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Physicochemical Properties of Amphoteric β-Lactam Antibiotics. IV.[1] First- and Second-Order Degradations of Cefaclor and Cefatrizine in Aqueous Solution and Kinetic Interpretation of the Intestinal Absorption and Degradation of the Concentrated Antibiotics[2]

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A kinetic study on the degradations of cefaclor and cefatrizine was carried out at 35 °C as a function of pH and initial drug concentration by the use of high-performance liquid chromatography. At constant pH and temperature, the degradation followed pseudo-first-order kinetics at the initial concentration of 5 mM. The shape of the rate-constant–pH profile of cefaclor resembled those for cefatrizine and other aminocephalosporins. At neutral pH, cefaclor was degraded *via* intramolecular nucleophilic attack of the α-amino group on the β-lactam moiety. The intramolecular reaction rate was very similar to that in the cases of cefatrizine and cephaloglycine, but was ten times faster than those for cefadroxil, cephalexin, and cephradine under the same conditions.

Accelerated degradations of the highly reactive aminocephalosporins, cefaclor and cefatrizine, were observed at higher drug concentrations than 10 mM. By simultaneously assaying the disappearances of the α-amino group and the antibiotic itself, it has been confirmed that both antibiotics undergo self-aminolysis in solution through a nucleophilic attack of the free side-chain amino group in one molecule upon the β-lactam bond of a second molecule, forming a dimer. Since the degradation rates of cefaclor and cefatrizine were found to be larger than those of other amino-β-lactam antibiotics, the second-order degradation rate process should be considered in the analysis of their *in situ* intestinal absorption rates at high concentrations. The above kinetic data were applied to the intestinal absorption of both antibiotics in rats. The disappearance rate of the antibiotics from the intestinal perfusate was successfully interpreted in terms of a combination of Michaelis–Menten absorption, first-order absorption, first-order degradation and second-order dimerization.

Keywords——cefaclor; cefatrizine; β-lactam antibiotics; aminocephalosporin; stability; intermolecular reaction; pH effect; second-order kinetics; rat intestinal absorption; rat intestinal degradation

Introduction

Cefaclor (CCL)[3] and cefatrizine (CFT)[3,4] are newly developed oral β-lactam antibiotics with broad-spectrum activity. Pharmacokinetic studies of CCL and CFT in man[4-7] have shown that their urinary recoveries are inferior to those of cefadroxil (CDX) and cephalexin (CEX), although they are more active than CEX against some bacterials.[4,8,9]

In our previous papers,[1,10-12] the physicochemical properties affecting the bioavailability, such as solubility, dissolution rate, and stability of amino-β-lactam antibiotics in aqueous solution, were reported. These studies included the five clinically used cephalosporins, CDX, CFT, CEX, cephaloglycine (CEG), and cephradine (CED). Among them, CEG and CFT have the highest reactivity at physiological pH, which may result in the reduced urinary recovery. Instability of CCL has been reported,[3] but no detailed kinetic study

on the stability has appeared in the literature. Polymerization is known to occur in concentrated solutions of aminopenicillins such as ampicillin, amoxicillin, and epicillin,[13] but there is no information on the polymerization reactions of aminocephalosporins.

Previously, we reported that the disappearance rates of amino-β-lactam antibiotics from the luminal solution perfusing through the rat small intestine followed mixed-type kinetics involving saturable absorption, nonsaturable absorption, and first-order degradation processes.[14] In this case, the absorption rate was estimated by subtracting the *in vitro* first-order degradation rate from the total disappearance rate. In order to clarify the intestinal absorption mechanisms of CCL and CFT, which have large degradation rates *in vitro*, a detailed kinetic study of the degradation is required to facilitate analysis of the *in situ* absorption rates.

This work deals with the kinetics and mechanism of the first-order and second-order degradations of CCL and/or CFT. The data obtained were applied to an *in situ* absorption kinetic study of both antibiotics at various concentrations in the intestinal perfusion solution.

