netics of the proposed carrier-mediated transport of amphoteric β-lactam antibiotics will be presented in subsequent papers.

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Intestinal Absorption Mechanism of Amphoteric β-Lactam Antibiotics II: Michaelis–Menten Kinetics of Cyclacillin Absorption and Its Pharmacokinetic Analysis in Rats

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Abstract □ The absorption of cyclacillin at pH 7.0 by the rat small intestine was investigated using *in situ* perfusion. At the lowest dose of 95 μg/ml, the antibiotic disappearance was rapid and followed first-order kinetics, with the disappearance being 85% at 100 min. At the intermediate concentrations of 770 and 1200 μg/ml, the disappearance after 100 min was 69 and 54%, respectively, and semilogarithmic plots clearly showed convex curvatures. At the highest concentration of 30 mg/ml, cyclacillin disappeared slowly from the perfusate, in an apparent first-order fashion. The disappearence was 26% after 100 min of perfusion and was similar in extent at 5.2 mg/ml. This concentration–time profile was satisfactorily fitted to the simultaneous Michaelis–Menten and first-order kinetic equations. The area under the blood concentration *versus* time curve (*AUC*) after a single intraduodenal dose of cyclacillin was almost consistent with the *AUC* after the equivalent intravenous dose (10 mg/kg). Additional evidence from a pharmacokinetic analysis of steady-state blood concentrations after constant infusion of cyclacillin through the portal vein and the small intestinal lumen indicated that cyclacillin absorption by the rat intestinal tissue at relatively low concentrations (<1 mg/ml) followed solely Michaelis–Menten kinetics. Cyclacillin may be transported by certain types of carrier-mediated mechanisms.

Keyphrases □ Cyclacillin—intestinal absorption kinetics *in situ*, blood levels in rats after intravenous, intraportal, and intraduodenal administration □ Absorption kinetics—rat intestinal loops, blood cyclacillin levels after intravenous, intraportal, and intraduodenal administration □ Kinetics, absorption—blood cyclacillin levels after intravenous, intraportal, and intraduodenal administration, *in situ* rat intestinal loops □ Antibiotics, amino-β-lactam—cyclacillin, *in situ* intestinal absorption kinetics, blood levels after intravenous, intraportal, and intraduodenal administration to rats □ Pharmacokinetics—cyclacillin, intestinal absorption *in situ*, blood levels after intravenous, intraportal, and intraduodenal administration to rats

Previous *in situ* absorption studies (1–4) utilizing rat intestinal loops showed that the percentage disappearance of amino-β-lactam antibiotics, such as amoxicillin, cyclacillin, cephalexin, cephradine, and cefadroxil, was extremely large at a low dose but was markedly reduced at a high dose. Accumulated results from the past 10 years

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(1–13) suggest that the GI absorption of amino-β-lactam antibiotics does not obey the so-called lipid theory but that certain carrier-mediated mechanisms underlie their absorption.

Among these classes of β-lactam antibiotics, cyclacillin revealed a remarkable dose-dependent disappearance from the rat gut lumen (2, 4). Recently, the presence of one or more carrier systems was proposed for the active transport of cyclacillin in the rat intestinal mucosa (8, 10). However, when various cyclacillin doses were administered orally to mice (14), rats (15), dogs (16), and humans (17), the peak blood levels were almost parallel to the dose. The question now arises as to whether the dose-dependent disappearance from the intestinal lumen solution is due to absorption into the bloodstream, to gut wall accumulation, or to both absorption and degradation in the gut lumen and at the mucosal surface.

The present study on cyclacillin absorption by the rat intestine was undertaken to elucidate: (*a*) the kinetics of disappearance from the recirculating intestinal perfusion solution, (*b*) the ratio of the area under the blood concentration–time curve (*AUC*) after the intraduodenal administration to that after an intravenous dose, and (*c*) the relationship between steady-state blood levels after constant infusion into the portal vein and those after single intestinal perfusion of cyclacillin at various doses.

EXPERIMENTAL

Materials—Cyclacillin anhydrate (983 μg/mg) was used as received[1]. All other reagents and solvents were the best grade available.

Animals—Male albino Wistar rats weighing 220 ± 4 g and 214 ± 20 g were used in the measurement of *AUC* and the other experiments, respectively. They were fasted for 20 hr prior to the experiment, but water was given freely. The rats were anesthetized with urethan (1.5 g/kg ip) ~1 hr prior to surgery.

***In Situ* Recirculating Perfusion Method**—The procedure employed was essentially the same as that described previously (2). The bile duct was ligated in all recirculating perfusion experiments. The intestine was cannulated with a glass cannula at the pylorus and jejunum ends for ~30 cm and prewashed with 100 ml of pH 7.0 isotonic phosphate buffer. After washing with 20 ml of the pH 7.0 isotonic buffer containing cyclacillin, 6 ml of this antibiotic solution was recirculated continuously to produce a final volume of ~9 ml. The pH of the lumen solution was maintained at pH 7.0 by means of a pH-stat[2]. Samples withdrawn periodically from the perfusate were filtered to remove solid materials, diluted if necessary, and analyzed as described under *Analysis*. The total sampling volume was within 6% of the perfusion solution.

