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RESEARCH ARTICLES

Comparative Stability of Cephalosporins in Aqueous Solution: Kinetics and Mechanisms of Degradation

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Abstract □ The acidic, neutral, and alkaline degradations of six therapeutically useful cephalosporins (cephalothin, cephaloridine, cephaloglycin, cephalexin, cephradine, and cefazolin), 7-aminocephalosporanic acid, 7-aminodeacetoxycephalosporanic acid, and some 7-substituted derivatives were followed by high-pressure liquid chromatographic, UV spectrometric, iodometric, and hydroxamic acid assays. The pH–rate profiles were determined at 35° and $\mu = 0.5$. The acidic degradation pathway for the 3-acetoxymethyl and 3-pyridinylmethyl derivatives was the specific hydrogen-ion-catalyzed hydrolysis of the β-lactam bonds. The β-lactam hydrolyses of these antibiotics exhibited half-lives of about 25 hr at pH 1.0 and 35°. The acetyl functions of 3-acetoxymethylcephalosporins were hydrolyzed eight times faster than their β-lactam moieties to yield the corresponding deacetyl intermediates, which were rapidly converted to the lactones. Deacetoxycephalosporins were fairly acid stable; *e.g.*, cephalexin and cephradine were about 25 times more stable than cephalothin, cephaloridine, and cephaloglycin and about 180 times more stable than ampicillin at pH 1.0. In the neutral degradation of 3-acetoxymethyl compounds, the competitive reactions of the direct water attack and intramolecular catalysis by the side-chain amido upon the β-lactams were proposed. The pH–rate profiles near pH 8 for cephaloglycin, cephalexin, and cephradine could be explained by the intramolecular–nucleophilic attack of the side-chain α-amino group upon the β-lactam carbonyls to produce diketopiperazine-type compounds. The reactivity of the cephalosporins in the hydroxide-ion-catalyzed degradation was influenced significantly by the C-3 methylene substituents.

Keyphrases □ Cephalosporins—stability in aqueous solutions, kinetics and mechanisms of degradation, effect of pH □ Stability—cephalosporins in aqueous solutions, kinetics and mechanisms of degradation, effect of pH □ Degradation—cephalosporins in aqueous solutions, kinetics and mechanisms, effect of pH □ Antibiotics—cephalosporins, stability in aqueous solutions, kinetics and mechanisms of degradation, effect of pH □ Structure–activity relationships—various cephalosporins, stability in aqueous solutions, kinetics and mechanisms of degradation, effect of pH

Penicillins and cephalosporins have been known to undergo remarkably facile cleavage of their β-lactam bonds in aqueous solution. Interest in the kinetics of cephalosporin degradation arose from previous studies (1–5) on penicillins which showed that their instability may affect possible chemical reactions involved in penicillin allergy (6) as well as the formulation of pharmaceutical dosage forms (7).

The kinetic approaches to the degradation of cephalosporins have been utilized in a few instances. These studies include the degradation of cephalosporin C in a wide pH range at 25° (8) and of several cephalosporins at pH 10 and 35° (9). However, many questions regarding the acidic, neutral, and alkaline degradations of cephalosporins have not been completely answered. The present studies on therapeutically useful cephalosporins and other semisynthetic compounds (Table I) were begun with the expectation that a systematic and quantitative approach to the degradation would help answer some questions. Some preliminary aspects were reported earlier (10).

EXPERIMENTAL

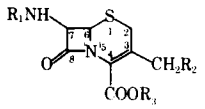
Materials—Cephalothin sodium, cephaloridine, cephaloglycin, deacetylcephalothin sodium[1], and cephradine[2] were used as supplied. Cefazolin sodium was purchased commercially[3]. The substituted phenylcephalosporins (I*a*–I*e*), substituted phenyldeacetoxycephalosporins (II*a*–II*d*), and 7-benzenesulfonamidocephalosporanic acid (III) were prepared as sodium salts by the acylation of 7-aminocephalosporanic acid and 7-aminodeacetoxycephalosporanic acid, in the manner described by Flynn (11). The UV and IR spectra of these synthesized cephalosporins confirmed the presence of an intact β-lactam ring. Deacetylcephalothin lactone was obtained by the method of Neidleman *et al.* (12).

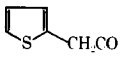
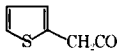
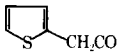
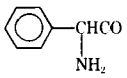
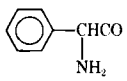
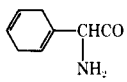
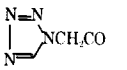
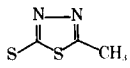
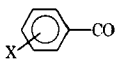
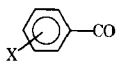
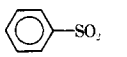
All other chemicals used were the highest commercial grade available.

Kinetic Procedures—An accurately weighed cephalosporin was

[1] Shionogi & Co., Osaka, Japan.
[2] Sankyo Co., Ltd., Tokyo, Japan.
[3] Fujisawa Pharmaceutical Co., Osaka, Japan.

Table I—Properties of Investigated Cephalosporins



Cephalosporin	Substituents R_1	R_2	R_3	Analytical Method	pKa[a]
Cephalothin		$OCOCH_3$	Na	HPLC, UV, iodometry	pKa_1 = 2.22
Deacetylcephalothin		OH	Na	HPLC	—
Cephaloridine			—	HPLC, UV, iodometry	pKa_1 = 1.67
Cephaloglycin		$OCOCH_3$	H	HPLC, UV, iodometry	pKa_1 = 1.91, pKa_2 = 6.90
Cephalexin		H	H	HPLC, UV, iodometry, hydroxamic acid assay	pKa_1 = 2.56, pKa_2 = 6.88
Cephradine		H	H	HPLC, UV, iodometry, hydroxamic acid assay	pKa_1 = 2.53, pKa_2 = 7.30
Cefazolin			Na	HPLC, UV, iodometry	pKa_1 = 2.54, pKa_3 = 1.70[b]
Substituted phenylcephalosporin (I) [*a*, 4-CH_3; *b*, H; *c*, 4-Cl; *d*, 4-NO_2; *e*, 3,5-$(NO_2)_2$]		$OCOCH_3$	Na	UV, iodometry	—
Substituted phenyldeacetoxycephalosporin (II) (*a*, 4-CH_3; *b*, H; *c*, 4-Cl; *d*, 4-NO_2)		H	Na	UV, iodometry, hydroxamic acid assay	—
7-Benzenesulfonamidocephalosporin (III)		$OCOCH_3$	Na	UV, iodometry	—
7-Aminocephalosporanic acid	H	$OCOCH_3$	H	UV, iodometry	pKa_1 = 2.02, pKa_2 = 4.42
7-Aminodeacetoxycephalosporanic acid	H	H	H	UV, iodometry, hydroxamic acid assay	pKa_1 = 2.95, pKa_2 = 4.87

[a] Values determined potentiometrically at 35° and μ = 0.5. These values were computed from the equations of Glasstone and Hammel (20) for the data corresponding to 25–75% of neutralization and were the averages from at least three experiments, never deviating more than ±0.05 pK unit. pKa_1, pKa_2, and pKa_3 refer to 4-carboxylic acid, 7-ammonium or α-ammonium of the 7-side chain, and other dissociable groups, respectively. [b] Kinetically determined.

dissolved in (in experiments at low pH, aliquots from stock solution were diluted with) acid, alkaline, or an appropriate buffer solution preheated at a desired temperature to produce a final cephalosporin concentration of about 5×10^{-5}–5×10^{-3} *M* according to an employed analytical method or the solubility. The fast reacting solutions in acid and alkaline media were studied in 100-ml volumetric flasks. Where the half-life was more than 1 day, the reaction solution was sealed in 5-ml ampuls. The reactions were conducted in a constant-temperature water bath at 35 ± 0.1°. Samples were withdrawn at suitable time intervals, diluted with distilled water, if necessary, and analyzed.

All reactions were run under pseudo-first-order conditions with catalysts in excess and followed first-order kinetics. The kinetic parameters describing degradation of cephalosporins were determined by a combination of appropriate analytical methods. The pseudo-first-order rate constants were obtained by least-squares analysis.

The reaction solution pH was maintained at the desired value by the use of a pH-stat[4] or appropriate buffer systems. The buffer solutions used were hydrochloric acid–potassium chloride, phosphoric acid–monobasic sodium phosphate, acetic acid–sodium acetate, monobasic–dibasic sodium phosphates, sodium bicarbonate–sodium carbonate, boric acid–sodium borate, and dibasic–tribasic sodium phosphates. The ionic strength of each solution was adjusted to 0.5 by addition of potassium chloride. The pH values of the reaction solutions were measured at the experimental temperature initially and at the end of the experiment on a pH meter[5] standardized with standard buffer solutions of pH 4, 7, and 9 at the same temperature. No significant changes in pH were observed.