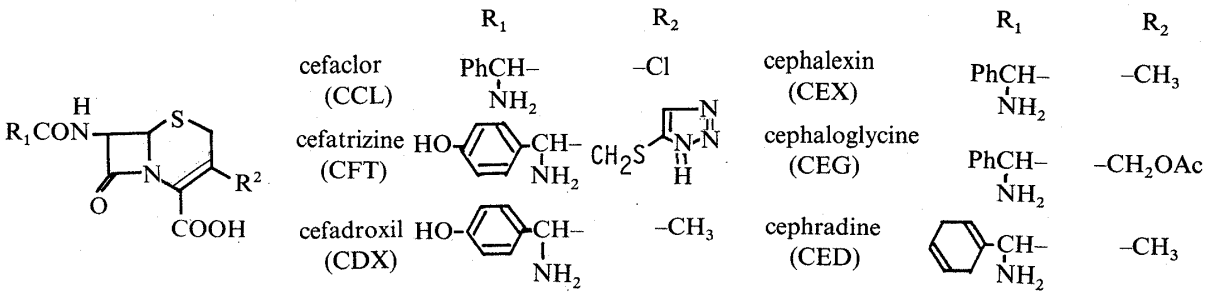

Chart 1

Experimental

Materials——CCL monohydrate (960 μg/mg) and CFT propylene glycol (832 μg/mg) were supplied by Shionogi Pharmaceutical Co., Osaka, Japan and Banyu Pharmaceutical Co., Tokyo, Japan, respectively.

All other chemicals were of reagent grade and were used without further purification.

Procedures: Degradation Kinetics——The reactions were carried out in aqueous solutions at 35 ± 0.1 °C and an ionic strength of 0.5, in the same way as reported for CDX,[12] unless otherwise stated. The degradation was initiated by dissolving an accurately weighed amount of the drug in an appropriate buffer solution or 0.5 M potassium chloride solution preheated to the desired temperature. When the pH of the solution was maintained by means of a pH stat (TTT2 titrator and ABU12b autoburet, Radiometer, Copenhagen, Denmark) during the kinetic run, 0.5 M KCl solution containing 1×10^{-4} M disodium edetate was used in order to avoid possible heavy metal-catalyzed degradation. The amount of sodium hydroxide (0.5—2 M) necessary to keep the pH constant was less than 10% of the reaction volume in all kinetic runs. Samples were withdrawn at suitable time intervals, cooled in an ice bath, and diluted with 0.2 M acetate buffer (pH 4.0) to prevent possible degradation during the analysis.

The concentration of residual intact cephalosporin was analyzed by a high-performance liquid chromatographic (HPLC) method. In addition to the HPLC determination of overall degradation, other aliquots were taken for analysis of the decrease in the concentration of the primary amino group in cephalosporin during the degradation by a colorimetric assay in the same manner as used for the CDX degradation study.[12]

Determination of Ionization Constants——The apparent pK_a value for the dissociation of the α-ammonium moiety of CCL was determined under the conditions of the kinetic study by the potentiometric titration method described previously.[12] The pK_a value obtained was 7.08 ± 0.08.

Analytical Procedures: HPLC——The liquid chromatograph (TRIROTAR-II, Japan Spectroscopic Co., Tokyo, Japan) was equipped with a detector set at 254 nm and a 4.0×300-mm stainless steel column prepacked with octadecylsilane chemically bonded on porous silica gel (μBondapak C_{18}, Waters Associates, Milford, Mass., U.S.A.). The mobile phase was an acetonitrile–methanol–0.01 M ammonium acetate mixture; the contents of acetonitrile and methanol were 3% and 0.3% for CCL, and 1.5% and 1% for CFT, respectively. The chromatography was performed at ambient temperature, and the samples were eluted at a flow rate of 1 or 2 ml/min to obtain appropriate retention times. A plot of the peak height of an antibiotic *versus* concentration was linear in the concentration range of 10—

100 μg/ml. The sample peak heights were converted to concentrations by comparison with a standard curve which was obtained daily.

Colorimetric Assay——The procedure used for CDX was adopted for CCL degradation.[12]

***In Situ* Recirculating Perfusion Method**——Male albino Wistar rats, 200±25 g, were fasted for 20 h prior to the experiment, but water was given freely. The rats were anesthetized with urethan (1.5 g/kg, *i.p.*) 1 h prior to surgery. The procedure employed for the intestinal perfusion experiment was essentially the same as that described previously.[14] In order to avoid the accelerated degradation by phosphate-catalyzed reaction, 0.01 M isotonic phosphate buffer, which contains the lowest concentration of phosphate giving effective pH control, was employed.

Non-linear Regression Analysis and Computer Simulation——The non-linear least-squares treatment of the kinetic data by use of the NONLIN program[15] and numerical solution of the differential equation by NUMINT in NONLIN were run on a FACOM-170F digital computer at the Data Processing Center, Kanazawa University.

Results and Discussion

Degradation Kinetics of CCL at Low Concentration in Aqueous Solution

(a) Reaction Order and Observed Rate Constants——The degradation of 5 mM CCl followed pseudo-first-order kinetics at constant pH, temperature, and ionic strength of 0.5 at pH 7.5—11.5. This result is illustrated by the linear log concentration *versus* time plots at 35 °C in Fig. 1.

