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Research Article

Characterization of the Oral Absorption of β-Lactam Antibiotics. I. Cephalosporins: Determination of Intrinsic Membrane Absorption Parameters in the Rat Intestine *In Situ*

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The oral absorption of five cephalosporin antibiotics, cefaclor, cefadroxil, cefatrizine, cephalexin, and cephradine, has been studied using a single-pass intestinal perfusion technique in rats. Intrinsic membrane absorption parameters, "unbiased" by the presence of an aqueous permeability (diffusion or stagnant layer), have been calculated utilizing a boundary layer mathematical model. The resultant intrinsic membrane absorption parameters are consistent with a significant carrier-mediated, Michaelis–Menten-type kinetic mechanism and a small passive component in the jejunum. Cefaclor colon permeability is low and does not exhibit concentration dependent behavior. The measured carrier parameters (±SD) for the jejunal perfusions are as follows: cefaclor, J^*_{max} = 21.3 (±4.0), K_m = 16.1 (±3.6), P^*_m = 0, and P^*_c = 1.32 (±0.07); cefadroxil, J^*_{max} = 8.4 (±0.8), K_m = 5.9 (±0.8), P^*_m = 0, and P^*_c = 1.43 (±0.10); cephalexin, J^*_{max} = 9.1 (±1.2), K_m = 7.2 (±1.2), P^*_m = 0, and P^*_c = 1.30 (±0.10); cefatrizine, J^*_{max} = 0.73 (±0.19), K_m = 0.58 (±0.17), P^*_m = 0.17 (±0.03), and P^*_c = 1.25 (±0.10); and cephradine, J^*_{max} = 1.57 (±0.84), K_m = 1.48 (±0.75), P^*_m = 0.25 (±0.07), and P^*_c = 1.06 (±0.08). The colon absorption parameter for cefaclor is P^*_m = 0.36 (±0.06, where J^*_{max} (mM) is the maximal flux, K_m (mM) is the Michaelis constant, P^*_m is the passive membrane permeability, and P^*_c is the carrier permeability. Aminocephalosporin perfusion results indicate that jejunal absorption in the rat occurs by a nonpassive process, with some of the compounds possessing a small but statistically significant passive component, while the colon permeability is low and follows a simple passive absorption mechanism.

KEY WORDS: colon; concentration dependent absorption; intrinsic permeability; jejunum; nonpassive absorption; unstirred layer.

INTRODUCTION

Characterizing the oral absorption mechanism of the β-lactam antibiotics has been of considerable interest in recent years (1–11). Cephalosporin perfusion studies in the rat small intestine, using various *in vitro* and *in situ* techniques, have sometimes yielded conflicting results. For example, cephalexin has been reported to be transported by both passive (7,8) and nonpassive processes (11). Disparate cephalexin absorption data may have been the result of an experimentally introduced aqueous "bias," a consequence of a diffusion boundary layer in the intestine. Unstirred layers have been long recognized as barriers to diffusion (12). Both the passive permeability and the nonpassive membrane transport parameters are dependent on the flow rate and length perfused (13–18), therefore they can be "biased" by experimental conditions. For nonpassively absorbed drugs the maximal rate of transport, J_{max}, remains unaffected by aqueous bias; however, an effective unstirred layer results in the determination of a higher apparent Michaelis constant, K_m^{app} (17). In this situation nonpassive absorption may appear as passive if K_m^{app} shifts to a value that exceeds the drug perfusate concentration. A modified boundary layer method for analysis of the results of an intestinal perfusion experiment was recently developed (13) to calculate the intrinsic, steady-state absorption parameters for the case of nonpassive absorption following Michaelis–Menten plus passive kinetics. Since the extent of aqueous bias on P_{eff} is dependent on perfusion hydrodynamics and it is not always possible to maintain constant hydrodynamic conditions from experiment to experiment, it is preferable to use intrinsic absorption parameters, especially if the permeability results are to be compared.

A potential problem with non-steady-state perfusion methods is the effect of the binding of drug to intestinal mucin, which may result in artificially low outlet concentrations and higher permeabilities. Recently, these effects have been studied (20,21) in the rat with differing results. Niibuchi *et al.* (20) demonstrated that two well-absorbed β-lactams, cephradine and cephalexin, had a low affinity for both the soluble (S) and the insoluble (I) mucin fractions, whereas Miyazaki *et al.* (21) reported that these compounds were strongly bound to both fractions. However, β-lactam flux using steady-state perfusion methods is not affected by

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mucin binding (22) since the glycoprotein binding sites of mucin are either saturated (irreversible binding) or at local equilibrium with free drug (reversible binding). Non-steady-state methods, notably initial rate and closed-loop methods (23), are expected to be affected since the non-steady-state diffusion coefficient is strongly influenced by the concentration dependence of mucin binding (24). In this report, the modified boundary layer method is applied to the steady-state uptake of five cephalosporin antibiotics.