Deuterium Oxide Solvent Isotope Effects—Solvent isotope effects on the degradation rates of cephalothin were determined at 35° in 98% deuterium oxide by the use of an autotitrator[4] to maintain a constant pD. The pD values were taken as the pH meter[5] reading plus the proper correction at 35° (13).

Analytical Procedures—*Liquid Chromatography*—High-pressure liquid chromatography (HPLC) was used to follow the kinetics of the degradation of several cephalosporins. The liquid chromatograph[6] was equipped with a UV detector[7] set at 254 nm and a stainless steel column, 2 mm i.d. For cephalothin, cephalexin, and cephradine, a strong anion-exchange resin[8] was used as the stationary phase. The mobile phase was aqueous 0.02 *M* NaH_2PO_4 adjusted to pH 8.5 with sodium hydroxide. For cephaloridine, cephaloglycin, and cefazolin, reversed-phase chromatography[9] was used with a mobile phase of 30% methanol–70% 0.05 *M* ammonium carbonate.

Samples were eluted at 100 kg/cm^2 at room temperature for ion-exchange chromatography and at 150 kg/cm^2 at 50° for reversed-phase chromatography, resulting in flow rates of about 1 and 0.3 ml/min, respectively.

Five microliters of degradation solution (5×10^{-4}–5×10^{-3} *M* total antibiotic) at suitable intervals was injected with the flow stopped. Peak heights were measured, and the concentrations were calculated from the calibration curves (obtained daily). The calibration curves

[4] Radiometer pH stat–titrimeter assembly consisting of TTT2 titrator, SBR2 titrigraph, and SBU12b autoburet or TTT1 titrator, PHM 26 pH meter, SBR2 titrigraph, and SBU12b autoburet.
[5] Radiometer PHM 26 pH meter or TTT2 titrator.
[6] Shimadzu-Dupont model 830.
[7] Shimadzu UV 202 recording spectrophotometer.
[8] SAX on Zipax, 1 m, Dupont.
[9] ODS on Sorbax, 0.25 m, Dupont.

of the peak height against the concentration of the antibiotics were satisfactorily linear.

Spectrophotometric Method—Since all intact cephalosporins gave the UV absorption band near 260 nm due to O=CNC=C linkage, the disappearance of this band absorption is characteristic of β-lactam opening, either chemically or enzymatically (14). The UV spectra[10] of the reaction solutions were monitored periodically.

Iodometric Method—It has been known that iodometric analysis is also applicable for the determination of intact cephalosporins as well as penicillins (15, 16). The procedure employed was essentially the same reported for the determination of penicillin G (17).

From the degradation solution (5×10^{-3} *M* total cephalosporin), two samples of 2 ml were pipetted into separate conical flasks. To the first sample, 5 ml of 1 *N* NaOH was added. After standing for 20 min at room temperature, 5 ml of 0.2 *M* phthalate buffer solution (pH 4.5), 5 ml of 1 *N* HCl, and 10 ml of 0.01 *N* iodine were added. The flask was kept for 20 min in darkness at room temperature. Back-titration with 0.01 *N* thiosulfate gave *a* ml of 0.01 *N* iodine consumed by this sample. The second sample was treated with 5 ml of pH 4.5 phthalate buffer solution and 10 ml of 0.01 *N* iodine for 20 min in darkness at room temperature and then back-titrated with 0.01 *N* thiosulfate. The iodine uptake corresponded to *b* ml. The difference, $(a - b)$ ml, represents the amount of intact cephalosporin present in the degradation solution.

Solutions containing known amounts of cephalosporin and its alkaline degradation products were prepared so that the total concentration became 5×10^{-3} *M*. These solutions were analyzed according to the analytical method. The excellent linear relationship between the extent of iodine consumption, $(a - b)$ ml, and the concentration of intact cephalosporin in solutions containing degradation products was obtained, although the iodine consumption depended upon the time treated with 1 *N* NaOH and the pH of the test solution.

Hydroxamic Acid Method—The method for the assay of remaining β-lactam of deactoxycephalosporins was adapted from a literature procedure (18). To 2 ml of reaction solution (about 5×10^{-3} *M* cephalosporin) in a 20-ml volumetric flask were added 2 ml of an aqueous 3 *M* solution of hydroxylamine hydrochloride and 2 ml of aqueous 3.5 *N* NaOH. After the mixture was allowed to stand for 3 min at room temperature, 2 ml of aqueous 3.5 *N* HCl and then 1 ml of an aqueous 35% ferric ammonium sulfate–0.1 *N* H_2SO_4 solution were pipetted into the flask. The solution was diluted to 20 ml with distilled water, and the resultant color was read at 510 nm[11] after exactly 3 min.

pKa Determination—The apparent pKa values of the tested cephalosporins were determined potentiometrically (19) at an ionic strength of 0.5 at 35°. The initial concentrations of cephalosporins were in the range of approximately 2×10^{-3}–2×10^{-2} *M* according to their solubility. For cephaloglycin, cephalexin, cephradine, 7-aminocephalosporanic acid, and 7-aminodeacetoxycephalosporanic acid, the titration with acid was a process of proton association (proton gain) from zwitterion to cation; the titration with base was a process of proton dissociation (proton loss) from zwitterion to anion (Scheme I).

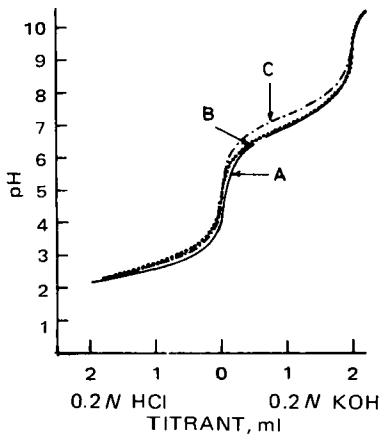
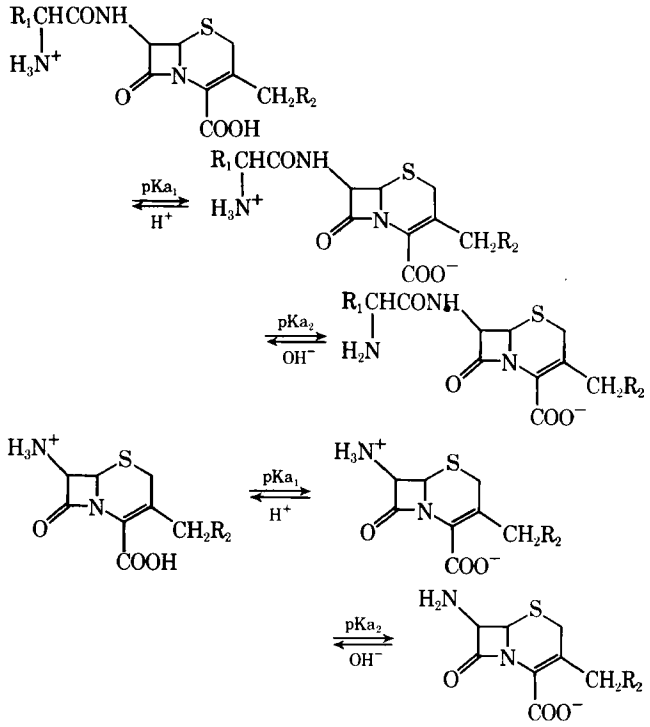


Figure 1—*Titration curves of 1.0 × 10⁻² M amphoteric cephalosporins at 35° and μ = 0.5. Key: A, cephaloglycin; B, cephalexin; and C, cephradine.*

[10] Shimadzu multipurpose recording spectrophotometer model MPS-5000 or Hitachi recording spectrophotometer model 323.

[11] Hitachi spectrophotometer model 101.



Scheme I

Figure 1 shows the typical titration curves obtained from the titration of these amphoteric cephalosporins, cephaloglycin, cephalexin, and cephradine, with hydrochloric acid and potassium hydroxide solutions. The apparent pKa values, computed by the equations of Glasstone and Hammel (20), are listed in Table I, where pKa_1, pKa_2, and pKa_3 refer to the dissociation of the 4-carboxylic acid, the conjugated acid of the 7-amino or 7-(α-amino) group, and another dissociation group, respectively. Each pKa value is an average of the data obtained in most cases to 25–75% of neutralization.