Determination of *AUC* after Intravenous or Intraduodenal Administration—A 30-cm intestinal loop was prepared by ligation between the pylorus and jejunum. Cyclacillin solution was prepared with physiological saline, and 10-mg/kg doses were given intravenously through the femoral vein (0.5 ml) or intraduodenally (1 ml). Aliquots (0.2 ml) of blood samples taken periodically from the jugular vein were assayed by the microbiological method after being hemolyzed with an equivalent volume of distilled water. The total weight of sampled blood was within 0.7% of the rat body weight.

Tissue Accumulation after Intraduodenal Administration—After completion of 1- or 5-hr experiments for *AUC* determination, the intestinal area of the 30-cm intestinal loop was isolated, tearing off the mesentery, and the serosal surface then was blotted with filter paper. The intestine was cut into small slices and homogenized in a polytef homogenizer with saline to give 10% (w/v, wet weight) homogenate. An aliquot of the homogenates then was analyzed.

Constant Intraportal Infusion—To obtain a steady-state blood level after constant infusion into the portal vein, antibiotic solutions of 5, 10, 15, and 20 mg/ml prepared with saline were infused at 0.003 ml/min into rats whose bile duct had been cannulated with a polyethylene tube to exclude bile. Aliquots (0.2 ml) of blood samples withdrawn periodically during 7-hr experiments were analyzed after being hemolyzed.

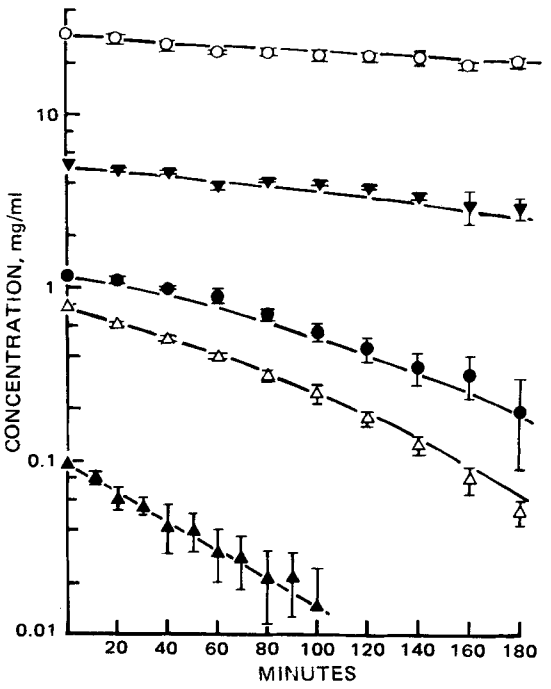


Figure 1—*Time courses for cyclacillin disappearance from isotonic phosphate buffer perfused through the rat small intestine as a function of dose. The points represent the mean concentration, and the vertical bars represent the standard deviations from three or six experiments. The curves are model-predicted concentrations based on nonlinear least-squares fitting of data to Eq. 1. Key: ▲, 95 μg/ml; △, 770 μg/ml; ●, 1200 μg/ml; ▼, 5200 μg/ml; and ○, 30 mg/ml.*

Appearance in Blood after *In Situ* Single Intestinal Perfusion—The rats were treated similarly as in the *in situ* recirculating perfusion experiments, but the bile duct was cannulated with a polyethylene tube to exclude bile. Cyclacillin solutions (0.1, 0.2, 0.5, and 1.0 mg/ml, prepared with pH 7.4 isotonic phosphate buffer) were perfused from the pylorus end at a flow rate of 2 ml/min and collected at the jejunum end. Aliquots (0.2 ml) of blood samples taken periodically during 7-hr absorption experiments were analyzed after being hemolyzed. The total weight of sampled blood was within 1% of the rat body weight.

Analysis—The residual cyclacillin in the recirculating perfusion solution was determined by both high-performance liquid chromatographic (HPLC) and microbiological assays.

The liquid chromatograph[3] was equipped with a UV detector[4] set at 210 nm. A strong cation-exchange resin[5] packed into a 4.6 × 500-mm stainless steel column was used as the stationary phase. The mobile phase was aqueous 0.1 *M* KH_2PO_4 adjusted to pH 4.0–4.2 with sodium hydroxide solution. Samples were eluted at a flow rate of 2 ml/min. The recirculating solution (10–50 μl) was withdrawn at suitable time intervals, injected *via* a variable-loop injector on flow after appropriate dilution, if necessary, and filtrated with a 0.45-μm filter[6]. The peak heights were measured, and the concentrations were calculated from calibration curves obtained daily.

