Intestinal Absorption Mechanism of Amphoteric β-Lactam Antibiotics I: Comparative Absorption and Evidence for Saturable Transport of Amino-β-lactam Antibiotics by *In Situ* Rat Small Intestine

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Abstract □ The disappearance of various β-lactam antibiotics from *in situ* rat small intestinal loops was studied at pH 7.4. For monobasic penicillins, despite the wide variety of apparent partition coefficients in isobutyl alcohol–water, the disappearance from the jejunal loops was almost 30% (±5% *SD*). On the other hand, the disappearance of amphoteric derivatives of penicillins and cephalosporins having very low lipid solubility varied widely between 12 and 80%. The peak blood levels after intraduodenal administration to the rats correlated well with the extent of disappearance of amphoteric penicillins from the intestinal loops. Absorption studies utilizing *in situ* intestinal loops were performed at variable dose ranges to yield a clear dose-dependent disappearance. It is suggested that certain carrier-mediated transport systems underlie the absorption mechanisms of amphoteric β-lactam antibiotics.

Keyphrases □ Aminopenicillins—intestinal absorption mechanism proposed, compared with monobasic penicillins, *in situ* rat intestinal loops □ Aminocephalosporins—intestinal absorption mechanism proposed, compared with monobasic penicillins, *in situ* rat intestinal loops □ Absorption, intestinal—mechanism proposed for saturable transport of amphoteric amino-β-lactam antibiotics, *in situ* rat intestinal loops □ Antibiotics, amino-β-lactam—compared with monobasic penicillins, intestinal absorption mechanism proposed, *in situ* rat intestinal loops

Aminopenicillins, such as ampicillin, amoxicillin, and cyclacillin, and aminocephalosporins, such as cephalexin and cephradine, are acid stable and are absorbed after oral administration (1, 2). Much interest has been focused on the mechanism of the GI absorption of these antibiotics.

BACKGROUND

Many studies on the GI absorption of β-lactam antibiotics and their amino derivatives have been conducted to assess animal models (3, 4) or to compare their bioavailability (1, 5–7) rather than to elucidate the absorption mechanism. Recently, Tsuji *et al.* (8) reported that the GI absorption rates of monobasic penicillins in rats deviated significantly from the pH-partition hypothesis, and they explained this deviation on the basis of passive transport of the undissociated molecule through both the aqueous diffusion layer barrier adjacent to the GI membrane surface and the lipid barrier of the GI membrane. With the two-compartment diffusion mechanism, the structure–absorption rate relationship of this series of penicillins and cephalosporins, such as cephalothin and cefazolin, was established (9).

However, the GI absorption of aminopenicillins and aminocephalosporins cannot be predicted from this mechanism whereby the antibiotics are absorbed from the GI tract by passive diffusion of the unionized species. Amino-β-lactam antibiotics must have a zwitterionic structure to be ionized completely and to exhibit very low lipid solubility under GI conditions. Possible mechanisms were proposed for the membrane permeability of amphoteric penicillins and cephalosporins (10–21), but the mechanism by which amino-β-lactam antibiotics are absorbed is not clearly understood.

The present investigation was undertaken to determine the difference in absorption characteristics between monobasic and amphoteric β-lactam antibiotics. The comparative absorption of aminopenicillins, including ampicillin, amoxicillin, cyclacillin, and epicillin, and of three aminocephalosporins, cephalexin, cephradine, and cefadroxil, was studied in rats by the *in situ* GI loop method. Several monobasic penicillins also were included for comparison. The *in vitro* physicochemical properties, including comparative aqueous stabilities, solubilities, and dissolution rates, of the amphoteric β-lactam antibiotics (22, 23) as well as preliminary observations on the intestinal absorption were reported previously (15, 16, 21).

EXPERIMENTAL

Materials—Ampicillin anhydrate[1] (1015 μg/mg), amoxicillin trihydrate[2] (850 μg/mg), cyclacillin anhydrate[1] (983 μg/mg), epicillin anhydrate[3] (pure powder), cephalexin monohydrate[4] (925 μg/mg), cephradine monohydrate[3] (952 μg/mg), and cefadroxil monohydrate[5] (947 μg/mg) were used as supplied. The monobasic penicillins were the same as those employed previously (8). The standard solution of the penicilloic acids of ampicillin, amoxicillin, and epicillin was prepared according to a reported procedure (24).