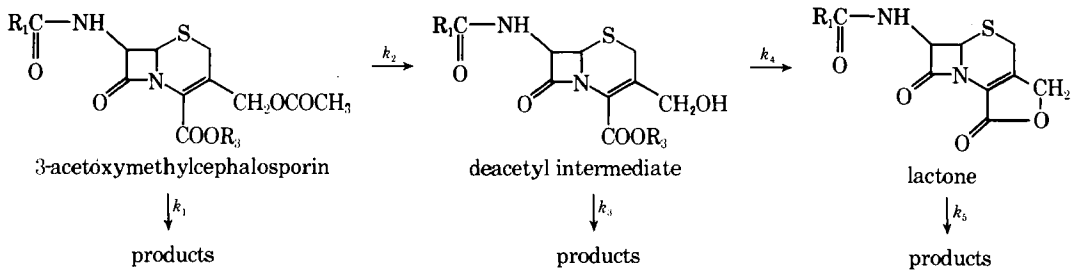
RESULTS

HPLC Studies and Rate Constants—*Degradation Kinetics of 3-Acetoxymethylcephalosporins*—3- Acetoxymethylcephalosporins such as cephalothin and cephaloglycin have another possible site, in addition to the β-lactam moiety, susceptible to degradation. This site is the ester group of the acetoxy side chain. In fact, acidic and alkaline degradations of cephalosporin C (8, 21), cephalothin (12, 22), and cephaloglycin (23) lead to the formation of the corresponding deacetyl derivatives and their lactones as the hydrolytic intermediates. However, except for cephalosporin C (8), no quantification of these hydrolytic reactions has appeared.

The kinetics for cephalothin degradation were followed at various pH values by HPLC. Typical chromatograms of basic (pH 10.00), neutral (pH 5.00), and acidic (pH 2.00) reaction mixtures, sampled at about 50–60% degradation of cephalothin, are shown in Fig. 2. The peaks designated 1, 2, and 3 are the parent compound, deacetylcephalothin, and its lactone, respectively. The peaks designated U were not identified.

Figure 3 shows the typical time course, expressed as mole percent of the initial concentration (C_0) of the substrate, for the disappearance of cephalothin and simultaneously for the formation and disappearance of deacetylcephalothin in the degradation of cephalothin at pH 10.00 and 35°. At relatively low pH, no accumulation of deacetylcephalothin was observed during the reaction due to the rapid lactonization to deacetylcephalothin lactone (Fig. 2).

Scheme II, expressed as a general formula, is valid in the entire pH range, where k_1, k_3, and k_5 correspond to the rates of β-lactam cleavage in the nucleus. In neutral and basic pH regions, the k_4 reaction process is negligible, and the following equations can be ob-



Scheme II

tained for the degradation of cephalothin:

$$[CET]/C_0 = e^{-(k_1+k_2)t} \quad \text{(Eq. 1)}$$

$$[DAT]/C_0 = \frac{k_2}{(k_1 + k_2) - k_3}[e^{-k_3 t} - e^{-(k_1+k_2)t}] \quad \text{(Eq. 2)}$$

where [CET] and [DAT] represent the concentrations at time t of cephalothin and deacetylcephalothin, respectively. The values $k_{app} = k_1 + k_2$ were obtained from the slopes of the apparent first-order plots of the disappearance of cephalothin (Fig. 3). The rate constants k_2 and k_3 were determined from the analog computer fitting of the $[DAT]/C_0$ data.

Analysis of the data at pH 10.00 gave $k_1 = 0.217\ hr^{-1}$, $k_2 = 0.346\ hr^{-1}$, and $k_3 = 0.210\ hr^{-1}$. Treatment of data at any other pH verified the general relationships $k_1/k_2 = 1.1$ and $k_2/k_1 = 1.6$, which are similar to those reported for cephalosporin C ($k_1 = k_3$ and $k_2/k_1 = 4$) (8). The lactonization of deacetylcephalothin became significant below pH 4. For example, at pH 2.00 and 35°, the value of k_4 was $0.708\ hr^{-1}$. Because of this rapid lactonization rate, the extremely low concentration of deacetylcephalothin and the appreciable production of deacetylcephalothin lactone were observed during the degradation of cephalothin (Fig. 2).

The degradation pathway for cephaloglycin was confirmed by HPLC analysis to be as shown in Scheme II.

Degradation Kinetics of Deacetoxycephalosporins—It is expected that the degradation of deacetoxycephalosporins such as cephalexin and cephradine occurs only at the β-lactam moiety.

The chromatographic changes of the degraded solutions of cephalexin and cephradine exhibited the same pattern for all solutions. Figure 4 illustrates the data in 0.3 *M* borate buffer at pH 8.02 and 35°. The semilogarithmic plots of the data obtained at various pH values were reasonably linear (Fig. 5), indicating that the degradation of cephalexin and cephradine follows simple first-order kinetics. The pseudo-first-order rate constants, k_{obs}, were obtained from the slopes.

Determination of Rate Constants by UV Spectroscopy, Iodometry, and Hydroxamic Acid Assay—During the kinetic runs, all cephalosporins studied lost their characteristic UV absorbance at about 260–270 nm. Typical spectral changes as a function of time are illustrated for the degradation of cephalothin (at pH 10.00, maintained by the use of a pH-stat), cephaloglycin (at pH 8.00, maintained by the use of a pH-stat), and cefazolin (in 0.24 *M* acetate buffer of pH 3.53) at 35° and $\mu = 0.5$ in Figs. 6, 7, and 8, respectively.

Plots of $(A_t - A_\infty)$ at λ_{max} *versus* time t were reasonably linear according to the first-order expression:

$$\ln (A_t - A_\infty) = \ln (A_0 - A_\infty) - k_{obs}t \quad \text{(Eq. 3)}$$

where A_t, A_0, and A_∞ are the absorbances at time t, zero, and infinity, respectively. All reactions were followed for at least one or two half-lives, and the values of the pseudo-first-order rate constant, k_{obs}, were obtained by plots of Eq. 3 or by the method of Guggenheim (24). Several typical first-order plots for the degradation of cephalothin, cephaloglycin, and cefazolin according to Eq. 3 are shown in Fig. 9.

For 3-acetoxymethylcephalosporins, the obtained k_{obs} values were approximately equal to the k_1 values (Scheme II) determined by HPLC. Since the absorptivity of the corresponding deacetyl intermediates with an intact nucleus did not differ appreciably from the parent compounds (12, 23, 25, 26), this agreement suggests that the rates for β-lactam cleavage of these deacetyl intermediates are almost the same as those for the parent acetoxy derivatives. This speculation was shown to be reasonable for cephalothin and for cephalosporin C (8).

The pseudo-first-order rate constants, k_{obs}, were also determined by the iodometric titration method and/or hydroxamic acid assay. Typical first-order plots determined iodometrically for the degra-

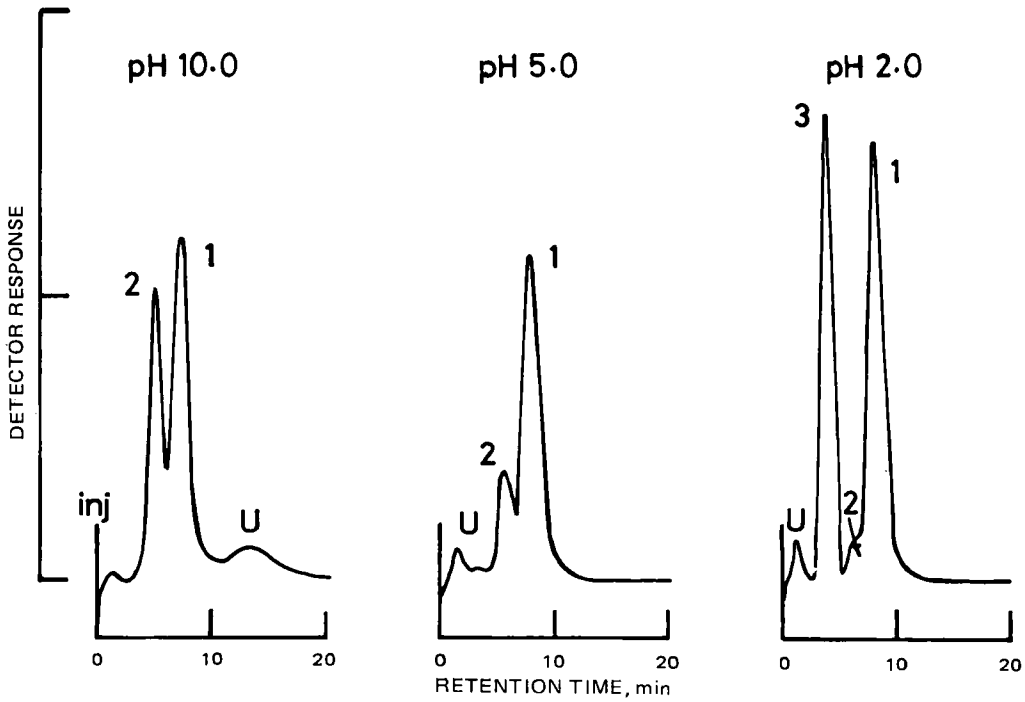


Figure 2—*High-pressure liquid chromatograms of 50–60% degraded cephalothin at 35° and several pH values. Key: 1, cephalothin; 2, deacetylcephalothin; 3, deacetylcephalothin lactone; and U, unknown peak.*

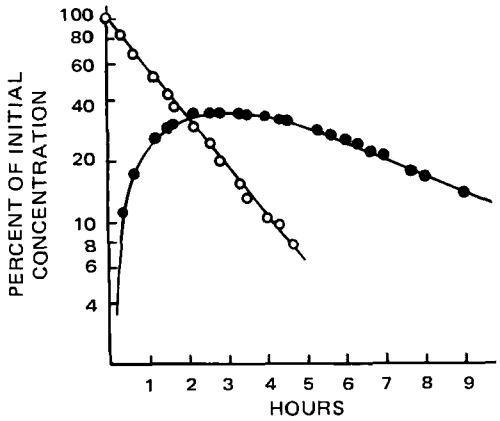


Figure 3—*Time courses for cephalothin (○) and deacetylcephalothin (●) during the degradation of cephalothin at pH 10.00 (pH was maintained by the use of a pH-stat), 35°, and μ = 0.5. The lines were generated using an analog computer programmed according to Scheme II (k_1 = 0.217/hr, k_2 = 0.346/hr, and k_3 = 0.210/hr); the points are experimental values measured by HPLC.*

dation of cephalothin at pH 10.00 and 5.00 and at several temperatures are shown in Fig. 10.