The recirculating intestinal perfusion solution, intestinal tissue homogenates, and hemolyzed blood samples were analyzed by the microbiological paper disk method employing *Sartina lutea*[7]. For the latter two samples, standards were established by using fresh pooled 10% gut homogenates and fresh pooled blood, both from control rats.

The accuracies of the two analytical methods were as described previously (4).

[1] Takeda Chemical Industries, Osaka, Japan.
[2] A pH-stat titrator assembly consisting of a TTT2 titrator and ABU12b autoburet, Radiometer, Denmark.
[3] Model FLC-A700, Japan Spectroscopic Co., Tokyo, Japan.
[4] Model UVIDEC-100, Japan Spectroscopic Co., Tokyo, Japan.
[5] Zipax SCX, DuPont Instruments, Wilmington, Del.
[6] Sartorius-Membranefilter GmbH, Göttingen, West Germany.
[7] IFO 12708, Institute for Fermentation, Osaka, Japan. The strain was derived from ATCC 9341.

Table I—Percentage of Residual Antibiotic in the Lumen Solution Perfused through the Rat Small Intestine[a] at Various Doses as a Function of Time

Minutes	Initial Concentration of Cyclacillin, μg/ml				
	95	770	1200	5200	30000
0	100.0	100.0	100.0	100.0	100.0
10	84.3 ± 7.7	—	—	—	—
20	64.0 ± 10.2	78.6 ± 1.4	92.4 ± 4.8	92.5 ± 1.5	93.2 ± 6.4
30	57.7 ± 7.5	—	—	—	—
40	44.4 ± 13.9	65.0 ± 2.9	82.2 ± 2.1	89.6 ± 2.4	85.8 ± 7.1
50	41.7 ± 10.4	—	—	—	—
60	31.2 ± 11.3	52.3 ± 3.9	74.7 ± 6.8	76.4 ± 6.3	78.6 ± 1.0
70	28.9 ± 10.0	—	—	—	—
80	22.0 ± 9.8	41.6 ± 4.7	58.0 ± 4.5	80.5 ± 1.3	78.3 ± 4.4
90	22.1 ± 8.8	—	—	—	—
100	15.2 ± 9.9	31.2 ± 3.7	45.8 ± 6.1	76.4 ± 2.0	73.9 ± 6.1
120	—	22.6 ± 2.1	37.0 ± 6.0	72.9 ± 1.8	72.9 ± 4.7
140	—	15.7 ± 1.9	28.6 ± 5.9	64.7 ± 3.1	71.2 ± 7.8
160	—	10.0 ± 1.8	25.3 ± 7.2	56.3 ± 12.2	63.4 ± 3.4
180	—	6.4 ± 1.0	15.8 ± 8.5	54.2 ± 7.4	65.4 ± 5.1

[a] $n = 3$ for all doses except the 95-μg/ml dose ($n = 6$).

RESULTS AND DISCUSSION

Concentration–Time Profile for *In Situ* Disappearance from Recirculating Perfusate—Semilogarithmic plots of the disappearance of cyclacillin from the rat small intestinal lumen at pH 7.0 are given in Fig. 1. The analytical results determined by both HPLC and biological assay for samples of initial concentrations of 95, 1200, and 5200 μg/ml were in good agreement. The percentages of residual antibiotic at various doses as a function of time are summarized in Table I.

At the lowest concentration (95 μg/ml), cyclacillin disappearance was rapid and followed apparent first-order kinetics, with the disappearance being 85% at 100 min. At the intermediate concentrations of 770 and 1200 μg/ml, the disappearance after 100 min was 69 and 54%, respectively, and the semilogarithmic plots clearly showed convex curvatures. At the highest concentration (30 mg/ml), cyclacillin disappeared slowly from the perfusate in an apparent first-order fashion. The disappearance was 26% after 100 min of perfusion and was similar in extent at 5200 μg/ml. This concentration–time profile, showing a dramatic decrease in the disappearance rate with an increase in the initial cyclacillin concentration, indicates that the disappearance rate of this antibiotic from the rat intestinal perfusate can be described in the mixed kinetic terms of Michaelis–Menten and first order:

$$\frac{dC}{dt} = -\frac{V_{max}C}{K_m + C} - (k_1 + k_2)C \qquad \text{(Eq. 1)}$$

where C is the cyclacillin concentration remaining in the perfusate at time t, V_{max} is the maximum rate of disappearance, K_m is the Michaelis–Menten constant, and k_1 and k_2 are the first-order rate constants of absorption and degradation, respectively, as defined previously (3).