(b) Degradation Rate–pH Profiles——The kinetics and mechanism of degradation of amphoteric cephalosporins were reported previously.[1,11,12] The pH-dependence of the overall first-order rate constants, k_{pH}, of CCL degradation is illustrated in Fig. 2. The rate constants used in construction of this graph were obtained from the intercepts of the plots of k_{obs} *versus* total buffer concentration at various pH values. The results obtained by using a pH-stat were also incorporated. Figure 2 shows that above pH 10 the observed rate of degradation increased rapidly and uniformly with increasing pH.

At pH values near pK_a, there was an ascending sigmoid dependence of k_{pH} on pH, followed by a rapid rate decrease as the pH decreased. This inflection indicates that the dissociation equilibria of the side chain amino group influenced the degradation rate. For comparison, the pH–k_{pH} profiles for CFT,[1] CDX,[12] CEX,[11] and CED[11] obtained under the same kinetic conditions are redrawn in Fig. 2. The pH-dependence of k_{pH} for CCL above pH 6 shows the same shape as those for all other aminocephalosporins. The shape around pK_{a2} in the pH–rate profiles of the aminocephalosporins can be accounted for in terms of the mechanism of intramolecular nucleophilic attack of unprotonated side-chain amino groups on the β-lactam moiety (Chart 2). Since in the pH range studied CCL exists in two different ionic forms, a zwitterion ($IH^{\pm}$) and an anion (I^-), the total rate constant can be expressed by Eq. 1:

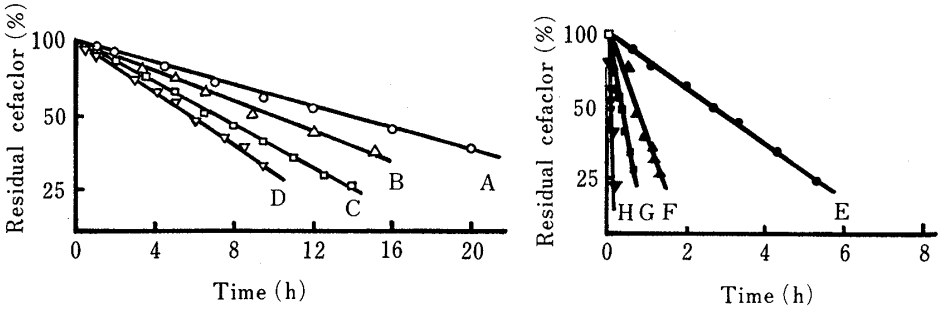

Fig. 1. Apparent First-Order Plots for the Degradation of 5 mM CCL at Various pH Values, 35 °C, and an Ionic Strength of 0.5, Determined by HPLC Assay

A, pH 7.5 (○); B, pH 8.0 (△); C, pH 8.5 (□); D, pH 9.0 (▽); E, pH 10.0 (●); F, pH 10.5 (▲); G, pH 11.0 (■); H, pH 11.5 (▼). The pH of the reaction solution was maintained with a pH-stat.

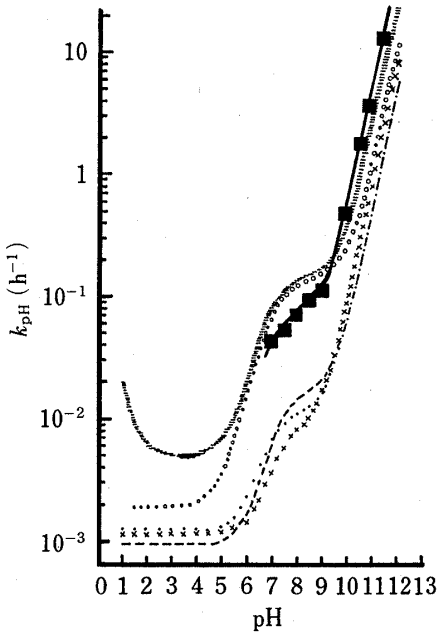

Fig. 2. log k_{pH}–pH Profiles for the Degradation of CCL (■) at 35 °C and an Ionic Strength of 0.5

The solid line represents the curve calculated from Eq. 1 with the constants listed in Table I; the points are the experimental values. The other lines refer to the log k_{pH}–pH profiles for CED (····), CEG (------), CEX (× × ×), CDX (– – – –), and CFT (ooo) at 35 °C and an ionic strength of 0.5 (from refs. 1, 11, and 12).