The rate constants determined by the different analytical methods at the same pH and 35° are listed in Table II. The values evaluated from UV spectrophotometric analysis are in relatively good agreement with those determined by Indelicato *et al.* (9) under the same conditions. Although agreement among the rate constants determined by the different analytical methods was not always obtained, the differences did not prevent the determinations of the specific rate constants for the interpretation of their pH–rate dependencies (Figs. 11–15) and for a discussion of the relative stabilities. From the present results and previous discussions (8, 9, 16) of analytical methods for the cephalosporin stability tests, it appears that UV spectrophotometric and chemical methods such as iodometry and colorimetry are acceptable for good estimations of the β-lactam opening rate of the starting cephalosporins. However, HPLC seems to be the most desirable analytical method.

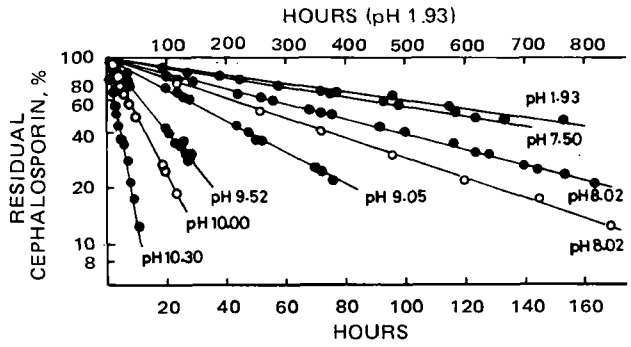


Figure 5—*Apparent first-order plots followed by HPLC for the degradation of cephalexin (○) and cephradine (●) at various pH values, 35°, and μ = 0.5. Key: pH 1.93, 0.06 M phosphate buffer; pH 7.50, pH-stat; pH 8.02, 0.3 M borate buffer; pH 9.05, 0.18 M borate buffer; pH 9.52, 0.08 M carbonate buffer; pH 10.00, pH-stat; and pH 10.30, pH-stat.*

Dependence of Rate on Temperature—Arrhenius plots of the observed first-order rate constants, k_{obs}, for the degradation of cephalothin at two different pH values are given in Fig. 16. The energy of activation, E_a, calculated from the slope of the line was 22.6 kcal/mole at pH 5.00. The calculated apparent energy of activation at pH 10.00 was 28.5 kcal/mole. However, the heat of ionization of water was included in this value. By employing the value of 13.1 kcal/mole as the heat of water (27), the net energy of activation of the degradation of cephalothin in the basic solution, 15.4 kcal/mole, was obtained.

Log k_{pH}–pH Profile—At constant pH and in the presence of excess buffer, the degradation of all types of cephalosporins at low concentration (below 5×10^{-3} *M*) followed pseudo-first-order kinetics and gave rate constants k_{obs} and k_1 for their β-lactam cleavage. General acids and bases served as effective catalysts for the degradation of cephalosporins, although these catalytic effects were not always observed. Typical plots for borate buffer catalytic effects on the rates of cephradine are shown in Fig. 11, giving a reasonable straight line at constant pH. Extrapolation of such plots to zero buffer concentration provides, as intercepts, the values of the pseudo-first-order rate constants, k_{pH}, corresponding to the nonbuffer-cat-

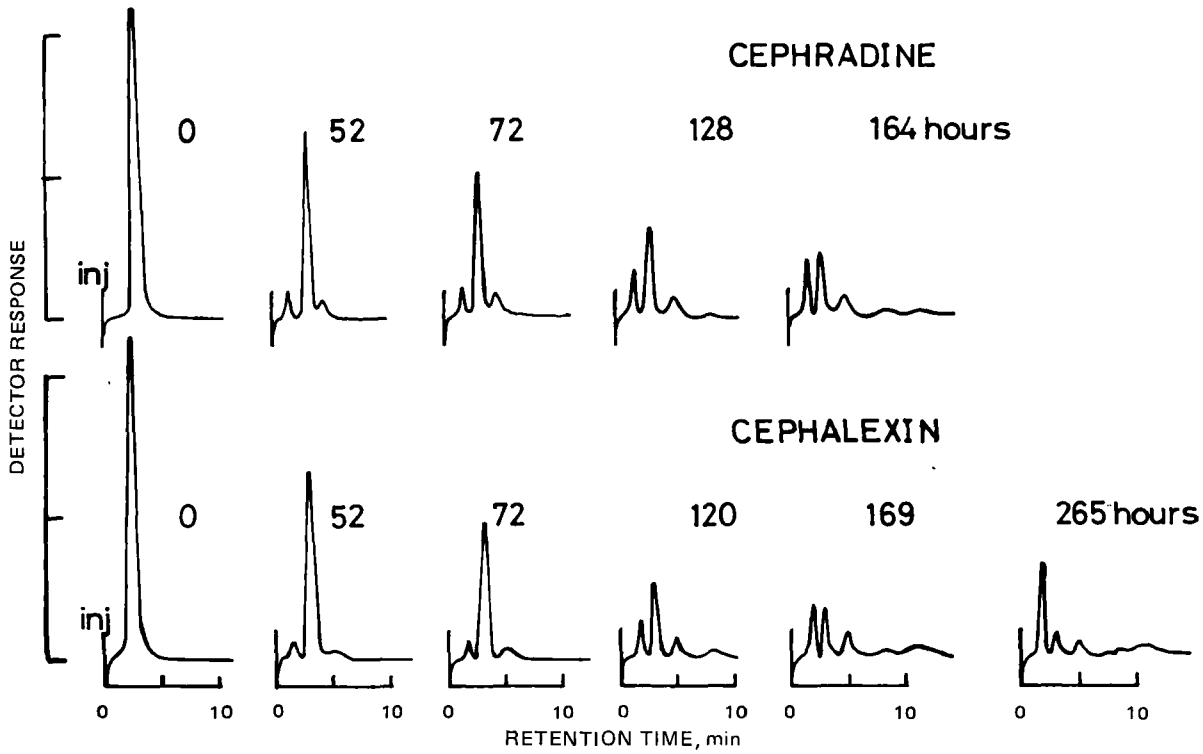


Figure 4—*HPLC changes for the degradations of cephalexin and cephradine in 0.3 M borate buffer (pH 8.02) at 35° and μ = 0.5 with time in hours (listed on graph).*

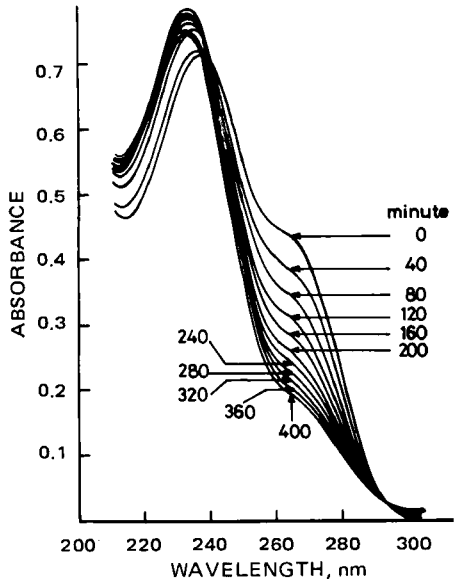


Figure 6—*Spectral changes for the degradation of 5.0 × 10^{-5} M cephalothin at pH 10.00, 35°, and μ = 0.5 with time in minutes (listed on graph). The reaction pH was maintained by the use of a pH-stat.*

alyzed degradation of the cephalosporin nucleus. The k_{pH} values were also determined by the use of a pH-stat.