EXPERIMENTAL

Materials

Cefadroxil, cephalexin (987 mg/g), cephradine (910 mg/g), citric acid, dibasic sodium phosphate, monobasic potassium phosphate, and polyethylene glycol 4000 were from Sigma Chemical Co., St. Louis, Mo.); cefaclor (956 mg/g, Lot No. 206BV6) was from Eli Lilly and Company, Indianapolis, Ind.; cefatrizine-1,3-propylene glycolate (845 mg/g) was from SmithKline Beckman Corp., Philadelphia, Pa., and Cebris, Sermoneta, Italy; and ^{14}C-polyethylene glycol was from duPont–New England Nuclear, Boston, Mass. All buffer and mobile-phase components were analytical [or high-performance liquid chromatography (HPLC)] grade and used as received.

Perfusion Experiments

Perfusion procedures have been detailed in an earlier report (25). The perfusion solution consisted of a citric acid–dibasic sodium phosphate buffer (pH 6.5), 0.01% (w/v), PEG 4000 with a tracer amount of its ^{14}C isotope, and a β-lactam antibiotic. The perfusate (pH 6.5, 300 ± 5 mmol/kg water) was maintained at 37°C by a water bath (Tek-Pro, American Dade, Miami, Fla.). The osmolality was measured using either a Model 5002 automatic osmometer (Precision Systems, Inc., Sudsbury, Mass.) or a Model 5500 vapor pressure osmometer (Wescor Inc., Logan, Utah).

All experiments were performed on male Charles River rats, 250–400 g, age 60 to 80 days. The rats were fasted overnight, 12 to 18 hr before each experiment. Water was given ad libitum. Anesthesia was induced by a 50% (w/v) intramuscular injection of urethane (1.5 g/kg). The colon or jejunum was located and a 10- to 15-cm segment was perfused. The jejunum was cannulated at 2 to 4 cm below the ligament of Treitz and 6 to 15 cm distal to the first incision. The intestinal segment was perfused using a constant-infusion pump (Harvard Apparatus, Model 931, South Natick, Mass.) for 90 to 105 min. The perfusion flow rate was approximately 0.25 ml/min. Steady state was achieved in approximately 30 min, after which four to six samples were taken at 10- to 15-min intervals. All samples were simultaneously quenched at the experiment's completion with 6% (w/v) citric acid solution. After the last sample was taken, the length was measured by placing a piece of string along the intestine and measuring the string with a ruler.

Analytical Methods

The samples were analyzed by liquid scintillation counting and by high-performance liquid chromatography (HPLC). Samples were counted using a Beckman LS-9000 counter (Beckman Instruments, Inc., Fullerton, Calif.) with automatic quench correction. A 0.5-ml sample was mixed with 15 ml of scintillation fluid (Ready-Solv, Hp/b, Beckman Instruments, Inc.) before counting. Samples were counted using a single-channel technique. Background radioactivity was corrected for using a blank. The HPLC instrumentation consisted of a pump (Beckman 112 solvent delivery module, Beckman Instruments, Inc., Berkeley, Calif.), a Waters Intelligent Sample Processor (WISP, Model 710B, Waters Associates, Milford, Mass.), an ultraviolet detector (Kratos Spectroflow 783 absorbance detector, Kratos Analytical Instruments, Ramsey, N.J.), and a reverse-phase column (μ-Bondapak C_{18}, Waters Associates, Milford, Mass.). The mobile phase consisted of methanol and pH 5 citric acid–dibasic sodium phosphate buffer (22:78). Samples were analyzed using ultraviolet detection (λ = 254 nm) and eluted at a flow rate of 1.5 ml/min.

Stability

The stability of the cephalosporins (1 m*M*) was tested in perfusate with an apparent first-order half-life (pH 6.5, 37°C) of 2.6, 1.6, 2.2, and 1.6 hr for cefatrizine, cefadroxil, cephalexin, and cephradine, respectively. Additionally, the stability of cefatrizine was studied in perfused and unperfused buffer (pH 6.5, 37°C; Fig. 1), with no significant difference between the two. Given the usual residence time of approximately 1.5 min[3] in the perfused intestinal segment, less than 0.02% degradation is expected for the least stable cephalosporin.