All other chemicals were reagent grade and were utilized without further purification, except for imidazole which was purified by double recrystallization from benzene followed by thorough washing with ether.

Animals—Male albino Wistar rats, 200 ± 25 g, 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* Experiments on Absorption from Rat GI Loops**—*Disappearance from Small Intestinal Loops*—A midline incision was made to expose the small intestine, and the bile duct was ligated. The intestinal lumen was washed gently with 50 ml of pH 7.4 isotonic phosphate buffer[6] (25). Six milliliters of air was introduced to expel residual buffer and then was gently removed. In this experiment, two loops (each 5 cm long) were prepared by double ligation. The first loop was made 2 cm from the pylorus, and 1 cm of intestine separated the consecutive loop in the duodenum. For the study in the jejunum, the consecutive loops were prepared 15 cm from the pylorus. A dose of antibiotic was dissolved in pH 7.4 isotonic phosphate buffer, and 1 ml was injected into each intestinal loop. All studies lasted 1 hr.

At the end of the experiment, the remaining antibiotic solution was collected and the intestine was washed thoroughly with isotonic buffer. The solutions were combined to make up the desired volume. The samples were analyzed after filtration with a 0.45-μm membrane filter[7] to remove solid materials.

Disappearance from Ligated Areas of Stomach, Small Intestine, and Large Intestine—The rat alimentary tract was divided into four portions to locate the specific area for the absorption of amino-β-lactam antibiotics as follows: stomach, upper small intestine (20-cm length below the pylorus), lower small intestine (20-cm length above the ileum), and large intestine (10-cm length below the cecum). These regions were separated by ligation, and 2 mg of cyclacillin or cefadroxil in 1 ml was injected into each region. The drug was dissolved in pH 2.0 isotonic citrate buffer (25) or saline for the experiments in the stomach or intestinal regions, respectively. After 1 hr, the ligated GI segments were removed. Three rats were used. Other experimental procedures were the same as already described.

Blood Level and Tissue Accumulation after Stomach and Intraduo-

[1] Takeda Chemical Industries, Osaka, Japan.
[2] Fujisawa Pharmaceutical Co., Osaka, Japan.
[3] Sankyo Co., Tokyo, Japan.
[4] Shionogi Co., Osaka, Japan.
[5] Bristol Myers Co., Tokyo, Japan.
[6] The pH was measured with a PHM26 pH-meter, Radiometer, Copenhagen, Denmark.
[7] Sartorius-Membranefilter GmbH, Göttingen, West Germany.

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Table I—Values for pKa, Apparent Partition Coefficient in Isobutyl Alcohol–Water at pH 7.4, and Disappearance from Rat Intestinal Loops 1 hr after Administration of Various β-Lactam Antibiotics

Antibiotic	Dissociation Constant		Partition Coefficient in Isobutyl Alcohol–Water	Dose, mg/ml	Disappearance from *In Situ* Jejunal Loop, % ± *SD*	
	pK_1	pK_2			Upper	Lower
Penicillin V	2.79[a]	—	1.10[a]	5	26.9 ± 4.0 (3)[b]	27.4 ± 1.8 (3)
Phenethicillin	2.80[a]	—	1.55[a]	5	37.9 ± 4.0 (3)	33.8 ± 1.0 (3)
Propicillin	2.76[a]	—	3.02[a]	5	30.9 ± 5.5 (4)	32.5 ± 0.6 (3)
Oxacillin	2.73[a]	—	1.78[a]	5	27.2 ± 3.0 (5)	17.9 ± 1.7 (3)
Cloxacillin	2.78[a]	—	2.40[a]	5	22.1 ± 1.4 (4)	21.3 ± 1.5 (4)
Dicloxacillin	2.76[a]	—	4.07[a]	5	28.9 ± 3.7 (4)	29.9 ± 1.2 (3)
Floxacillin	2.76[a]	—	2.57[a]	5	27.0 ± 0.8 (3)	28.1 ± 2.4 (3)
Ampicillin	2.67[a]	6.95[a]	0.373	2	12.9 ± 5.5 (4)	11.8 ± 2.6 (4)
Amoxicillin	2.67[a]	7.11[a]	0.130	2	19.7 ± 1.4 (4)	17.0 ± 2.9 (5)
Cyclacillin	2.64[a]	7.18[a]	0.866	2	79.5 ± 3.4 (4)	79.7 ± 12.1 (5)
Epicillin	2.77[c]	7.17[c]	0.438	2	21.2 ± 6.9 (4)	20.8 ± 4.9 (5)
Cefadroxil	2.70[d]	7.22[d]	0.0813	2	66.2 ± 9.1 (4)	70.8 ± 3.2 (4)
Cephalexin	2.67[e]	6.96[e]	0.193	2	40.2 ± 13.6 (4)	36.9 ± 12.4 (4)
Cephradine	2.63[e]	7.35[e]	0.268	2	55.4 ± 6.5 (3)	52.0 ± 12.5 (3)