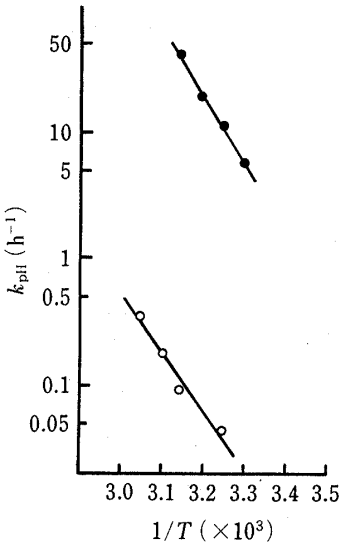

Fig. 3. Arrhenius Plots of the Apparent First-Order Rate Constants k_{pH}, for CCL Degradation at pH 7.0 (○) and 11.50 (●), and an Ionic Strength of 0.5

The pH values were maintained by the use of a pH-stat.

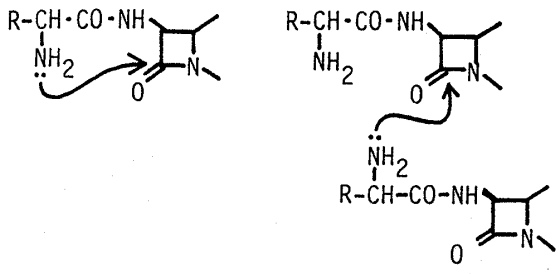

Chart 2

TABLE I. Rate Constants[a] for Degradation of Aminocephalosporins at 35 °C and an Ionic Strength of 0.5

Cephalosporin	$10^2 k_b$ h^{-1}	$10^{-2} k_{OH}$ $M^{-1} h^{-1}$
Cafaclor	8.0	19.1
Cefatrizine[b]	14.4	5.84
Cefadroxil[b]	1.61	2.54
Cephalexin[b]	1.01	2.64
Cephradine[b]	0.740	3.98
Cephaloglycin[b]	13.5	13.1

a) Defined in Eq. 1. b) Ref. 1.

$$k_{obs} = k_b\left(\frac{K_a}{a_H^+ + K_a}\right) + k_{OH}\frac{K_W}{a_H^+} \quad (1)$$

where k_b represents the first-order rate constant for the spontaneous degradation of the ionic species of CCL; k_{OH} represents the second-order rate constant for specific hydroxide-ion-catalyzed degradation of all species; K_w is the autoprotolytic constant. Incorporation of $K_a = 8.32 \times 10^{-8}$ and $K_w = 2.09 \times 10^{-14}$ at 35 °C[16] into Eq. 1 gave the best-fit parameters of $0.080 \pm 0.005\,h^{-1}$ for k_b and $1910 \pm 140\,M^{-1}h^{-1}$ for k_{OH} for CCL by using the NONLIN computer program.[15] The curve in Fig. 2 was calculated for CCL from Eq. 1 by the use of these parameters. These values are listed in Table I together with those for other aminocephalosporins.[1,11,12] The degradation rates of several aminocephalosporins in the neutral and basic

regions depend on the nature of the substituent at the 3-position, and this can be attributed to the difference in the long-range inductive effect on the electrophilicity of the β-lactam carbonyl carbon atom toward nucleophiles such as hydroxide ion and amines, and/or the effectiveness as a leaving group of the 3-methylene moiety.[1,11] Since the Cl substituent is highly electron-withdrawing, CCL can be classified as an unstable cephalosporin.

(c) Temperature Dependency——Rate constants for the overall disappearance of CCL were obtained in the temperature range between 35 and 60 °C at pH 7.00 and 11.50. The pH of the reaction solution was maintained constant by means of a pH-stat. The Arrhenius plots are shown in Fig. 3. From these data, the apparent activation energies at pH 7.00 and pH 11.50 were determined to be 21.4 and 24.7 kcal/mol, respectively.

The activation energy at pH 7.0 reflects the intramolecular reaction initiated by the attack of the α-amino group on the β-lactam moiety.

At pH 11.50, the net activation enthalpy, $\Delta H^{\neq}$, of the hydroxide ion-assisted degradation of CCL was calculated to be 11.3 kcal/mol at 35 °C, by subtracting the heat of ionization of water (13.1 kcal/mol)[16] from the apparent activation energy (24.4 kcal/mol).