DATA ANALYSIS

Water Transport

Intestinal water transport was monitored by comparing the total radioactivity of the inlet and outlet samples. ^{14}C-

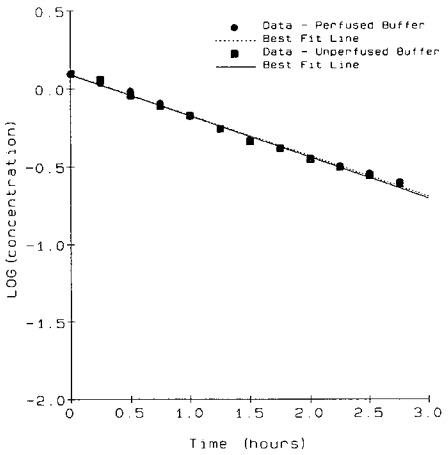

Fig. 1. Plot of the stability of cefatrizine (1 m*M*) in perfused and unperfused buffer (pH 6.5, 37°C).

[3] Mean residence time = V_i/Q, where V_i is the volume of the intestine and Q is the volumetric flow rate. For this calculation V_i = 0.3 ml and Q = 0.2 ml/min.

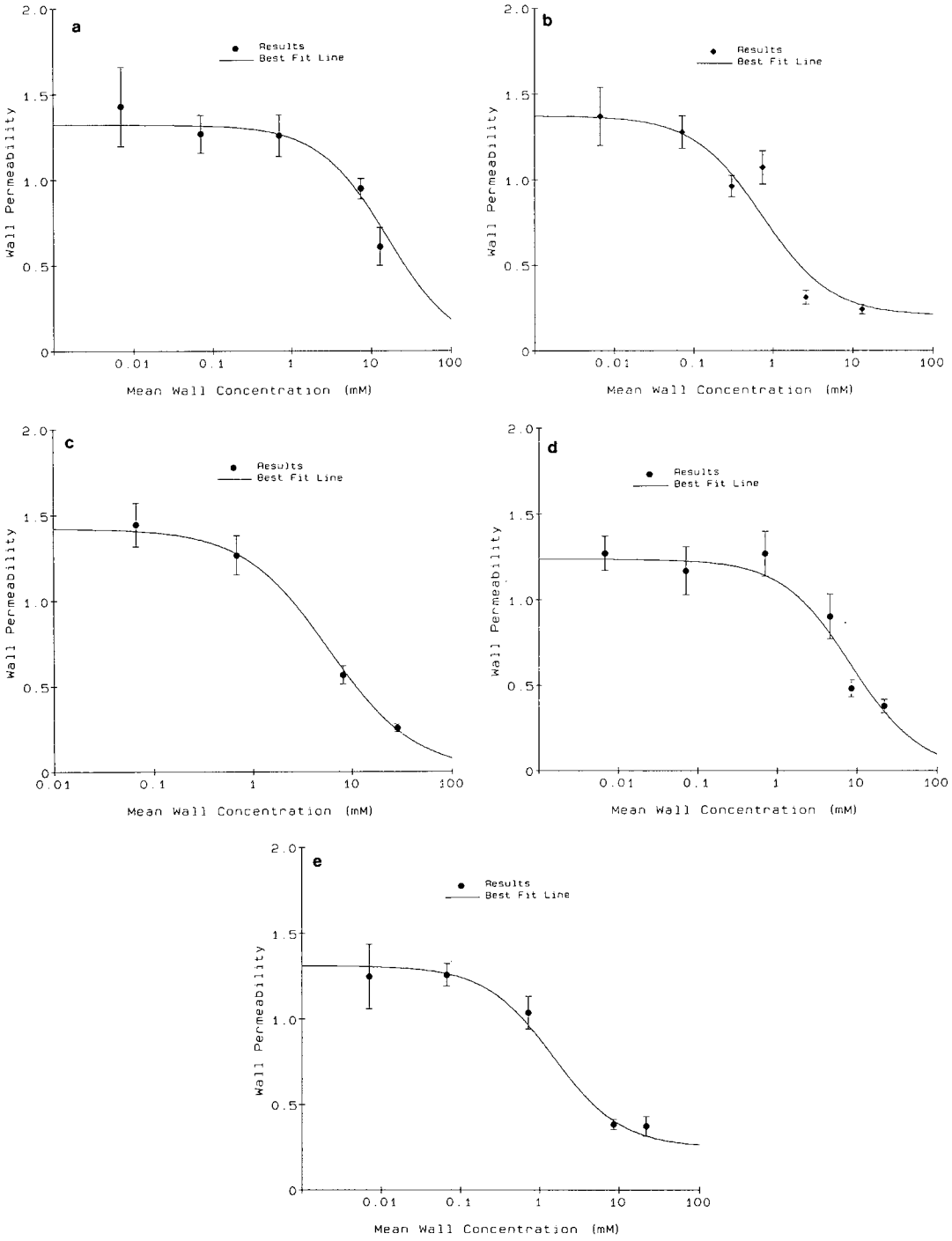

Fig. 2. Plots of the intrinsic wall permeability versus the wall concentration of (a) cefaclor, (b) cefatrizine, (c) cefadroxil, (d) cephalexin, and (e) cephradine, respectively. Permeabilities are reported as the mean ± SE.

Polyethylene glycol 4000 was used as the nonabsorbable marker. The percentage of water transport per centimeter length perfused for each sample was calculated from

$$\% \text{ water transport/cm} = \frac{100}{L}\frac{A_i - A_f}{A_f} \quad (1)$$

where A_i and A_f are the initial and final counts per minute of ^{14}C-polyethylene glycol 4000. Water transport below 0.5%/cm of intestinal length is considered normal and experiments with water transport >0.5%/cm were not used in the determination of the kinetic parameters. The outlet concentrations were corrected for this volume change using Eq. (1).

Estimation of Unbiased Membrane Parameters

"Unbiased" membrane parameters are estimated using a modified boundary layer model recently developed by Johnson and Amidon (13). The dimensionless effective wall permeability, P^*_{eff}, is calculated from steady-state perfusion results:

$$P^*_{eff} = \frac{1 - (C_m/C_0)}{4\,Gz} \quad (2)$$

where C_0 and C_m are the inlet and outlet perfusate concentrations, respectively, and the Graetz number, Gz, is

$$Gz = \frac{\pi DL}{2Q} \quad (3)$$

where D is the diffusion coefficient, L is the length of the intestine, and Q is the fluid flow rate. The aqueous permeability, P^*_{aq}, can be estimated from

$$P^*_{aq} = (A\,Gz^{1/3})^{-1} \quad (4)$$

where A is a constant and is calculated from a film model approximation to the boundary layer result:

$$A = 10.00\,Gz + 1.01, \quad 0.004 \leq Gz < 0.01$$
$$A = 4.50\,Gz + 1.07, \quad 0.01 \leq Gz < 0.03$$
$$A = 2.5\,Gz + 1.13, \quad 0.03 \leq Gz$$

The concentration at the intestinal wall, C_w, and the intrinsic wall permeability, P^*_w, are then calculated (13) as follows:

$$C_w = C_0\left(1 - \frac{P^*_{eff}}{P^*_{aq}}\right) \quad (5)$$

and

$$P^*_w = \frac{P^*_{eff}}{[1 - (P^*_{eff}/P^*_{aq})]} \quad (6)$$

P^*, a dimensionless permeability, is defined as $P^* = PR/D$, where R is the radius of the intestine and D is the estimated aqueous diffusion coefficient.

For a combination of carrier and passive uptake mechanisms the wall permeability is assumed to be of the form

$$P^*_w = \frac{J^*_{max}}{K_m + C_w} + P^*_m \quad (7)$$

where J^*_{max} is the maximal flux, K_m is the intrinsic Michaelis constant, and P^*_m is the intrinsic passive membrane permeability.

The carrier permeability, which is the first-order limiting carrier permeability, is defined as