[a] Data at 37° and $\mu = 0.5$ from Refs. 22 and 30. [b] The experimental number is given in parentheses. [c] Data at 35° and $\mu = 0.5$ from Ref. 22. [d] Unpublished results at 37° and $\mu = 0.5$ from this laboratory. [e] Data at 37° and $\mu = 0.5$ from Ref. 23.

denal Administration—Antibiotic solutions of ampicillin, amoxicillin, epicillin, and cyclacillin, prepared by dissolution in 1 ml of saline, were given to rats at a dose of 10 mg/kg. A stomach tube was employed for administration into the stomach in rats whose pylorus was ligated. For intraduodenal administration, the drug solution was injected into the proximal duodenum in rats whose pylorus was ligated.

To determine the amount of antibiotic absorbed, blood was taken from the jugular vein at appropriate time intervals and assayed by a microbiological method and/or fluorometry after being hemolyzed with an equivalent volume of distilled water.

The accumulation in the small intestinal tissue during the *in situ* absorption experiments was examined for cyclacillin. After the 1-hr experiments, part of the whole intestine 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 a 10% (w/v, wet weight) homogenate. An aliquot of the homogenates then was analyzed.

Analysis of Loop Samples—The residual antibiotic and degradation product, the penicilloic acid, were assayed simultaneously by fluorometric determination for ampicillin and amoxicillin (26, 27) and also for epicillin. The fluorescence intensity was measured[8] with reference to that of quinine sulfate prepared with 0.1 *N* H_2SO_4. The assay for ampicillin was applied to epicillin, but the emission and excitation wavelengths were changed to 440 and 330 nm, respectively.

Aqueous mixtures of epicillin and its penicilloic acid in three proportions were analyzed, and the average recoveries were 106 ± 3 (*SD*)% for the intact drug and 103 ± 6% for the penicilloic acid. For cyclacillin, a spectrophotometric assay method (28) using imidazole mercuric chloride reagent was employed after acylation of the amino group with acetic anhydrate at pH 9.0. The compositions of the reagents were as described for ampicillin (28, 29). This method was employed only when the sample was diluted more than 25 times with the saline and in the concentration range of 15–80 μg/ml to prevent the influence of mucus and secretion products. The accuracy of this procedure was within 2% ($n = 5$).

High-performance liquid chromatography (HPLC) was utilized[9] for the determination of cephalexin, cephradine, cefadroxil, cyclacillin (only <10 μg/ml), and several monobasic penicillins. For the amino-β-lactam antibiotics, the carrier was 3–7% acetonitrile–0.01 *M* ammonium acetate, and the column was octadecylsilane chemically bonded on totally porous silica gel prepacked into a 4.0 × 300-mm[10] or a 4.6 × 250-mm[11] stainless steel column. For cyclacillin, the carrier was 0.1 *M* phosphate buffer of pH 4.0–4.2, and the column was strong cation-exchange resin[12] packed into a 4.6 × 500-mm stainless steel column. For the monobasic penicillins, the carrier was 0.02 *M* KH_2PO_4 and the column was anion-exchange resin[13] prepacked into a 2.1 × 500-mm stainless steel column. The detector was a UV spectrophotometer[14] set at 210 and 254 nm for the penicillins and cephalosporins, respectively.

An aliquot (50–100 μl) of the appropriately diluted solution was injected *via* a variable loop injector on flow, and the peak heights were measured. The calibration curves for the peak heights against the antibiotic concentration were satisfactorily linear and were prepared daily. The accuracy of the HPLC analysis from many trials was within 3%.