Effect of Initial Concentration on the Stability of CFT and CCL

As reported previously and described in the previous section, the primary α-amino group in the C-7 side chain of aminocephalosporins is capable of attacking the β-lactam moiety intramolecularly.[1,11] Since CCL and CFT are both unstable cephalosporins, the α-amino group of each antibiotic may react intermolecularly with the intact β-lactam of another molecule as confirmed in the case of ampicillin.[13] Therefore, the stability kinetics of the two antibiotics were investigated at 35 °C and various pH values as a function of initial antibiotic concentration while the solution pH was maintained at the desired value by means of a pH-stat without the use of buffer solution. The time-courses of the disappearance at various initial concentrations from 41 to 170 mM are shown in Fig. 4. These antibiotics showed greater instability as the antibitoic concentration was increased. It was reported for aminopenicillins that rapid degradation depending on the initial antibiotic concentration can be attributed to the occurrence of dimerization by the nucleophilic attack of the free α-amino group on the β-lactam of a second molecule.[13] Assuming that similar dimerization occurs with the present aminocephalosporins, the overall degradation rate may be written by Eq. 2.

$$-\frac{dC}{dt}=\left(k_b\frac{K_a}{a_H^+ + K_a}+k_{OH}\frac{K_W}{a_H^+}\right)\cdot C+k_2C^2 \qquad (2)$$

where C represents the concentration of the intact antibiotic, and k_2 is the second-order rate constant.

To clarify the involvement of the α-amino group in this dimerization reaction of CCL and CFT, the decrease in the concentration of the primary amino groups in CCL and CFT solution during degradation was followed by means of the trinitrobenzenesulfonic acid assay. Figure 5 shows plots of the disappearance of amino groups and β-lactam antibiotics at pH 7.0 and 10.0, at 35 °C. Because loss of amine is attributed to both intramolecular reaction (k_b) and the dimerization (k_2), the rate law for the overall loss of α-amino groups can be formulated as:

$$-\frac{dA}{dt}=k_b\frac{K_a}{a_H^+ + K_a}A+\frac{1}{2}k_2A^2 \qquad (3)$$

where A refers to the concentration of α-amino groups of the antibiotic. The values of k_2 for CCL and CFT were obtained by NONLIN by fitting Eqs. 2 and/or 3 to the experimental data in Figs. 4 and/or 5 and are listed in Table II. The solid lines in these figures represent the curves calculated for total disappearance of antibiotics and apparent disappearance of amino groups by incorporating various calculated parameters into Eq. 2 or 3. The good agreements

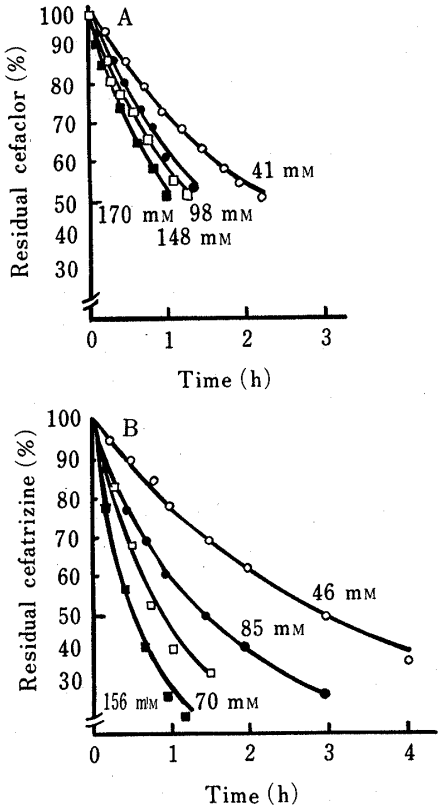

Fig. 4. Degradation of CCL (A, pH 9.0) and CFT (B, pH 8.0 (○, ●) and 10.0 (□, ■)) at Various Initial Concentrations at 35 °C

The pH values were maintained by the use of a pH-stat. The solid lines represent the curves calculated from Eq. 2 with the constants listed in Table II; the points are the experimental values determined by HPLC. The concentrations refer to the initial concentration.

TABLE II. Rate Constants[a] for Degradation of Aminocephalosporins at 35 °C

Cephalosporin	pH	Initial concentration (mM)	k_2 $M^{-1}h^{-1}$
Cefaclor	7.0	25	1.41
		43	2.13
		78	1.55
	8.0	46	2.64
		95	3.45
	8.5	48	4.05
	9.0	41	6.11
		98	4.81
		148	3.73
		170	4.19
	9.5	48	6.53
	10.0	45	2.79
		101	11.0
	10.3	48	26.9
Cefatrizine	8.0	46	2.97
		85	5.80
	10.0	70	10.9
		156	10.6

a) Defined in Eq. 2.