Figure 12 shows log k_{pH} *versus* pH for the degradation of cephalothin and cephaloridine at 35° and μ = 0.5. Figure 13 shows the log k_{pH}–pH profiles for I, II, and III. There were three important pH regions: one where a hydrogen-ion-catalyzed reaction took place, a pH-independent region, and a region where the reaction was hydroxide ion catalyzed. The degradation rates of these cephalosporins were not influenced by the dissociation of 4-carboxylic acid groups (pKa = ~2–3; Table I), whereas all types of penicillins are apparently influenced by the dissociation of the 3-carboxylic acid group (pKa = ~2.7), as illustrated for penicillin G (28) in Fig. 12. These observations imply that the degradation rates of both undissociated and dissociated cephalosporins are of almost the same magnitude. Therefore, the

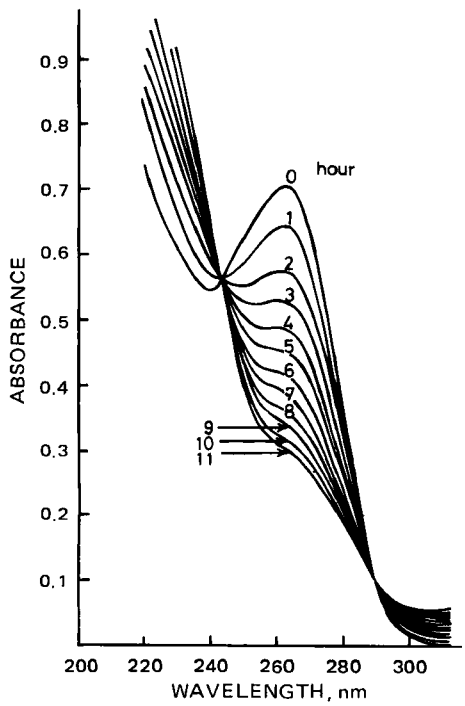


Figure 7—*Spectral changes for the degradation of 1.0 × 10^{-4} M cephaloglycin at pH 8.00, 35°, and μ = 0.5 with time in hours (listed on graph). The reaction pH was maintained by a pH-stat.*

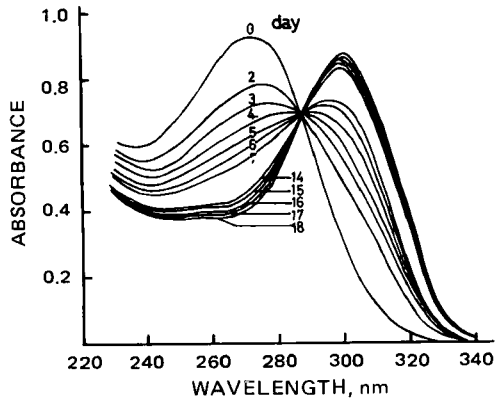


Figure 8—*Spectral changes for the degradation of 5.0 × 10^{-5} M cefazolin in 0.24 M acetate buffer of pH 3.53 at 35° and μ = 0.5 with time in days (listed on graph).*

apparent first-order rate constant at a given pH can be expressed as the following rate law:

$$k_{pH} = k_H a_H + k_0 + k_{OH}(K_w/a_H) \qquad \text{(Eq. 4)}$$

where k_H and k_{OH} are the second-order rate constants for the hydrogen-ion-catalyzed degradation and hydroxide-ion-catalyzed degradation, respectively; k_0 is the first-order rate constant for spontaneous or water-catalyzed degradation; and a_H is the activity of hydrogen ion as measured by the glass electrode. The value for the autoprotolysis constant of water, K_w, at 35° is 2.09 × 10^{-14} (27). These rate constants that produced the best fits of the observed rate–pH profiles are given in Table III. Figure 12 shows the plateau rate of cephalothin at 35° in deuterium oxide; this rate ($k_0^{D_2O}$ = 1.17 × 10^{-2} hr^{-1}) was almost the same as that in water ($k_0^{H_2O}$ = 1.09 × 10^{-2} hr^{-1}). For comparison, the corresponding rate constants recalculated from the literature (references are cited in Ref. 1) and determined (1) in this laboratory for several penicillins are recorded in Table III.

Figure 12 also includes the log k_{pH}–pH profile for the degradation of cefazolin. The profile bent over at pH values lower than 2 and indicated that a dissociation constant, K_{a_3}, affected the degradation rate, most probably in the thiadiazole moiety. The log k_{pH}–pH profile for cefazolin was fitted by:

$$k_{pH} = k_a\left(\frac{a_H}{K_{a_3} + a_H}\right) + k_0 + k_{OH}(K_w/a_H) \qquad \text{(Eq. 5)}$$

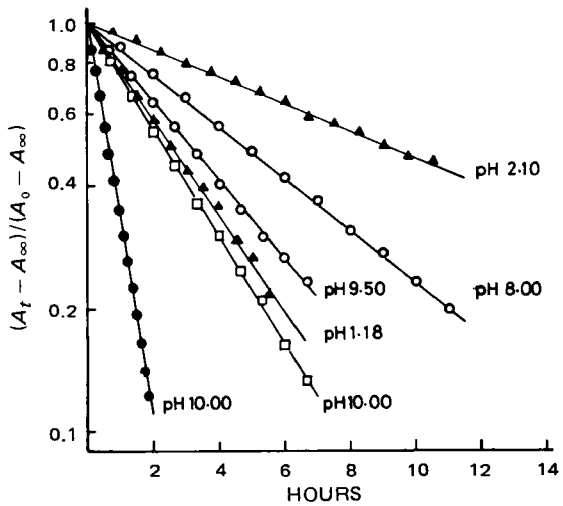


Figure 9—*Apparent first-order plots followed spectrophotometrically for the degradation of cephalothin (□), cephaloridine (●), cephaloglycin (○), and cefazolin (▲) at various pH values, 35°, and μ = 0.5. The reaction pH was maintained by a pH-stat. The absorbance values, A_t, were measured at 265 nm for cephalothin, 260 nm for cephaloridine, 263 nm for cephaloglycin, and 273 nm for cefazolin; A_0 and A_∞ are the absorbances at zero time and at infinite time, respectively.*

Table II—Comparison of the Rate Constants[a] Determined by the Different Analytical Methods at the Same pH Values and 35°

Cephalosporin	pH[b]	10^2k, hr^{-1} HPLC	UV[c]	Iodometry	Hydroxamic Acid[d]	Reported Value[e]
Cephalothin	5.00	1.20[f]	—	1.14	—	—
	9.84	20.2[f]	21.8	—	—	—
	10.00	21.7[f]	30.0	13.9	—	34.9
	10.25	49.1[f]	40.7	21.2	—	—
Cephaloridine	10.00	106	108	103	—	67.7
Cephaloglycin	8.00	15.4	12.0	14.6	—	—
	9.50	—	21.5	18.4	—	—
	10.00	71.8	69.0	—	—	60.1
Cephalexin	8.00	—	0.80	0.92	—	—
	10.00	7.14	14.5	8.39	11.0	3.78[g]
Cephradine	10.00	—	6.70	7.00	7.08	—
	11.00	100	116	76.2	73.7	—
Cefazolin	1.18	16.4	26.8	—	—	—
	6.00	—	0.20	0.24	—	—
	9.70	—	13.7	13.5	—	—
	10.00	24.9	21.7	21.2	—	—

[a] All rate constants were obtained by least-squares analysis. [b] Reaction pH was maintained by the use of a pH-stat. [c] Rate constants were obtained from Eq. 3 or the Guggenheim method (24). [d] Rate constants were obtained from the Guggenheim method (24). [e] Rate constants were determined UV spectrophotometrically by Indelicato *et al.* (9). [f] k_1 values in Scheme II. [g] Rate constants were determined by the pH-stat alkalimetric titration method.

The values of the various rate constants and the kinetically determined pKa_3 value for cefazolin are given in Tables III and I, respectively. As compared with cephalothin and cephaloridine, cefazolin was two to five times more stable in neutral pH solutions and about 10 times more unstable at pH 1.0. The instability of cefazolin itself in acidic media may result in the β-lactam cleavage and/or the thiadiazole elimination, since the acidic degradation rates of this antibiotic followed the apparent rates of the loss of the starting material by UV spectroscopy and HPLC.

The rate–pH profiles for cephaloglycin, cephalexin, and cephradine, which contain α-amino groups in their side chains, have interesting shapes (Fig. 14). A linear increase of the log k_{pH} for cephaloglycin with decreasing pH below 2, to give a negative unity slope, indicated specific hydrogen-ion catalysis, and the k_{pH} became pH independent between pH 2 and 5. On the other hand, rate constants for cephalexin and cephradine were independent of pH below 5, indicating that the hydrogen-ion-catalyzed degradation was negligible compared with spontaneous degradation. The k_{pH} values for these cephalosporins increased with pH values above 5. The fact that there were inflections in the log k_{pH}–pH plots near pH 7 indicates that the dissociation equilibria of the α-amino groups (pKa_2 = 6.90, 6.88, and 7.30 for cephaloglycin, cephalexin, and cephradine, respectively) (Table I) influenced the degradation rates. Above pH 10, the positive unity slopes of their log k_{pH}–pH profiles reflects the specific hydroxide-ion-catalyzed degradation. Accordingly, the apparent first-order rate constants for the degradation of cephaloglycin, cephalexin, and cephradine can be formulated as:

$$k_{pH} = k_H a_H + k_0 + k_b \left(\frac{K_{a_2}}{K_{a_2} + a_H}\right) + k_{OH}(K_w/a_H) \quad \text{(Eq. 6)}$$

where k_b represents the spontaneous degradation of the anionic species of cephaloglycin, cephalexin, and cephradine. The lines of Fig. 14 were generated from Eq. 6 by the use of their rate constants listed in Table III and the dissociation constants, pKa_2, in Table I. Figure 14 also shows the log k_{pH}–pH profile at 35° and μ = 0.5 for ampicillin (29), which contains the α-amino group in the penicillin side chain; there was no facilitation of the degradation rate near the pKa of the α-ammonium group (pKa_2 = 7.05) (29).