Analysis of Blood Samples—Fluorometric procedures (26, 27) were utilized for ampicillin and amoxicillin. Cyclacillin, epicillin, and amoxicillin were assayed by the microbiological paper disk diffusion method employing *Sartina lutea*[15] in the usual manner. Calculation of the penicillin concentration was made from the respective calibration curves prepared using pooled blood from control rats. The accuracies of the two analytical methods, *i.e.*, fluorometry and microbiological assay, were within 5%. The analytical data obtained by the methods agreed with each other within the limits of experimental error when a sample of amoxicillin was tested.

Analysis of Cyclacillin from Tissue Homogenates—The microbiological assay was employed, and the calibration curve was prepared using pooled 10% gut homogenates from control rats.

Determination of Apparent Partition Coefficients—For the study on the distribution of amphoteric β-lactam antibiotics in isobutyl alcohol–water, the aqueous phase was prepared to give a constant Na^+ concentration of 0.1 *M* using phosphate buffer for the reasons described previously (30). This buffer component was determined so as to maintain a constant ionic strength of 0.15 and a pH of 7.4. A 1×10^{-3} *M* antibiotic solution was prepared with this solution. To minimize the volume exchange due to mutual miscibility, the aqueous and organic phases were saturated previously with each solvent.

Exactly 3–10 ml of each solution was transferred to a glass-stoppered flask and shaken for 2 hr to achieve complete equilibrium at 37 ± 0.1°. The two phases separated on standing for 1 hr, and the aqueous phase was centrifuged at 3000 rpm for 10 min. If necessary, after appropriate dilution with distilled water, the concentration in the aqueous phase was determined by HPLC. The apparent partition coefficient was calculated according to the equation described previously (30).

RESULTS AND DISCUSSION

The pKa values and apparent partition coefficients of the β-lactam antibiotics at pH 7.4 in isobutyl alcohol–water are summarized in Table I.

Disappearance of Various β-Lactam Antibiotics from *In Situ* Intestinal Loops—Values for the percentage disappearance of various penicillins and cephalosporins during 1 hr are shown in Table I.

Despite the fact that the seven monobasic penicillins grouped by phenoxy and isoxazole derivatives exhibit a wide variety of apparent partition coefficients in isobutyl alcohol–water, their disappearance from both the upper and lower jejunums was almost 30% (±5% *SD*). Previous data concerning the rat *in situ* intestinal recirculating method demonstrated (9) that the intestinal absorption of ionized monobasic β-lactam

[8] Model FP-4 fluorescence spectrophotometer, Japan Spectroscopic Co., Tokyo, Japan.
[9] Model FLC A-700, Japan Spectroscopic Co., Tokyo, Japan.
[10] μBondapak C_{18}, Waters Associates, Milford, Mass.
[11] Silica gel, SC-02, Japan Spectroscopic Co., Tokyo, Japan.
[12] Zipax SCX, DuPont Instruments, Wilmington, Del.
[13] Zipax AAX, DuPont Instruments, Wilmington, Del.
[14] Model UVIDEC-100, Japan Spectroscopic Co., Tokyo, Japan.
[15] IFO 12708, Institute for Fermentation, Osaka, Japan. The strain was derived from ATCC 9341.

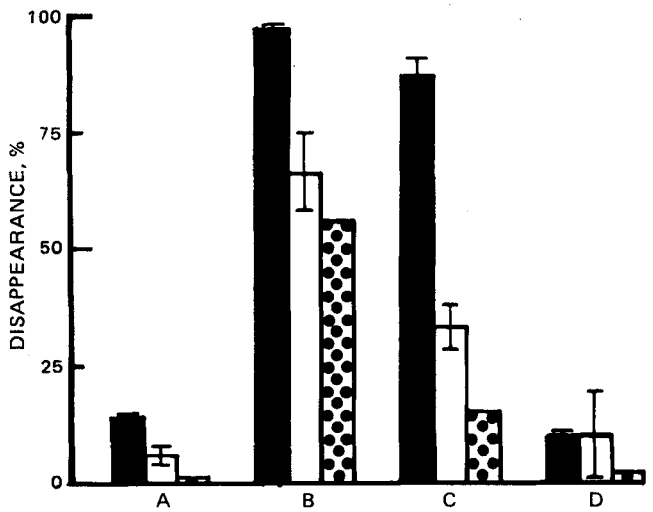

Figure 1—*Absorption of cyclacillin and cefadroxil from loops in the digestive tracts of rats. Key: closed columns, cyclacillin; open columns, cefadroxil; dotted columns, cephalexin; A, stomach; B, upper small intestine (20 cm below the pylorus); C, lower small intestine (20 cm above the ileocecum); and D, large intestine (10 cm below the cecum). The data for cephalexin are redrawn from Ref. 31.*