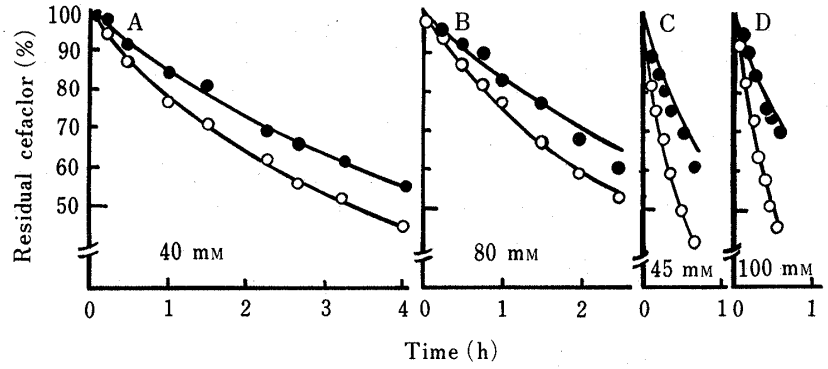

Fig. 5. Time Courses of Disappearance of Primary Amino Groups Determined by Colorimetric Assay and the Total Disappearance of CCL Determined by HPLC Assay during the Degradation of CCL at Various Initial Concentrations at 35 °C

The pH values were maintained by the use of a pH-stat. A, pH 7.0; B, pH 7.0; C, pH 10.0; D, pH 10.0. The solid lines represent the curves calculated from Eqs. 2—3 with the constants listed in Table II; the points are the experimental values. ●, primary amino groups; ○, CCL.

indicate that these equations adequately describe the concentration-dependent disappearance of CCL and CFT. The present results suggest that third-order degradation of both antibiotics is negligible at initial concentrations of below 200 mM and that second-order degradations of

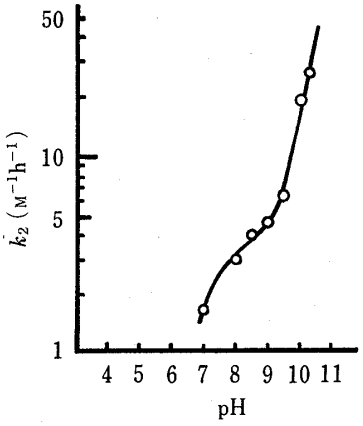

Fig. 6. log k_2–pH Profiles for the Degradation of CCL at 35 °C

The solid line represents the curve calculated from Eq. 4 and the constants given in the text; the points are the experimental values.

each antibiotic occur, yielding the dimer.

The pH dependence of the second-order rate constant, k_2, of CCL degradation at 35 °C and $\mu=0.5$ is shown in Fig. 6. The rate constant in the pH range studied is expressed by Eq. 4.

$$k_2=k_n\frac{K_a}{a_H^+ + K_a}+k_{sb}\frac{K_W}{a_H^+} \quad (4)$$

where k_n represents the specific rate constant for aminolysis and k_{sb} is that for specific base catalysis of the nucleophilic displacement reaction by unprotonated amino groups of other CCL molecules. The kinetic parameters obtained from NONLIN analysis of the data in Fig. 6 are as follows: $k_n=3.51\pm0.46\,M^{-1}h^{-1}$. $k_{sb}=6.34\times10^4\pm0.48\times10^4\,M^{-2}h^{-1}$.

Kinetics of Disappearance of the Antibiotics from the Rat Intestinal Lumen Solution (*In Situ* Recirculating Perfusion Method)

We reported that the disappearance rates of the various amino-β-lactam antibiotics from an *in situ* rat small intestinal perfusing solution followed mixed-type kinetics with saturable and nonsaturable processes.[14,17,18] The kinetic parameters have been determined over a wide range of antibiotic concentrations from 0.02 to 100 mM.[14,17,18] In order to determine the absorption kinetic parameters for CCL and CFT, the second-order degradation as well as first-order degradation should be considered, as discussed above, although the second-order degradation of the other aminocephalosporins in the perfusate during the absorption experiment was negligible.

To clarify the concentration dependency of the absorption of CCL and CFT, a detailed kinetic study was performed. Figure 7 shows the time courses of residual antibiotic at two or three different initial concentrations in the isotonic 0.01 M phosphate buffer used for intestinal perfusion study (the pH was maintained constant at 7.0 by using a pH-stat). By iterative nonlinear least-squares analysis of the data to be fitted to Eq. 2 using the NONLIN computer program, the first-order (k_1) and second-order (k_2) rate constants were found to be $0.0941\pm0.0022\,h^{-1}$ and $4.00\pm0.20\,M^{-1}h^{-1}$ for CCL, and $0.0824\pm0.0048\,h^{-1}$ and $9.35\pm0.57\,M^{-1}h^{-1}$ for CFT, respectively. The curves in Fig. 7 were generated from Eq. 2 by using the calculated kinetic parameters. The good agreements between the predictions and observations indicate that the degradations of CCL and CFT obey Eq. 2 under the conditions of the intestinal perfusion experiment. The value of k_2 in the above buffer solution of pH 7.0 at 37 °C was about twice that in non-buffer solution at pH 7.0 and 35 °C. This may be due to the catalytic effect of phosphate ion on the second-order reaction rather than to the temperature difference.