The pH–rate profiles obtained for 7-aminocephalosporanic acid and 7-aminodeacetoxycephalosporanic acid are shown in Fig. 15, indicating that specific hydrogen-ion-catalyzed, spontaneous, and specific hydroxide-ion-catalyzed degradations were proceeding, but the spontaneous reaction rates could be influenced by the dissociation of 7-ammonium groups. The rate enhancement of water attack on the β-lactam moiety may be attributed to the inductive effect of the protonated amino group at the α-position, as was observed (1) in the degradation of 6-aminopenicillanic acid (Fig. 15). The rate constants for 7-aminodeacetoxycephalosporanic acid were independent of pH below 3, where the compound was completely protonated, implying that the hydrogen-ion-catalyzed reaction of its protonated form is

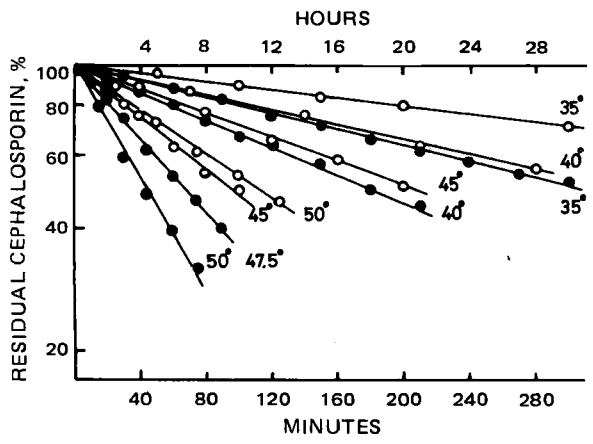


Figure 10—*Apparent first-order plots followed iodometrically for the degradation of cephalothin at various temperatures, pH 5.00 (○, upper scale), pH 10.00 (●, lower scale), and μ = 0.5. The reaction pH was maintained by a pH-stat.*

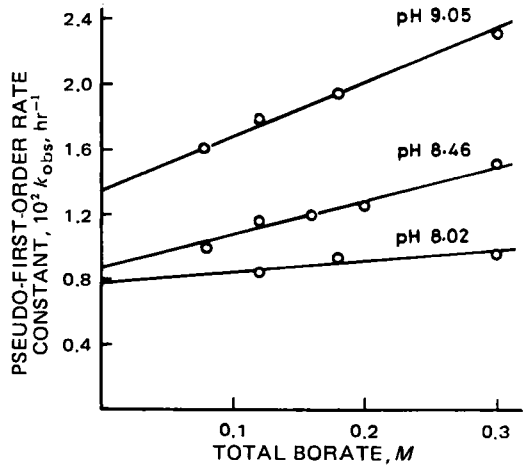


Figure 11—*Plots of the pseudo-first-order rate constants,* k_{obs}, *versus the total buffer concentration for the degradation of cephradine in borate buffer at 35° and μ = 0.5. The rate constants were determined from the HPLC analysis.*

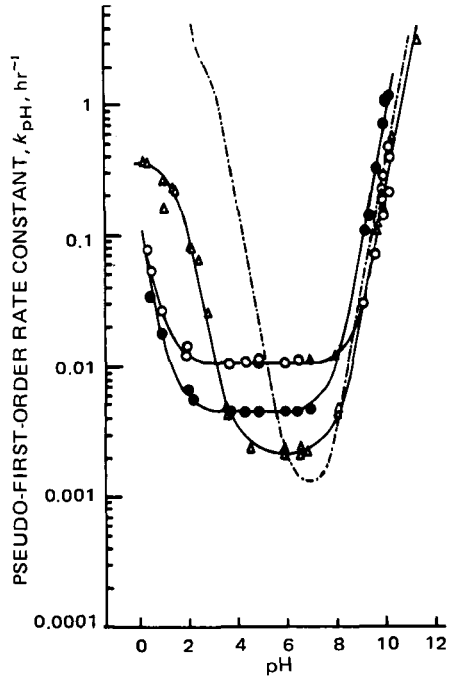


Figure 12—*Log* k_{pH}*–pH profiles for the degradation of cephalothin (○, in* H_2O*; ▲, in* D_2O*), cephaloridine (●), and cefazolin (△) at 35° and* $\mu = 0.5$*. The lines represent the curves calculated from Eq. 4 or 5 and the constants in Tables I and III; the points are the experimental values. The dashed-dotted line refers to the pH–rate profile for penicillin G at 35° and* $\mu = 0.5$ *(28).*

negligible. The reactions are kinetically described by:

$$k_{pH} = k_H a_H + k_c\left(\frac{a_H}{K_{a_2} + a_H}\right) + k_d\left(\frac{K_{a_2}}{K_{a_2} + a_H}\right) + k_{OH}(K_w/a_H) \quad \text{(Eq. 7)}$$

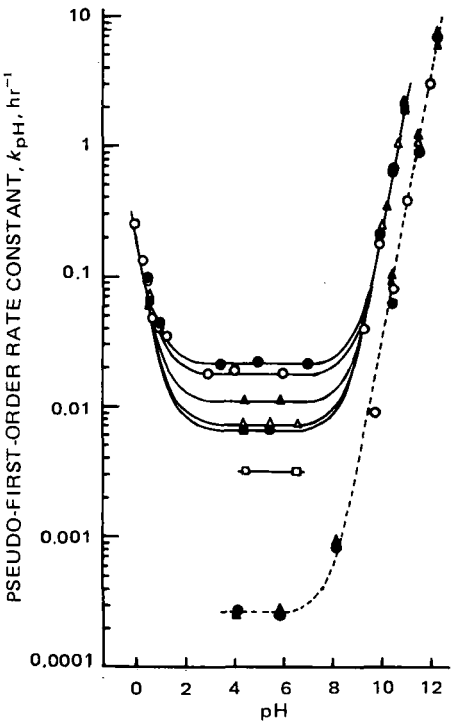


Figure 13—*Log* k_{pH}*–pH profiles for the degradation of I, II, and III at 35° and* $\mu = 0.5$*. The lines represent the curves calculated from Eq. 4 and the constants in Table III; the points are the experimental values. Key: —●—, Ia; —○—, Ib; —▲—, Ic; —△—, Id; —■—, Ie; - - -●- - -, IIa; - - -○- - -, IIb; - - -▲- - -, IIc; - - -△- - -, IId; and —□—, III.*

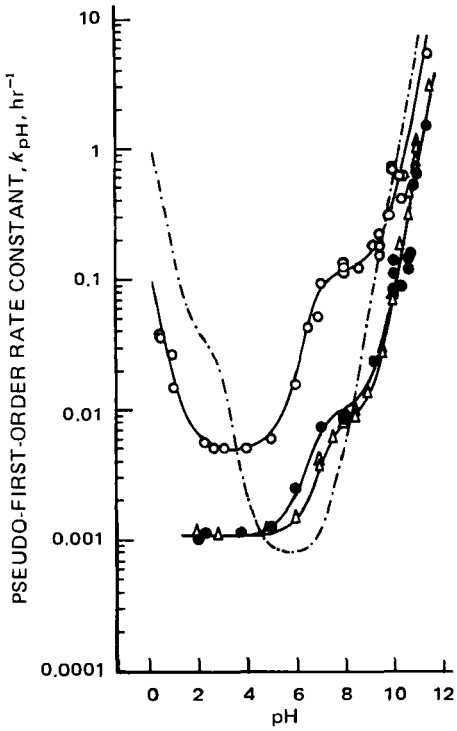


Figure 14—*Log* k_{pH}*–pH profiles for the degradation of cephaloglycin (○), cephalexin (●), and cephradine (△) at 35° and* $\mu = 0.5$*. The lines represent the curves calculated from Eq. 6 and the constants in Tables I and III; the points are the experimental values. The dashed-dotted line refers to the pH–rate profile for ampicillin at 35° and* $\mu = 0.5$ *(29).*

The various rate constants are given in Table III, including those for 6-aminopenicillanic acid (1).