Iterative nonlinear least-squares analysis of the data in Fig. 1 to be fitted to Eq. 1, using a NONLIN computer program (18), provided the following parameters[8,9]: V_{max} = 502 ± 31 μg/ml/hr or 1.47 ± 0.09 mM hr^{-1}, K_m = 420 ± 27 μg/ml or 1.23 ± 0.08 mM, and $k_1 + k_2$ = 0.111 ± 0.014 hr^{-1}. During the fitting procedure, the concentration was weighted by the reciprocal of its square.

There were negligible concentrations of the penicilloic acid of cyclacillin on HPLC during the absorption experiments, which was consistent with previous observations using the *in situ* intestinal loop method (2, 4). The *in vitro* first-order degradation rate constant of this antibiotic was determined as 0.014 hr^{-1} in the intestinal washing perfusate at 37°, indicating cumulative products of only 3% penicilloic acid from the initial dose after a 2-hr experiment. Subtraction of the first-order degradation rate constant (k_2) of 0.014 hr^{-1} from the best fitting first-order rate constant of 0.111 hr^{-1} yielded 0.097 hr^{-1} as the net first-order absorption rate constant (k_1). This value was very close to values of other amino-β-lactam antibiotics, amoxicillin (k_1 = 0.062 hr^{-1}) (2) and cephradine (k_1 = 0.082 hr^{-1}) (3), as well as to the average first-order absorption rate constant for monobasic penicillins (0.076 hr^{-1}) (19) determined in similar experiments.

The solid lines in Fig. 1 were generated using Eq. 1 with the NONLIN-fitted parameters, indicating that Eq. 1 gives an excellent description of the experimental data for the cyclacillin disappearance from the rat *in situ* intestinal perfusate.

Pharmacokinetic Evidence for Saturable Absorption by Blood Concentration Analysis—In the disappearance of cyclacillin from the intestinal perfusate by Michaelis–Menten kinetics, it is important to distinguish between net transport across the GI tract into the bloodstream and competitive enzymatic degradation in the lumen and/or at the mucosal surface.

The contribution of each kinetic term in Eq. 1 to the total disappearance was calculated as a function of constant antibiotic concentration. At concentrations of >5 mg/ml, the first-order kinetic term, $k_1 + k_2$, may play the major role in the disappearance; with those of <1.0 mg/ml, the total disappearance is expected to be virtually due to Michaelis–Menten kinetics.

AUC *after Intraduodenal Dose Compared with Intravenous Dose*—Figure 2 shows the mean blood concentration–time profiles of cyclacillin

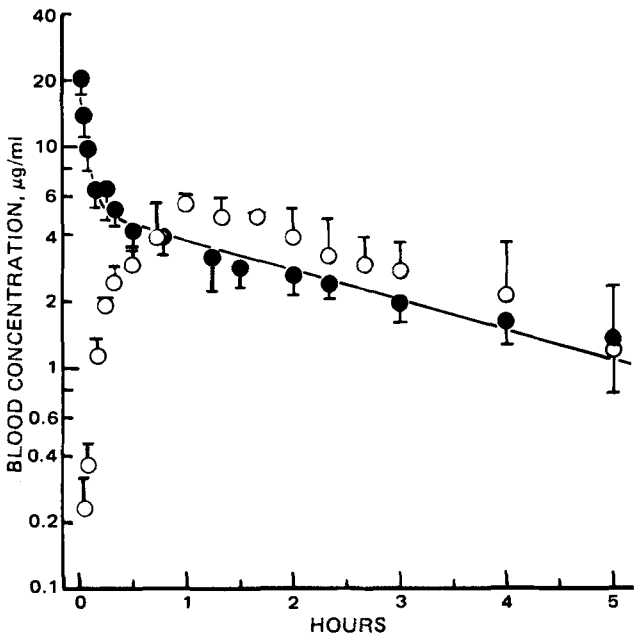
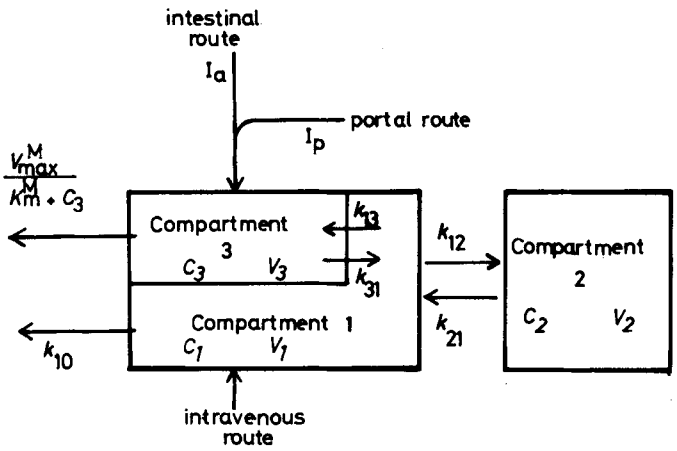


Figure 2—*Mean blood concentration of cyclacillin (10 mg/kg) following intravenous (●) or intraduodenal (○) administration in rats. The vertical bars represent the standard deviations from three experiments. The curve is the nonlinear least-squares line for the two-compartment open model.*

[8] Previously, we employed only the Michaelis–Menten kinetic term for the data fitting and reported V_{max} = 842 ± 28 μg/ml/hr or 2.47 ± 0.08 mM hr^{-1} and K_m = 654 ± 40 μg/ml or 1.91 ± 0.12 mM (2). Additional experiments were conducted at the highest concentration of 30 mg/ml. Incorporation of a first-order kinetic term gave the best fit to all data of both the previous (2) and present studies.