antibiotics followed apparent first-order kinetics with competing apparent first-order degradation to their β-lactam opening products. Between pH 6.5 and 9, absorption and the accompanying degradation were almost insensitive to changes in the intestinal lumen pH and initial drug concentration and to the lipophilicity of the molecules (9). The present results are consistent with those findings, suggesting that the disappearance of monobasic penicillins from the rat intestinal loops was entirely attributable to both absorption and degradation.

On the other hand, the percentage disappearance of amphoteric derivatives having rather low lipophilicity varied widely, between 12 and 80% during 1 hr at a 2-mg/ml dose (Table I). The degradation products (penicilloic acids) from aminopenicillins were determined at the end of the absorption experiment to be 1.6, 1.7, and 1.8% for ampicillin, amoxicillin, and epicillin, respectively. No detectable degradation products were observed with cyclacillin at this dose. The *in vitro* degradation of cephalexin, cephradine, and cefadroxil in the intestinal washings with isotonic phosphate buffer during 1 hr was 4.0, 4.7, and 2.1%, respectively. These findings of slight degradation of amophoteric β-lactam antibiotics strongly indicate that the high percentage disappearances of cyclacillin (80%), cephalexin (40%), cephradine (50%), and cefadroxil (70%) essentially were attributed to intestinal membrane transport resulting in accumulation by the gut tissue and/or transference into the vascular system.

These results suggest that some specialized transport mechanism may contribute to the absorption of zwitterionic antibiotics accompanying the simple diffusion transport (9) responsible in the absorption of ionized species of monobasic β-lactam antibiotics.

Absorption and Tissue Accumulation of Cyclacillin and Cefadroxil from Different Areas of Rat Alimentary Tract and Comparative Blood Levels after Intraduodenal Administration of Aminopenicillins—Figure 1 compares the percentage disappearances of cyclacillin and cefadroxil during 1 hr from four areas of the rat GI tract after injection into the ligated loop. The highest disappearance occurred from the small intestine, with almost all of the cyclacillin and 33–66% of the cefadroxil doses disappearing from the lumen. However, only ~10% of each dose disappeared from the stomach and large intestine. In a similar absorption study on cephalexin (redrawn in Fig. 1), Maekawa *et al.* (31) demonstrated that the total disappearance during 1 hr from the stomach, upper small intestine, lower small intestine, and large intestine of rats was 1.0, 56.2, 15.6, and 2.4%, respectively. These data are comparable with the present results for cyclacillin and cefadroxil. The accumulation of cyclacillin in the intestine was negligible (3%) during the *in situ* absorption experimental period.

Figure 2 shows the mean blood concentrations after intraduodenal administrations of aminopenicillins at a dose of 10 mg/kg. The peak blood concentrations for ampicillin, amoxicillin, and epicillin appeared after 1 hr, but cyclacillin was observed in the bloodstream within 0.5 hr after administration. The rank order of blood levels after intraduodenal administration was: cyclacillin > amoxicillin > epicillin ≥ ampicillin. The blood concentration of cyclacillin was far higher than the concentrations of the other penicillins ($p < 0.01$).

Ampicillin demonstrated a significantly lower blood level than amoxicillin ($p < 0.05$). The peak serum concentration after a single oral dose with sufficient water in humans was in the order (5, 6): cyclacillin > amoxicillin > ampicillin > epicillin, which is almost identical with that in rats. When the aminopenicillins were administered into the rat stomach, they were not detectable in the serum by microbiological assay, indicating that the aminopenicillins were scarcely absorbable from the stomach. The *in vitro* degradation of ampicillin, amoxicillin, epicillin, and cyclacillin after 1 hr at pH 2 and 35° was evaluated from the data of the degradation kinetics (22, 32) to be 5.8, 4.7, 2.9, and 7.2%, respectively. The disappearance of cyclacillin from the stomach (Fig. 1) thus may be due to its acid degradation. The present results support the claim of Lode (33) that absorption of ampicillin and cephalexin in humans occurred in the small intestine and not the stomach.