DISCUSSION

Acidic Degradation Mechanism and Relative Stability of Cephalosporins and Penicillins—The acidic degradation rates of penicillins are known to depend on the side-chain structure (7, 30). Penicillin G is extremely acid unstable with a half-life of only 0.7 min at pH 1 and 35° (28); ampicillin is the most acid stable (29), with the difference in acid stability between the two penicillins being about 300-fold. The side-chain reactivity of the penicillin molecule is attributed to the rearrangement initiated by the attack of the side-chain amido carbonyl upon the β-lactam to produce the corresponding penicillenic and penillic acids (6).

In contrast to the penicillin reactivity, 3-acetoxymethylcephalosporins (R_2 = $OCOCH_3$; cephalothin and cephaloglycin) undergo degradation of their β-lactams at almost the same rate regardless of the side-chain structure, R_1. The fact that there was no significant contribution of the side chain to the acidic degradation rates indicates that the reactions exclusively proceed *via* a simple and specific hydrogen-ion-catalyzed hydrolysis of the β-lactam bond rather than the acid-catalyzed intramolecular attack of the side-chain amido on the β-lactam moiety.

β-Lactams of these cephalosporins exhibit half-lives of about 25 hr at pH 1.0 and 35° and are about seven times more stable than ampicillin under the same conditions. However, their acetyl functions are hydrolyzed eight times faster than the β-lactam moiety to yield the corresponding deacetyl compounds, which are easily converted to the lactones (Scheme II). Calculated half-lives for the loss of the original 3-acetoxymethylcephalosporins are about 7 hr at pH 1.0 and 35°.

The acidic degradations of cephalexin, cephradine, II, and 7-aminodeacetoxycephalosporanic acid were pH independent even in strong acid solution. These deacetoxycephalosporins were fairly acid stable; *e.g.*, cephalexin and cephradine were about 25 times more stable than the other cephalosporins and approximately 180 times more stable than ampicillin at pH 1.0.

Competitive Degradations of Direct Water Attack and Intramolecular Catalysis by Side-Chain Amido Group—A com-

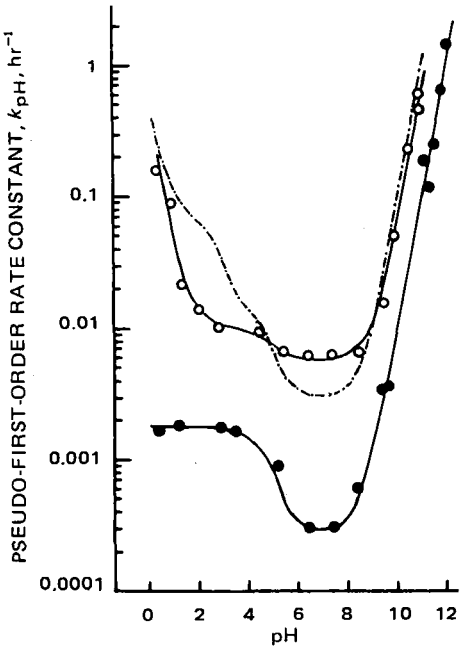


Figure 15—*Log* k_{pH}*–pH profiles for the degradation of 7-aminocephalosporanic acid (○) and 7-aminodeacetoxycephalosporanic acid (●) at 35° and* $\mu = 0.5$*. The lines represent the curves calculated from Eq. 7 and the constants in Tables I and III; the points are the experimental values. The dashed-dotted line refers to the pH–rate profile for 6-aminopenicillanic acid at 35° and* $\mu = 0.5$ *(1).*

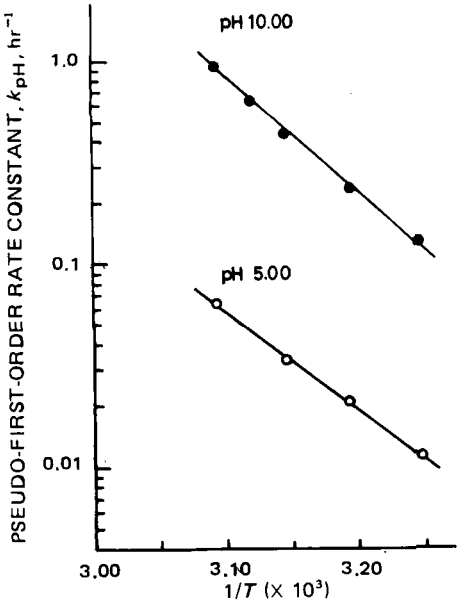


Figure 16—*Arrhenius plots for the apparent first-order rate constants,* k_{pH}*, for cephalothin at pH 5.00 and 10.00 and* $\mu = 0.5$*.*

parison of the degradation rates of several cephalosporins in neutral and basic regions illustrates an apparent dependence on the nature of both substituents, R_1 and R_2.

The most striking feature of the pH–rate profiles shown in Figs. 12 and 13 is the large plateau, extending from approximately pH 3 to 8, with the rates of the degradations being pH independent in this range. The chromatography of the neutral degradation solution of cephalothin, although resulting in a somewhat different elution pattern from that obtained in the alkaline degradation, showed the absence of products with appreciable absorptivity at 254 nm except for small amounts of deacetylcephalothin (Fig. 2). These chromatographic observations, together with agreement of the degradation rates measured by HPLC, UV spectroscopy, and the iodometric method, imply that the principal degradation in the flat region for cephalothin and probably for other 3-acetoxymethylcephalosporins should be the β-lactam cleavage of their nucleus.

The β-lactam opening rate of cephalothin ($k_0 = 1.09 \times 10^{-2}$ hr^{-1}) was about 10 times faster than the spontaneous water-catalyzed degradation rates of penicillins (k_0 = about $1–2 \times 10^{-3}$/hr, regardless of their side-chain structure) (Table III). The enhanced plateau rate is interpretable in terms of the competitive reaction of the hydrolytic cleavage of the β-lactam bond by the direct attack of water (Scheme III) and the degradation caused by intramolecular participation of the neighboring side-chain amido group (Scheme IV) as suggested previously (10).

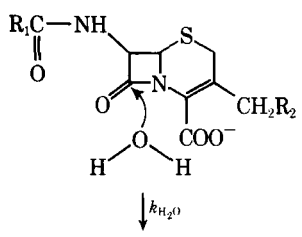
If the neutral degradation of cephalosporins involves such an intramolecular reaction (Scheme IV), the introduction of an electron-donating substituent into the 7-acylamino moiety should increase the susceptibility of the β-lactam bond to nucleophilic attack of the side-chain amido while an electron-withdrawing substituent should decrease it. The result obtained with a series of substituted phenylcephalosporins (Ia–Ie) showed that the order of decreasing k_0 was the same as the predicted order, the variation of k_0 being about fourfold. The benzenesulfonyl compound (III) prevented the plateau rate as expected and exhibited the minimum k_0 value of 3.3×10^{-3}/hr, which corresponds to a k_{H_2O} value of the direct water reaction (Scheme III) for 3-acetoxymethylcephalosporins.

The ρ value of −0.7 was obtained from the Hammett plot of k_{intra} ($= k_0 - k_{H_2O}$) for I. The negative value is consistent with the intramolecular participation of the side-chain amido group in spontaneous rearrangement of the substituted phenylcephalosporins (k_0 value in Table III). The deuterium solvent isotope effect for cephalothin ($k_0^{H_2O}/k_0^{D_2O} = 0.93$) strongly indicates no significant participation of water in the transition state, which would rule out the large contribution of the direct water reaction to the total plateau rate.

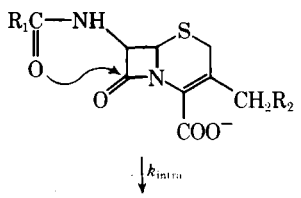
The plateau rate of cephalosporin C ($k_0 = 5.0 \times 10^{-3}$/hr at 25°) (8) was slightly faster than that of cephalothin ($k_0 = 4.0 \times 10^{-3}$/hr at 25°), suggesting that the intramolecular degradation was also proceeding. Cephaloridine, in spite of the same 7-acylamido side chain as cephalothin, showed lower reactivity than cephalothin, probably due to the block of the nucleophilic attack to β-lactam by the 3-bulkyl substituent as suggested by the molecular model.

This report is the first to suggest the occurrence of the side-chain amido group participation in the spontaneous degradation of cephalosporin β-lactam. The spontaneous degradation by the side-chain amido attack on the β-lactam of undissociated penicillins (1, 17) was confirmed to produce the corresponding penicillenic acid (6). While such a penicillenate analog was not confirmed for cephalosporins, considerable kinetic evidence has been presented in support of Scheme IV producing, most probably, the penicillenate analog intermediate or an unstable compound with the oxazolone structure as one spontaneous degradation product.