$$P^*_c = \frac{J^*_{max}}{K_m} \quad (8)$$

Rewriting Eq. (7) in terms of the carrier permeability results in

$$P^*_w = \frac{P^*_c}{1 + (C_w/K_m)} + P^*_m \quad (9)$$

RESULTS AND DISCUSSION

The experimental data was fit to Eq. (7) or (9) using weighted nonlinear regression. The variance of P^*_w was not homogeneous over the concentration range studied; therefore, the data were weighted by the reciprocal variance of P^*_w. All data were initially fit to the three-parameter model (P^*_c or J^*_{max}, K_m, and P^*_m); however, when the value of P^*_m was not significantly different from zero, the model was reduced accordingly. The jejunal perfusion results and fitted curves are shown in Figs. 2a–e for cefaclor, cefatrizine, cefadroxil, cephalexin, and cephradine, respectively. A summary of the intrinsic jejunal and colon membrane parameters is listed in Table I. For all the compounds listed in Table I it is evident that for any particular aminocephalosporin the variation in P^*_c is considerably less than that for the corresponding J^*_{max} or K_m. As seen in Fig. 3, there was no significant difference ($p = 0.23$) in the wall permeability of cefaclor in the rat colon at 1 and 10 mM, suggesting a passive absorption mechanism in the colon. Although cefaclor's solubility in perfusate ($\simeq$10 mM) limited the range of concentrations that were studied, a significant difference in P^*_w at 1 and 10 mM is expected based on the K_m of cefaclor (Table I) and Fig. 2a. It is clearly demonstrated that all of the cephalosporins studied have a significant saturable absorption component in the jejunum, while absorption from the colon occurs by a passive process.

Table I. Summary of the Intrinsic Membrane Absorption Parameters Derived from the Nonlinear Regression Analysis Using the Models of Wall Permeability in Eq. (7) or (9)[a]

Compound	J^*_{max} (mM)	K_m (mM)	P^*_c	P^*_m
		Jejunum		
Cefaclor	21.3 (4.0)	16.1 (3.6)	1.3 (0.1)	0
Cefadroxil	8.4 (0.8)	5.9 (0.8)	1.4 (0.1)	0
Cephalexin	9.1 (1.2)	7.2 (1.1)	1.3 (0.1)	0
Cefatrizine	0.7 (0.2)	0.6 (0.2)	1.3 (0.1)	0.2 (0.03)
Cephradine	1.6 (0.8)	1.5 (0.8)	1.1 (0.1)	0.3 (0.1)
		Colon		
Cefaclor	—	—	—	0.36 (0.06)

[a] Reported values are fitted values (SD).

The effect of using P^*_{eff} rather than P^*_w is shown in Fig. 5 and Table II. The experimental design for these studies was such that the aqueous resistance was always 10 to 25% of

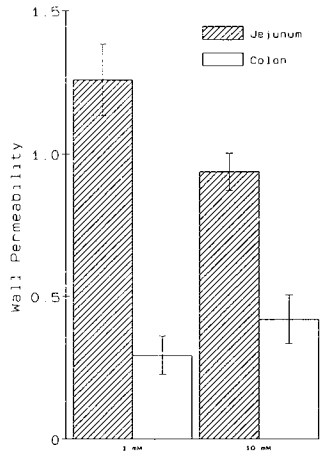

Fig. 3. Comparison of the permeability of cefaclor in the jejunum and colon at 1 and 10 mM. There is no significant difference between the colon permeabilities, whereas the difference in the jejunal data is significant ($P < 0.02$). Permeabilities are reported as the mean ± SE.

Table II. Comparison of the Effective Versus Intrinsic Membrane Absorption Parameters for Cephalexin[a]

	Effective	Intrinsic
J^*_{max} (mM)	10.8 (1.3)	9.1 (1.2)
K_m (mM)	13.0 (1.9)	7.2 (1.4)
P^*_c	0.8 (0.04)	1.3 (0.1)

[a] Reported values are fitted values (SD).

the membrane resistance (13), increasing the sensitivity of the experiment to the nonlinear membrane resistance. In other words, in a typical cephalosporin perfusion experiment the membrane resistance rather than the aqueous resistance limits absorption. Our experiments confirm the findings of Winne (17,18) and Dietschy (28) that lower permeabilities and a larger apparent Michaelis constant result from experimentally introduced aqueous "bias." As seen in Table II, even when absorption is membrane controlled, aqueous bias significantly affects the absorption parameters. This results in an underestimation of the wall permeability, especially at lower concentrations (near the first-order limit of permeability or the carrier permeability; Fig. 4), a rightward shift of the Michaelis constant result (Fig. 5), and a flattening of the effective permeability–concentration curve (Fig. 5), as compared to the intrinsic permeability–concentration curve making it more difficult to discern nonpassive absorption behavior. Of course, the magnitude of this effect is dependent on hydrodynamic conditions. Inconsistencies among the early β-lactam studies that used the non-steady-state closed-loop method (23) can be explained by the considerable effect the diffusion boundary layer has on the permeability coefficients and the Michaelis constant (17). With the closed-loop method the aqueous resistance limits absorption due to stagnant flow conditions, whereas in the steady-state single-pass perfusion technique the membrane represents the controlling resistance. In the case of Kimura *et al.* (9), where effective parameters were used and the highest concentration of cephalexin studied was 10 mM, it can be seen why nonpassive transport of cephalexin was not observed.