On this basis, the rat represents a suitable experimental animal for elucidation of the absorption mechanism of amino-β-lactam antibiotics in humans. Previous observations (31, 33) and the present results suggest that there is a specific area in the alimentary tract of mammals for the absorption of amino-β-lactam antibiotics. On the other hand, monobasic and lipophilic penicillins can be absorbed from both the stomach and small intestine below pH 6 (8, 9). The difference in permeability between amphoteric derivatives and monobasic ones may be attributed to a difference in the membrane transport mechanism. The latter types of β-lactam antibiotics may be absorbed by lipid membrane transport of the undissociated species through the barrier of the aqueous diffusion layer adjacent to the membrane surface (8, 9). The amino-β-lactam antibiotics, having very low lipid solubility (Table I) and being completely ionized in all alimentary tract areas, are unlikely to be absorbed by this mechanism.

Effect of Dose on Absorption by *In Situ* Intestinal Loops—To examine the mechanism responsible for absorption of amino-β-lactam antibiotics *in vivo*, the effect of the dose concentration on the percentage disappearance from the small intestinal lumen was investigated. If the percentage disappearance remains unaffected by the initial dose con-

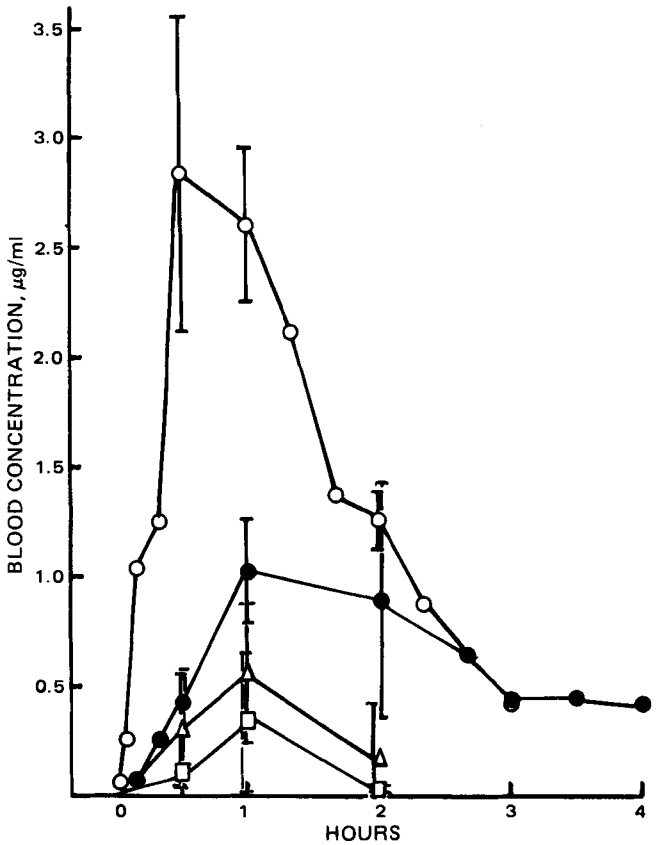

Figure 2—*Time courses of whole blood concentrations of aminopenicillins in rats after a 10-mg/kg dose in the duodenum. The mean levels and standard deviations (bars) for three animals are shown for 0.5, 1.0, and 2.0 hr. Key: □, ampicillin; ●, amoxicillin; ○, cyclacillin; and △, epicillin.*

Table II—Disappearance and Degradation Products of Aminopenicillins from Rat Intestinal Loops 1 hr after Administration