[9] The computer analysis was performed with a FACOM M-160 digital computer at the Data Processing Center, Kanazawa University.



Scheme I—Pharmacokinetic model for evaluation of the absorption rate of the drug.

(10 mg/kg) after intravenous and intraduodenal administrations in rats whose intestine had been ligated 30 cm from the pylorus to prevent possible loss of the antibiotic into the feces. The results are summarized in Table II.

The blood concentration (C_1) following intravenous bolus injection declined biexponentially in accordance with:

$$C_1 = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 2)}$$

The data were fitted to a two-compartment open model. The values of A, B, α, and β were estimated to be 23.3 ± 2.4 μg/ml, 5.29 ± 0.29 μg/ml, 18.7 ± 2.2 hr^{-1}, and 0.326 ± 0.031 hr^{-1}, respectively, using the NONLIN computer program (18), where the blood concentration was weighted as its reciprocal[9]. The solid line in Fig. 2 represents the best fit of the data.

Evaluation of the data from the intraduodenal dose of cyclacillin revealed that the terminal phase of the blood level–time profile represented a function of the β rate constant.

Both AUC values from the different administration routes were calculated according to the trapezoidal rule from time 0 to 5 hr, where pseudodistribution–equilibrium of cyclacillin might be attained. To each value was added the AUC value from 5 hr to time infinity, which was calculated using the terminal β rate constant. The total blood areas after intravenous and intraduodenal dosing were 18.48 ± 2.28 and 18.46 ± 2.83 μg hr/ml, respectively, and thus were the same within the limits of experimental error.

These results indicate that cyclacillin was absorbed completely by the ligated intestine following intraduodenal administration and that there was no appreciable first-pass metabolism and degradation of cyclacillin in the intestine during absorption of the 10-mg/kg dose. In an absorption experiment after 1 hr, the dose of cyclacillin remaining in the intestinal lumen was determined to be 6.5%, and the accumulation in the tissue was 8.0%; the total absorption was 85.5% after 1 hr. After 5 hr, the percentages of the residual cyclacillin in the intestinal lumen and the accumulation in the tissue were 0.7 and 2.3%, respectively, indicating almost complete cyclacillin absorption in 5 hr.

Absorption Kinetic Analysis of Steady-State Blood Concentration during Intraportal Vein Infusion and Single Intraduodenal Perfusion—Although no significant first-pass effect was observed for cyclacillin at an intraduodenal dose of 10 mg/kg, Kind *et al.* (20) reported that penicillins were subject to significant inactivation by the isolated rat liver. Excretion of penicilloic acid after oral administration of cyclacillin in humans was reported to be 15–19% for a 0.250–1-g dose (16). Therefore, the possible consequences of metabolism in the gut or liver must be considered since such processes sometimes are saturable.

With the pharmacokinetic treatment of Gibaldi and Feldman (21), a three-compartment open model may be adequate for considering the saturable first-pass effect after intraduodenal or intraportal administration (Scheme I). The present model consists of a central compartment being sampled (Compartment 1), a rapid equilibrium hepatoportal system (Compartment 3), which is included in the central compartment, and a slow equilibrium peripheral compartment (Compartment 2). The model is based on observations of apparent biexponential pharmacokinetics following an intravenous bolus dose as described earlier. Compartment 3 includes drug metabolism in the liver during the first passage of the drug into the circulation after hepatic route administration. In this model, C is the drug concentration in a given compartment and V is the com-

Table II—Mean Blood Concentrations (Micrograms per Milliliter) following Intraduodenal and Intravenous Administration of Cyclacillin (10 mg/kg) in Rats with a Ligated Intestine (n = 3–4)

Route	Minutes																	
	1.5	3	5	10	15	20	30	45	60	75	80	90	100	120	140	160	180	240	300
Intraduodenal	—	0.23 ± 0.09	0.36 ± 0.10	1.10 ± 0.25	1.88 ± 0.19	2.43 ± 0.42	2.94 ± 0.47	3.91 ± 1.66	5.60 ± 0.47	—	4.82 ± 1.03	—	4.79 ± 0.21	3.91 ± 1.31	3.19 ± 1.50	2.95 ± 0.95	2.70 ± 1.01	2.12 ± 1.51	1.17 ± 1.15
Intravenous	20.5 ± 3.4	13.8 ± 2.9	9.76 ± 2.1	6.35 ± 1.12	6.50 ± 1.95	5.22 ± 0.86	4.17 ± 0.72	3.84 ± 0.57	—	3.11 ± 0.95	—	2.85 ± 0.61	—	2.62 ± 0.52	2.39 ± 0.36	—	1.96 ± 0.38	1.60 ± 0.34	1.36 ± 0.59