The jejunal passive permeability of cefaclor was predicted to be zero from nonlinear regression analysis. It should be noted, however, that the reliability of the P^*_m estimate is questionable since the apparent solubility in the perfusing solution was approximately equal to K_m^{app}. As a result, the zero-order region of the permeability–concentration curve could not be well characterized. Since the results of the colon perfusions suggest a passive absorption mechanism, it is not unrealistic to expect a slight passive component in the jejunum.

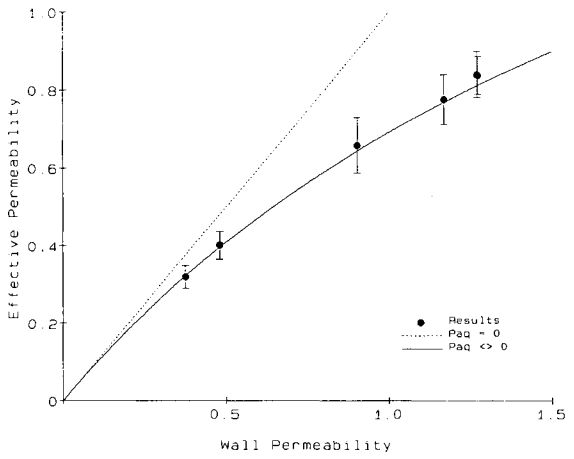

Fig. 4. Plot of the effective versus wall permeability of cephalexin. Permeabilities are reported as the mean ± SE.

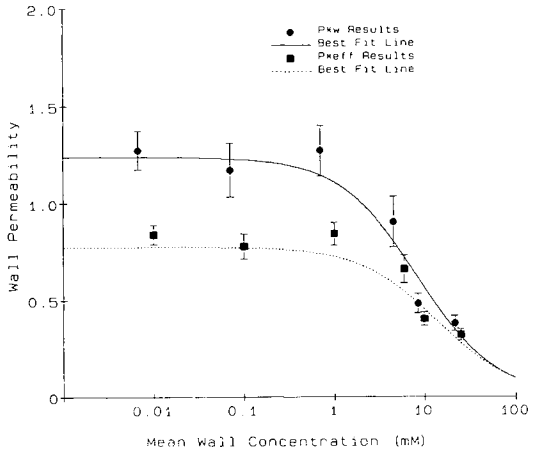

Fig. 5. Plot of the effective and wall permeability versus the effective or intrinsic cephalexin concentration. Permeabilities are reported as the mean ± SE.

In summary, a modified boundary layer model of the intestinal perfusion experiment has been used to determine the intrinsic membrane absorption parameters, P^*_{max}, K_m, P^*_c, and P^*_m of various cephalosporin antibiotics in the colon and jejunum of rats. Using intrinsic rather than effective absorption parameters is particularly important when studying a compound where the absorption mechanism is not strictly passive. Applying the modified boundary layer analysis to the data revealed that the primary mechanism of aminocephalosporin absorption in the jejunum is a nonpassive (carrier-mediated) process. On the other hand, the colon permeability of cefaclor was found to be low and independent of concentration. Intrinsic membrane absorption parameters are used to characterize the β-lactam absorption process in a hydrodynamically "unbiased," macroscopic sense. It should be noted that the resultant Michaelis constant, although hydrodynamically unbiased, represents a complex binding coefficient that may or may not be heterogeneous. These studies compliment the recent mechanistic work by Okano *et al.* (29) using membrane vesicles. The vesicle studies focus on a single step in the absorption process. On the other hand, the perfusion results provide parameters that pertain to the entire absorption process and consequently are the parameters to be used for estimating oral absorption in humans (30).

The consequence of a dose-dependent cephalosporin absorption mechanism on plasma level–time curves in the clinical setting may be significant. Doses that exceed the K_m of the antibiotic will be more slowly absorbed, resulting in the possibility that unabsorbed drug will pass into the colon, decreasing the fraction absorbed and causing gastrointestinal side effects. The magnitude of these effects is depen-

dent on several factors including the ratio of the dose to the K_m, gastric emptying, small intestinal transit time, and stability (pH). These factors and their effect on the fraction dose absorbed are considered in the following paper (30).

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