Antibiotic	Intestinal Area	20 μg/ml		2 mg/ml		5 mg/ml		20 mg/ml,
		Disappearance, % ± *SD*	Product, % ± *SD*	Disappearance, % ± *SD*	Product, % ± *SD*	Disappearance, % ± *SD*	Product, % ± *SD*	Disappearance, % ± *SD*
Ampicillin	1[a]	9.0 ± 3.2 (3)[b]	6.1 ± 0.5 (3)	7.3 ± 1.3 (3)	4.6 ± 2.5 (3)	—	—	—
	2[c]	5.8 ± 4.7 (3)	4.3 ± 0.5 (3)	9.2 ± 6.0 (3)	2.2 ± 0.8 (3)	—	—	—
	3[d]	10.9 ± 3.5 (4)	2.9 ± 1.0 (4)	11.2 ± 5.2 (4)	1.7 ± 0.9 (4)	—	—	—
	4[e]	8.4 ± 6.2 (4)	2.8 ± 1.1 (4)	10.4 ± 2.8 (4)	1.4 ± 1.3 (4)	—	—	—
Amoxicillin	1	28.6 ± 3.1 (4)	8.5 ± 4.2 (4)	12.1 ± 0.8 (3)	3.9 ± 2.8 (3)	—	—	—
	2	18.3 ± 2.1 (5)	4.7 ± 2.0 (5)	12.9 ± 1.9 (4)	1.4 ± 1.1 (4)	—	—	—
	3	21.7 ± 6.3 (5)	0.8 ± 0.8 (5)	18.8 ± 1.2 (4)	2.3 ± 1.0 (5)	—	—	—
	4	19.6 ± 6.6 (3)	0.5 ± 0.9 (3)	16.6 ± 2.7 (5)	1.0 ± 0.9 (5)	—	—	—
Cyclacillin	1	—	—	79.5 ± 8.0 (6)	—	42.0 ± 9.8 (4)	—	18.9 ± 5.3 (5)
	2	—	—	78.9 ± 7.5 (5)	—	48.9 ± 5.3 (4)	—	16.9 ± 3.5 (4)
	3	—	—	79.5 ± 3.4 (4)	—	53.0 ± 13.2 (4)	1.8	20.2 ± 5.6 (5)
	4	—	—	79.7 ± 12.1 (5)	—	47.7 ± 4.7 (4)	1.8	17.8 ± 6.2 (5)
Epicillin	1	29.9 ± 14.3 (4)	3.5 ± 3.1 (4)	22.1 ± 5.0 (4)	2.6 ± 1.5 (4)	—	—	—
	2	14.0 ± 9.4 (5)	9.6 ± 6.2 (5)	15.6 ± 3.2 (5)	2.4 ± 1.5 (5)	—	—	—
	3	20.3 ± 8.9 (5)	1.6 ± 1.7 (5)	19.1 ± 7.3 (8)	1.8 ± 1.8 (4)	—	—	—
	4	24.7 ± 12.3 (5)	4.3 ± 5.2 (5)	18.8 ± 9.7 (8)	1.8 ± 1.0 (4)	—	—	—

[a] Upper duodenum, 2 cm from the pylorus. [b] The experimental number is given in parentheses. [c] Lower duodenum, with 1-cm separation from 1. [d] Upper jejunum, 15 cm from the pylorus. [e] Lower jejunum, with 1-cm separation from 3.

centration, then the apparent first-order absorption might proceed as observed previously (8, 9) for monobasic β-lactam antibiotics. However, if this value is affected, the contribution of some specialized mechanism would be strongly suggested.

Tables II and III show the percentages of total disappearance after 1 hr in the four small intestinal segments of each 5-cm loop and at various dose concentrations. The degradation products (penicilloic acids) remaining in the lumen also were determined for ampicillin, amoxicillin, and epicillin by fluorometric assay and for cyclacillin by HPLC. Small amounts of the degradation products of these four antibiotics were found (Tables II and III).

The percentage disappearance of amoxicillin, cyclacillin, cephalexin, cephradine, and cefadroxil depended remarkably on the initial dose concentrations. These dose dependencies revealed by their general pattern that the percentage disappearance at a low dose was extremely large while that at a high dose was markedly reduced. A similar phenomenon also was observed for epicillin, although the difference between the percentage disappearance at 20 μg/ml and that at 2 mg/ml was not statistically significant. Unfortunately, with our experimental system, it was difficult to detect saturation phenomena in the disappearance of ampicillin. However, after modification of our study, Shindo *et al.* (19) successfully demonstrated evidence for saturable absorption of ampicillin using an 8-cm long rat intestinal loop *in situ*. They found that the absorption of [^{14}C]ampicillin, labeled at the phenylglycyl moiety, showed an apparent tendency for saturation at a dose above 3 mg/loop, and the transference into the blood was marked in the upper and middle intestine. Absorption from limited parts also was observed with amoxicillin, cephalexin, and cefadroxil (Tables II and III), while cyclacillin and cephradine were absorbed from almost all parts of the intestine to the same extent.