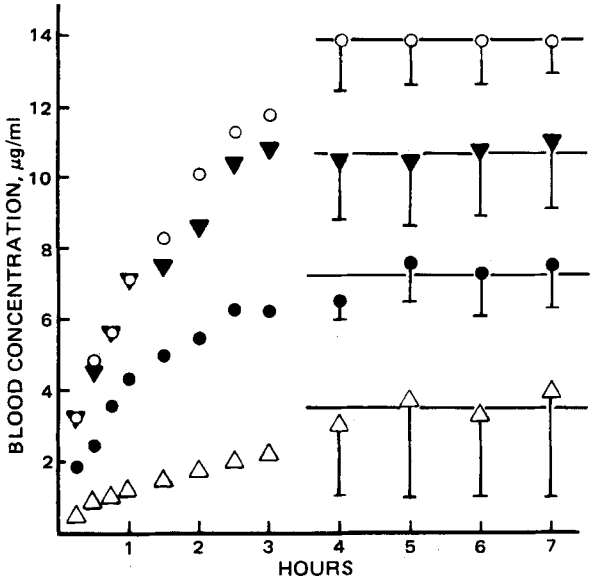


Figure 3—*Mean blood concentration of cyclacillin administered by constant portal vein infusion at a rate of 15.3 (△), 30.5 (●), 45.8 (▼), or 61.0 (○) μg/min. The vertical bars represent the standard deviations from three experiments.*

partment volume, k_{10} represents the first-order rate constant for renal elimination, and V^M_{max} and K^M_m represent the maximal rate and Michaelis constant for hepatic metabolism, respectively.

From this model, the rates of drug amount in each compartment are given by:

$$V_1\frac{dC_1}{dt} = -(k_{12}+k_{13}+k_{10})V_1C_1 + k_{21}V_2C_2 + k_{31}V_3C_3 \quad \text{(Eq. 3)}$$

$$V_2\frac{dC_2}{dt} = k_{12}V_1C_1 - k_{21}V_2C_2 \quad \text{(Eq. 4)}$$

$$V_3\frac{dC_3}{dt} = k_{13}V_1C_1 - k_{31}V_3C_3 - \frac{V^M_{max}C_3}{K^M_m + C_3} + \text{input} \quad \text{(Eq. 5)}$$

$$\text{input} = I_p \text{ or } I_a \quad \text{(Eq. 6)}$$

where I_a represents the absorption rate by the intestine and I_p is the infusion rate through the portal vein.

At the steady state of drug distribution and elimination during a constant intestinal perfusion or constant portal vein infusion, Eqs. 7–9 can be obtained by putting zero on the left side of Eqs. 3–6 and replacing C_i by C_i^{ss} (steady-state concentration) on the right sides of the equations:

$$-(k_{12}+k_{13}+k_{10})V_1C_1^{ss} + k_{21}V_2C_2^{ss} + k_{31}V_3C_3^{ss} = 0 \quad \text{(Eq. 7)}$$

$$k_{12}V_1C_1^{ss} - k_{21}V_2C_2^{ss} = 0 \quad \text{(Eq. 8)}$$

$$k_{13}V_1C_1^{ss} - k_{31}V_3C_3^{ss} - \frac{V^M_{max}C_3^{ss}}{K^M_m + C_3^{ss}} + \text{input} = 0 \quad \text{(Eq. 9)}$$

Rearrangement of Eqs. 7–9 gives:

$$\text{input} = k_{10}V_1C_1^{ss} + \frac{V^M_{max}C_3^{ss}}{K^M_m + C_3^{ss}} \quad \text{(Eq. 10)}$$

Combination of Eqs. 7 and 8 yields:

$$C_3^{ss} = \frac{(k_{13}+k_{10})V_1}{k_{31}V_3}C_1^{ss} \quad \text{(Eq. 11)}$$

where C_3^{ss} is proportional to C_1^{ss}. If the proportionality constant is K, then C_3^{ss} equals KC_1^{ss}.