Since intestinal absorption experiments were carried out in the initial antibiotic concentration range of 0.05—30 mM, the above-mentioned dimerization reaction should occur together with Michaelis–Menten and first-order absorption and first-order degradation,

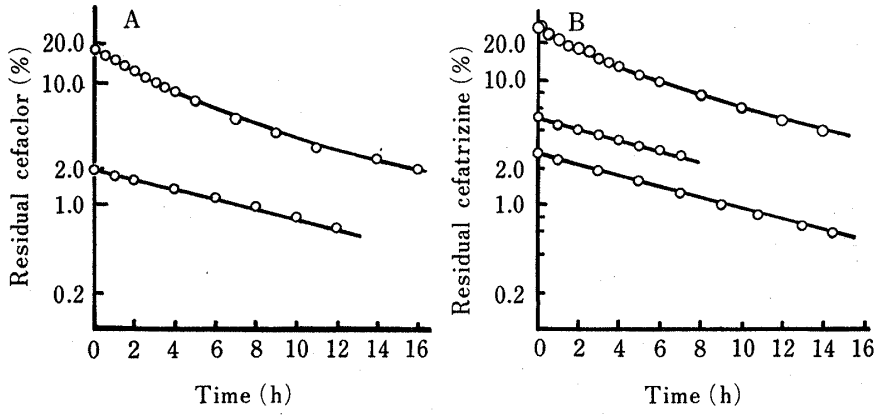

Fig. 7. Degradation of CCL (A) and CFT (B) at Various Initial Concentrations at pH 7.0 and 37 °C

The pH values were maintained by the use of a pH-stat. The reaction was carried out in 0.01 M isotonic phosphate buffer solution, as used for the *in situ* recirculating perfusion experiment. The solid lines represent the curves calculated from Eq. 2 with the constants given in the text; the points are the experimental values.

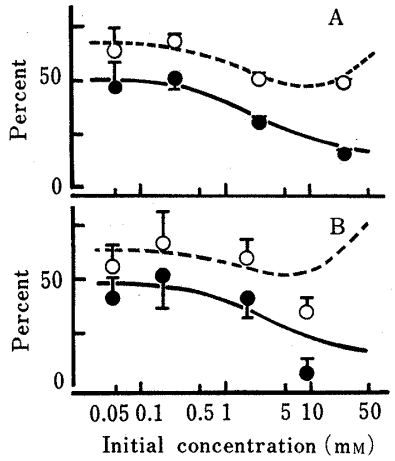

Fig. 8. Experimental Values (Symbols) and Calculated Curves for Total Disappearance (----) and Absorption (——) 2 h after Perfusion of the Indicated Initial Concentrations of CCL and CFT at pH 7.0 in the Rat Small Intestinal Perfusate Using the *in Situ* Recirculating Perfusion Technique

Symbols: percent total disappearance (○) and the percent absorption (●) calculated from Eq. 2 for CCL (A) and CFT (B).

TABLE III. Percentage of Residual Antibiotic After 2 h in the Lumen Solution Perfused through Rat Small Intestine at Various Concentrations

Initial concentration (mM)	Residual, %±S.D.	
	Cefaclor	Cefatrizine
0.048	49.4±10.0 (4)	65.7± 8.0 (4)
0.19	—	47.1±12.1 (4)
0.26	46.1± 3.6 (4)	—
1.9	—	53.0± 7.0 (4)
2.6	59.6± 2.5 (4)	—
9.3	—	72.9± 6.0 (4)
26.0	60.8± 1.5 (3)	—

S.D., standard deviation. The number of experiments is given in parentheses.

as previously determined for other amino-cephalosporins.[14,17,18] Therefore, the time course of the residual concentration of these two antibiotics in the perfusate should be described by Eq. 5 with the inclusion of a second-order degradation rate term:

$$-\frac{dC}{dt}=-\frac{V_{max}\cdot C}{K_m+C}-(k_a+k_1+k_2\cdot C)\cdot C \qquad (5)$$

where C is the drug concentration remaining in the perfusate at time t, V_{max} is the maximum rate of disappearance, K_m is the Michaelis constant, and k_a, k_1 and k_2 are the first-order absorption rate constant, and first-order and second-order degradation rate constants, respectively. The percentages of the residual antibiotic at various initial concentrations after

2 h in the perfusate of pH 7.0 were determined and the results are summarized in Table III. Fig. 8 shows the plots of the logarithm of initial concentration *versus* the percent disappearance of amino-β-lactam antibiotic.