Such remarkable dose-dependent absorption and the existence of specific areas of absorption suggest that the absorption of amino-β-lactam antibiotics may follow the same forms of specialized and saturable transport mechanisms since the competing degradation of β-lactam in the lumen solution is presumed to be small compared to the net absorption based on the facts discussed elsewhere.

Some investigators (10, 11, 13), using isolated rat gut, indicated the presence of an active transport mechanism of cephalexin and cyclacillin. Other studies (12, 14), however, showed by a similar *in vitro* technique that the β-lactam antibiotics, including cyclacillin (12), cephalexin (12), amoxicillin (14), and ampicillin (12, 14), were not transported by an active transport mechanism. Miyazaki *et al.* (14) demonstrated that, in both *in situ* and *in vitro* studies using rat small intestinal segments, the greater binding of amoxicillin than of ampicillin to the mucosa provided a possible explanation for the difference in body fluid concentration of these two drugs.

A recent report (20) indicated that cephradine, but not cephalexin and amoxicillin, was actively transported across the everted rat intestine. More recent findings clearly revealed that the absorption of amoxicillin (15, 17), cyclacillin (16, 17), cephalexin (21), and cephradine (21) from rat small intestinal perfusion solution was saturable. Kimura *et al.* (17) concluded that amoxicillin may be transported by a facilitated diffusion mechanism and that cyclacillin may be transported in part by active transport.

The present observations showing saturable absorption as a common phenomenon indicate that the same types of carrier-mediated transport mechanisms in the rat GI tract may underlie the absorption of amino-β-lactam antibiotics. Such a carrier system may be localized only in the small intestinal area and not in the stomach since no absorption was found in the latter part of the alimentary tract. Based on the present data for cyclacillin and cefadroxil and previous data (31, 33) for cephalexin, it is concluded that such a system is likely to make a small contribution in the large intestine.

Therefore, it is conceivable that carrier-mediated absorption is a common mechanism for the intestinal absorption of amino-β-lactam antibiotics, which are known to be effective *via* the oral route. The ki-

Table III—Disappearance of Aminocephalosporins from Rat Intestinal Loops 1 hr after Administration

Antibiotic	Intestinal Area	Disappearance of Administered Dose, % ± *SD*			Statistical Significance of Difference	
		2 mg/ml	10 mg/ml	20 mg/ml	$(p)^a$	$(p)^b$
Cefadroxil	1[c]	34.1 ± 5.9 (5)[d]	—	29.4 ± 6.2 (4)	—	NS
	2[e]	64.6 ± 7.4 (5)	—	35.1 ± 5.7 (4)	—	<0.001
	3[f]	66.2 ± 9.1 (4)	—	31.6 ± 5.7 (3)	—	<0.01
	4[g]	70.8 ± 3.2 (4)	—	36.8 ± 3.4 (3)	—	<0.001
Cephalexin	1	25.5 ± 5.4 (4)	—	22.2 ± 3.7 (3)	—	NS
	2	21.3 ± 6.4 (4)	—	28.9 ± 2.3 (3)	—	NS
	3	40.2 ± 13.6 (4)	—	24.7 ± 2.3 (3)	—	NS
	4	36.9 ± 12.4 (4)	—	21.5 ± 2.0 (4)	—	<0.05
Cephradine	1	47.7 ± 0.5 (3)	21.2 ± 4.4 (4)	17.0 ± 7.1 (3)	<0.01	<0.01
	2	51.4 ± 9.4 (4)	29.2 ± 9.7 (3)	17.3 ± 2.3 (3)	<0.05	<0.01
	3	55.4 ± 6.5 (3)	26.8 ± 1.4 (3)	24.1 ± 10.3 (3)	<0.01	<0.02
	4	52.0 ± 12.5 (3)	28.3 ± 1.6 (3)	22.3 ± 7.8 (3)	<0.05	<0.05

[a] Two-sample *t* test; disappearances of a 2- and 10-mg/ml dose. [b] Two-sample *t* test; disappearances of a 2- and 20-mg/ml dose. [c] Upper duodenum, 2 cm from the pylorus. [d] The experimental number is given in parentheses. [e] Lower duodenum, with 1-cm separation from 1. [f] Upper jejunum, 15 cm from the pylorus. [g] Lower jejunum, with 1-cm separation from 3.

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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