When a steady-state blood concentration, $[C_1^{ss}]_{I_a}$, is achieved after constant intestinal perfusion of drug, the absorption rate, I_a, can be expressed as:

$$I_a = \left(k_{10}V_1 + \frac{V^M_{max}KV_3}{K^M_m + K[C_1^{ss}]_{I_a}}\right)[C_1^{ss}]_{I_a} \quad \text{(Eq. 12)}$$

Similarly, the portal vein infusion rate can be described as:

$$I_p = \left(k_{10}V_1 + \frac{V^M_{max}KV_3}{K^M_m + K[C_1^{ss}]_{I_p}}\right)[C_1^{ss}]_{I_p} \quad \text{(Eq. 13)}$$

From Eqs. 12 and 13, it can be seen that I_a should be equal to I_p when the steady-state levels of $[C_1^{ss}]_{I_a}$ and $[C_1^{ss}]_{I_p}$ are the same. This relationship predicts that the constant rate of intestinal absorption, I_a, can be determined without knowledge of any pharmacokinetic parameters such as the elimination rates from the liver and kidneys and the distribution volumes of the compartments.

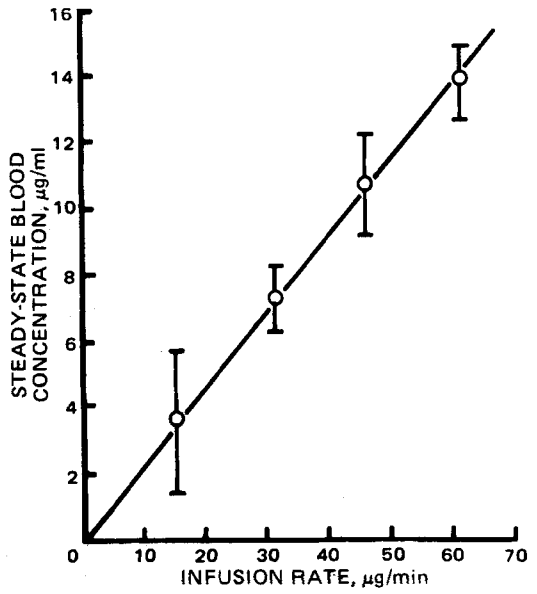


Figure 4—*Linear relationship between the steady-state blood concentration and portal vein infusion rate.*

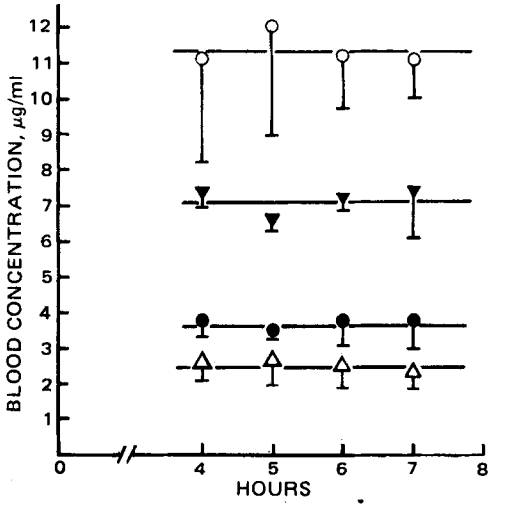


Figure 5—*Mean blood concentration of cyclacillin following single perfusion through the rat intestine at various drug concentrations. Key: △, 0.1 mg/ml; ●, 0.2 mg/ml; ▼, 0.5 mg/ml; and ○, 1.0 mg/ml. The vertical bars represent the standard deviation from three experiments.*

As shown in Fig. 3, the steady-state blood cyclacillin concentrations during a constant rate of infusion through the portal vein in rats were 3.57 ± 2.18, 7.29 ± 1.01, 10.7 ± 1.5, and 13.9 ± 1.0 μg/ml (mean ± *SD*) between 4 and 7 hr at infusion rates of 15.3, 30.5, 45.8, and 61.0 μg/min, respectively. Plots of $[C_1^{ss}]_{I_p}$ *versus* the infusion rate, I_p, are given in Fig. 4. These plots apparently are linear, yielding the following regression equation:

$$I_p = 4.33[C_1^{ss}]_{I_p} \quad \text{(Eq. 14)}$$

The clearances of cyclacillin by both the renal and hepatic routes in rats appear to be linear within the range of experimental blood levels.

Figure 5 shows the blood concentration–time profiles of cyclacillin during a constant single perfusion of cyclacillin solution of C_0 (micrograms per milliliter) at a rate of 2 ml/min through the rat small intestine. A steady state was attained after 4 hr. The average steady-state blood levels, $[C_1^{ss}]_{I_a}$, between 4 and 7 hr were 2.50 ± 0.52, 3.67 ± 0.47, 7.11 ± 0.71, and 11.37 ± 2.09 μg/ml for intestinal cyclacillin concentrations of 0.1, 0.2, 0.5, and 1.0 mg/ml, respectively. The absorption rates, I_a, were calculated

