman for the use of SAVOL.

REFERENCES

1. A. Adson, T. J. Raub, P. S. Burton, C. L. Barsuhn, A. R. Hilgers, K. L. Audus, and N. F. H. Ho. *J. Pharm. Sci.* **83**:1529–1536 (1994).
2. A. Adson, P. S. Burton, T. J. Raub, C. L. Barsuhn, K. L. Audus, and N. F. H. Ho. *J. Pharm. Sci.* **84**:1197–1204 (1995).
3. A. R. Hilgers, R. A. Conradi, and P. S. Burton. *Pharm. Res.* 7:902–910 (1990).
4. G. Wilson, I. F. Hassan, C. J. Dix, I. Williamson, R. Shah, and M. Mackay. *J. Controlled Release* **11**:25–40 (1990).
5. P. Artursson. *J. Pharm. Sci.* **79**:476–482 (1990).
6. J. N. Cogburn, M. Donovan, and C. Schasteen. *Pharm. Res.* **8**:210–216 (1991).
7. I. J. Hidalgo, T. J. Raub, and R. T. Borchardt. *Gastroenterology* **96**:736–749 (1989).
8. M. Rousset. *Biochimie* **68**:1035–1040 (1986).
9. M. Pinto, S. Leon-Robine, M. D. Appay, M. Kedinger, N. Triadou, E. Dussaulx, B. Lacroix, P. Assmann-Simon, K. Haffen, J. Fogh, and A. Zweibaum. *Biol. Cell.* **47**:323–330 (1983).
10. T. Teorell. *Prog. Biophys. Mol. Biol.* **3**:305–369 (1953).
11. R. S. Pearlman. Molecular Surface Area and Volume. In: W. J. Dunn, J. H. Block, R. S. Pearlman, editors. Partition Coefficient. Determination and Estimation. New York: Pergamon Press, 1986:3–20.
12. E. L. Cussler. Diffusion: Mass Transfer in Fluid Systems, Cambridge University Press, NY (1986).
13. E. M. Renkin. *J. Gen. Physiol.* **38**:225–243 (1955).
14. F. E. Curry. Mechanisms and thermodynamics of transcapillary exchange. In: E. M. Renkin, C. C. Michel, editors. The Cardiovascular System. Vol. IV. Microcirculation. Part 1. Bethesda, MD: *Am. Physiol. Society*, 1977:309–374.
15. P. A. Shore, B. B. Brodie, and C. A. M. Hogben. *J. Pharmacol. Exp. Ther.* **119**:361–369 (1957).
16. J. L. Madara and K. Dharmsathaphorn. *J. Cell Biol.* **101**:2124–2133 (1985).
17. K. H. Soergel *Gastroenterology* **105**:1247–1250 (1993).
18. N. F. H. Ho, D. P. Thompson, T. G. Geary, T. J. Raub, and C. L. Barsuhn. *Molec. Biochem. Parasitol.* **41**:153–166 (1990).
19. S. T. Ballard, J. H. Hunter, and A. E. Taylor. *Annu. Rev. Nutr.* **15**:35–55 (1995).