Although the values of percent disappearance of CCL and CFT from the perfusate were found to be almost equal in the concentration range from 0.05 to 30 mM, the absorption (percent) of these antibiotics, after correction for the degradation by using Eq. 2, was almost constant at low initial concentrations (below 0.5 mM) but significantly decreased as the concentration increased. These phenomena indicate that saturable absorption occurs for both antibiotics, as is the case for other amino-β-lactam antibiotics.[14,17,18] Iterative nonlinear least-squares analysis of the data to be fitted to Eq. 5, using the NONLIN computer programs,[15] provided the following parameter values: $K_m = 1.23 \pm 0.41$ mM and $V_{max} = 0.252 \pm 0.056$ mM h^{-1} for CCL, and $K_m = 1.68 \pm 1.28$ mM and $V_{max} = 0.307 \pm 0.15$ mM h^{-1} for CFT (the values of k_1 and k_2 were those determined in the *in vitro* experiment). The good agreement between the predictions and observations indicates that the disappearance of CCL and CFT from the lumen solution follows Eq. 5. The absorption mechanism has been discussed in detail elsewhere.[14]

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References and Notes

1) Part III: A. Tsuji, E. Nakashima, K. Nishide, Y. Deguchi, S. Hamano, and T. Yamana, *Chem. Pharm. Bull.*, **31**, 4057 (1983).
2) A part of this work was presented at the 103rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1983.
3) R. Jones, P. C. Fuchs, C. Thornsberry, A. L. Barry, T. L. Gavan, and E. H. Gerlach, *Am. J. Clin. Pathol.*, **72**, 578 (1979).
4) F. Leitner, R. E. Buck, M. Misiek, T. A. Pursiano, and K. E. Price, *Antimicrob. Agents Chemother.*, **7**, 298 (1975).
5) H. Lode, R. Stahlmann, and P. Koeppe, *Antimicrob. Agents Chemother.*, **16**, 1 (1979).
6) P. G. Welling, S. Dean, A. Selen, M. J. Kendall, and R. Wise, *Int. J. Clin. Pharmacol. Biopharm.*, **17**, 397 (1979).
7) B. R. Meyers, S. Z. Hirschman, G. Wormser, G. Gartenberg, and E. Srulevitch, *J. Clin. Pharmacol.*, **18**, 174 (1978).
8) F. Leitner, D. R. Chisholm, Y. H. Tsai, G. E. Wright, R. G. Deregis, and K. E. Price, *Antimicrob. Agents Chemother.*, **7**, 306 (1975).
9) P. Actor, J. V. Uri, L. Phillips, C. S. Sachs, J. R. Guarini, I. Zajac, D. A. Berges, G. L. Dunn, J. R. E. Hoover, and J. A. Weisbach, *J. Antibiot.*, **28**, 594 (1975).
10) T. Yamana and A. Tsuji, *J. Pharm. Sci.*, **65**, 1563 (1976).
11) *a*) A. Tsuji, E. Nakashima, S. Hamano, and T. Yamana, *J. Pharm. Sci.*, **67**, 1059 (1978); *b*) A. Tsuji, E. Nakashima, and T. Yamana, *ibid.*, **68**, 308 (1979).
12) A. Tsuji, E. Nakashima, Y. Deguchi, K. Nishide, T. Shimizu, S. Horiuchi, K. Ishikawa, and T. Yamana, *J. Pharm. Sci.*, **70**, 1120 (1981).
13) *a*) H. Bundgaard, *Acta Pharm. Suec.*, **13**, 9 (1976); *b*) *Idem, ibid.*, **14**, 47 (1977); *c*) *Idem, ibid.*, **14**, 67 (1977).
14) E. Nakashima, A. Tsuji, S. Kagatani, and T. Yamana, *J. Pharmacobio-Dyn.*, **7**, 452 (1984).
15) C. M. Metzler, "NONLIN, A Computer Program for Parameter Estimation in Nonlinear Situations," Technical Report 7292/69/7292/005, Upjohn Co., Kalamazoo, Mich.
16) H. S. Harned and W. J. Harmer, *J. Am. Chem. Soc.*, **55**, 2194 (1933).
17) *a*) A. Tsuji, E. Nakashima, I. Kagami, N. Honjo, and T. Yamana, *J. Pharm. Pharmacol.*, **29**, 707 (1977); *b*) A. Tsuji, E. Nakashima, I. Kagami, T. Asano, R. Nakashima, and T. Yamana, *ibid.*, **30**, 508 (1978); A. Tsuji, E. Nakashima, T. Asano, R. Nakashima, and T. Yamana, *ibid.*, **31**, 718 (1979).
18) A. Tsuji, E. Nakashima, I. Kagami, and T. Yamana, *J. Pharm. Sci.*, **70**, 772 (1981).