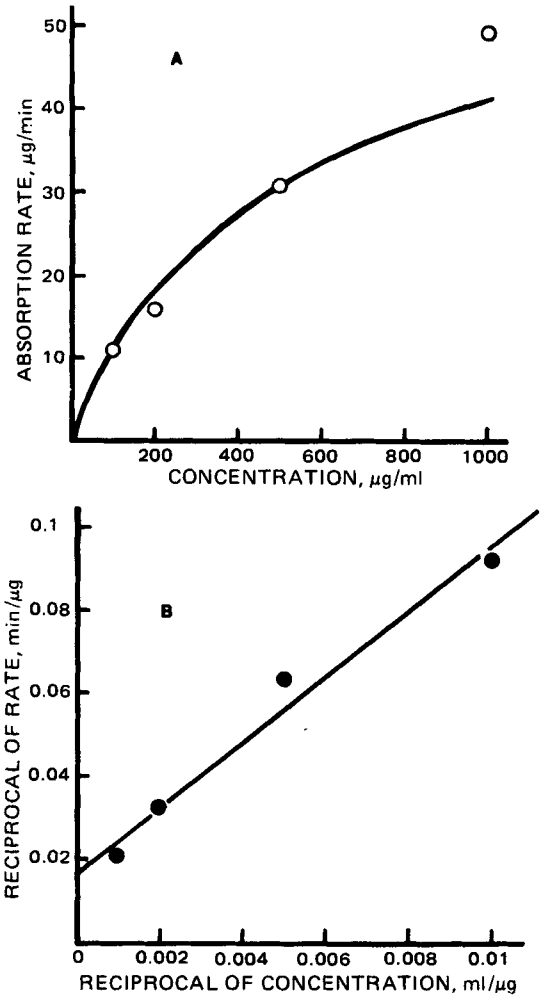


Figure 6—*A: Effect of drug concentration on the absorption rate of cyclacillin after rat intestinal single perfusion. B: Lineweaver–Burk plot of cyclacillin transport rate across the* in situ *rat intestinal wall to the bloodstream. The results are expressed as the means of three experiments;* V'_{max} *= 3640 μg/hr and* K_m *= 479 μg/ml.*

from Eq. 13 by substitution of $[C_1^{ss}]_{I_p}$ by $[C_1^{ss}]_{I_a}$ and are plotted *versus* C_0 in Fig. 6A. The relationship between the cyclacillin concentration perfused through the intestine, C_0, and the corresponding absorption rate I_a, demonstrated no proportionality but tended to be saturable. This finding is consistent with the fact that the disappearance of cyclacillin from the intestinal lumen in the *in situ* recirculating perfusion method followed Michaelis–Menten kinetics. If it is assumed that the net absorption of cyclacillin across the intestinal membrane is subject to a capacity-limited process at relatively low concentrations (<1.0 mg/ml), the steady-state absorption rate can be described solely by the Michaelis–Menten equation as follows:

$$I_a = \frac{V'_{max} C_0}{K_m + C_0} \qquad \text{(Eq. 15)}$$

where V'_{max} is the maximal rate (micrograms per hour). A Lineweaver–Burk plot of the reciprocal of the absorption rate ($1/I_a$) *versus* the reciprocal of the cyclacillin concentration ($1/C_0$) is shown in Fig. 6B. Using the least-squares method for the plot, the calculated values of V'_{max} and K_m were 3640 μg/hr and 479 μg/ml, respectively, which are comparable to the values evaluated from the kinetics of disappearance from the recirculating perfusate at the same perfusion rate of 2 ml/min (V'_{max} = 4520 ± 280 μg/hr[10] and K_m = 420 ± 27 μg/ml).

[10] This value was estimated by multiplying V_{max} by the intestinal recirculating perfusion volume of 9 ml.

CONCLUSION

The criticism has been made that GI absorption studies of drugs based on the rate or extent of disappearance from the lumen solution utilizing proper experimental techniques *in vivo* may not necessarily reflect the net absorption into the bloodstream due to such factors as drug metabolism in the intestinal lumen and at the mocusal surface. Previous studies (2, 4) demonstrated that cyclacillin exhibited a strongly dose-dependent disappearance from rat intestinal loops, suggesting some specialized transport system. However, this finding does not exclude possible enzymatic metabolism during the absorption experiments.

The present study provided conclusive evidence that the disappearance of cyclacillin from the intestinal lumen followed simultaneous Michaelis–Menten and first-order kinetics and that cyclacillin absorption into the bloodstream was rapid and complete by comparison of both *AUC* values after intravenous and intraduodenal administration. At the dose used (10 mg/kg), the observed Michaelis–Menten kinetic term is expected to play a major role in the total disappearance from the GI lumen. Additional evidence from a pharmacokinetic analysis of the steady-state blood concentrations after constant infusion of cyclacillin into the portal vein and through the small intestinal lumen indicated that the uptake of cyclacillin by the rat intestinal tissue at relatively low concentrations of <1 mg/ml followed solely Michaelis–Menten kinetics. These results suggest that cyclacillin may be transported in the rat intestine by certain carrier systems and moved into the bloodstream without significant loss of antibiotic activity.

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