However, Abraham (31) stated that stable compounds analogous to penicillenate do not appear to be formed in the cephalosporin



Scheme III



Scheme IV

Table III—Various Rate Constants for the Degradation of Cephalosporins and Penicillins at 35° and μ = 0.5

β-Lactam Antibiotic	k_H, M^{-1} hr^{-1}	10^3k_0, hr^{-1}	$10^{-2}k_{OH}$, M^{-1}hr^{-1}	10^2k_a[a], hr^{-1}	10^2k_b[b], hr^{-1}	10^3k_c[c], hr^{-1}	10^3k_d[c], hr^{-1}
Cephalosporins							
Cephalothin	0.172	10.9	10.6	—	—	—	—
Cephaloridine	0.134	4.40	38.8	—	—	—	—
Cephaloglycin	0.148	5.00	13.1	—	13.5	—	—
Cephalexin	—[d]	1.15	2.64	—	1.01	—	—
Cephradine	—[d]	1.10	3.98	—	0.740	—	—
Cefazolin	—[d]	2.15	11.4	37.0	—	—	—
Ia	0.238	21.0	9.08	—	—	—	—
Ib	0.206	18.7	8.56	—	—	—	—
Ic	0.186	11.4	8.45	—	—	—	—
Id	0.265	7.06	9.55	—	—	—	—
Ie	0.176	6.56	8.85	—	—	—	—
IIa	—[e]	0.270	1.43	—	—	—	—
IIb	—[e]	—[e]	1.33	—	—	—	—
IIc	—[e]	0.270	1.56	—	—	—	—
IId	—[e]	—[e]	1.52	—	—	—	—
III	—[e]	3.30	—[e]	—	—	—	—
7-Aminocephalosporanic acid	0.579	—	2.32	—	—	10.4	6.00
7-Aminodeacetoxycephalosporanic acid	—[d]	—	0.36	—	—	1.84	0.28
Penicillins[f]							
Penicillin G	601	0.90	11.9	341	—	—	—
Carbenicillin	52.2	2.04	12.1	183	—	—	—
Cloxacillin	35.6	0.94	13.4	21.0	—	—	—
Propicillin	30.7	0.89	17.3	13.3	—	—	—
Cyclacillin	4.61	2.49	11.0	4.33	—	—	—
Ampicillin	1.82	0.75	25.7	5.56	—	—	—
Substituted phenylpenicillins							
4-CH_3	—[e]	—[e]	17.0	2188	—	—	—
H	—[e]	—[e]	—[e]	977	—	—	—
4-Cl	—[e]	—[e]	18.3	399	—	—	—
3,4-$(Cl)_2$	301	1.45	16.2	104	—	—	—
4-NO_2	127	1.45	17.9	49.8	—	—	—
3,5-$(NO_2)_2$	15.0	1.55	17.4	5.98	—	—	—
6-Aminopenicillanic acid	0.608	—	3.15	—	—	11.4	3.03

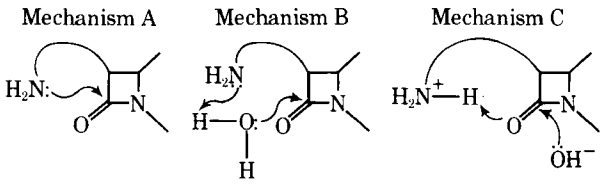
[a] Rate constant is defined in Eq. 5. For penicillins, k_a represents the rate constants for the uncatalyzed intramolecular rearrangement of the undissociated penicillins. [b] Rate constant is defined in Eq. 6. [c] Rate constant is defined in Eq. 7. [d] Not determined because of the large contribution of other kinetic terms. [e] Not determined. [f] Value was recalculated according to this work from the data summarized by Yamana *et al.* (1).

degradations. Instead, Jeffery *et al.* (32) isolated small amounts of thiazole from the neutral degradation solution of cephalosporin C at 37°. They (32) argued that this compound was formed by β-lactam opening and nucleophilic attack of sulfur on carbon of the acyl side chain. Although their arguments (31, 32) are inconsistent with our assumption based on the kinetic evidence, the possibility cannot be excluded that the thiazole may be produced by the nucleophilic attack of sulfur on the oxazolone moiety of the reactive intermediate resulting from Scheme IV. Since rapid fragmentation of β-lactam opening products of cephalosporins can occur in neutral aqueous solutions (31), confirmation of Scheme IV appears to be difficult from product analysis rather than from kinetic analysis.

If such a highly reactive intermediate can be produced from cephalosporins under physiological pH and temperature conditions, it may play a possible role in cephalosporin allergy, analogous to penicillenic acid in penicillin allergy (6). This possibility is now being investigated.

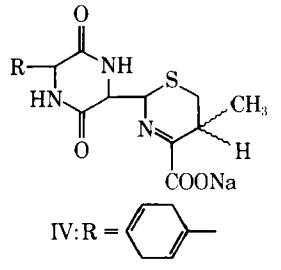
Intramolecular Nucleophilic Attack of Side-Chain α-Amino Group on β-Lactam—The pH–rate profiles for cephaloglycin, cephalexin, and cephradine degradations represent a unique shape in comparison with the profile of ampicillin, which has the same side-chain structure (Fig. 14). The results are consistent with a mechanism involving intramolecularly catalyzed degradation of the anionic species of these cephalosporin molecules at neutral pH, superseded in importance at high pH by hydroxide-ion attack on the anionic cephalosporins and in the lower pH region from 3 to 5 by direct water-catalyzed degradation of the β-lactams. At pH 8, about 10–20 times higher degradation rates of cephaloglycin, cephalexin, and cephradine were observed as compared with those of the corresponding cephalosporins not involving the side-chain α-amino function.

The mechanisms plausible to the neighboring α-amino group participation for the facilitation of β-lactam cleavage of cephaloglycin, cephalexin, and cephradine include intramolecular nucleophilic reaction (Mechanism A), intramolecular general-base catalysis (Mechanism B), and intramolecular general-acid and specific-base catalysis (Mechanism C).



These mechanisms are kinetically indistinguishable. However, if Mechanism A is participating in the degradations, the corresponding stable δ-lactams, diketopiperazines, may be produced. From an aqueous sodium carbonate solution of cephradine, Cohen *et al.* (33) recently isolated the diketopiperazine-type compound, IV, as the major alkaline degradation product. The authors (33) demonstrated the $\Delta^{4,5}$-double bond migration in the degradation of cephradine from NMR observation.

In this laboratory, the degradation of cephalexin was also followed by NMR spectroscopy according to Cohen *et al.* (33) and by HPLC. In deuterium oxide–sodium deuteroxide solution at pD 8.0 and 60°, the 3-methyl group in cephalexin showed a singlet at δ 1.84, which gradually decreased as the degradation proceeded, and a new singlet



IV: R =

Table IV—Effect of Solvent on the Degradation Rate of Cephaloglycin in 0.05 *M* Borate Buffer (pH 8.00) at 35° and $\mu = 0.5$

Solvent Composition	k_{obs}[a], hr^{-1}
Water	0.166
10% Dioxane–water	0.234
25% Dioxane–water	0.261
50% Dioxane–water	0.262

[a] Rate constants were determined spectrophotometrically.

for the secondary methyl group was observed at δ 1.22. When the degradation was performed in water at pH 8.0, the methyl proton signal appeared as a doublet (J = 8 Hz). The NMR spectral changes for cephalexin indicated the double bond migration probably to the 4,5-position during the degradation and exhibited the same behavior as found for cephradine (33). The HPLC studies also showed the same elution pattern between cephalexin and cephradine (Fig. 4).

With regard to cephaloglycin, no decrease in the degradation rates in aqueous dioxane at pH 8.00 was observed (Table IV), indicating no significant participation of water in this reaction.

Additionally, Indelicato *et al.* (9, 34) isolated similar $\Delta^{3,4}$-diketopiperazines, which were produced without migration of the double bond from the refluxed benzene solution of esters of cephaloglycin and cephalexin. Although these reactions were conducted in a nonaqueous solvent, the results (9, 34) serve to support that intramolecular nucleophilic attack of the α-amino group toward β-lactam is possible conformationally in cephalosporin molecules and may also proceed in aqueous media. The evidence obtained in the present study and previous findings (9, 33, 34) are consistent with Mechanism A rather than the kinetically equivalent alternatives.

In contrast, ampicillin did not exhibit such a rate enhancement of the β-lactam opening by the direct intramolecular attack of the side-chain α-amino group (Fig. 14). In spite of the structural similarity between cephalosporin and penicillin, this difference in reactivity is attributed to the steric hindrance of the *gem*-dimethyl group and the 3-proton to the attack of the α-amino group at the β-face as suggested by Indelicato *et al.* (9, 34).

According to the present study, under biological conditions at pH 7.4 and 37°, the β-lactam of cephalogylcin may be degraded nonenzymatically with a half-life of only about 5 hr. This intramolecular degradation process may proceed competitively with enzymatic deacetylation (35), yielding deacetylcephaloglycin.

Alkaline Degradation Mechanism and Relative Stability of Cephalosporins and Penicillins—The k_{OH} values (Table III) for cephalosporins and penicillins are approximately 10^2–10^3 times greater than those for simple and unfused β-lactams (k_{OH} = about 10^{-1} M^{-1} hr^{-1} at 35°) (36). Some investigators stated that the enhanced sensitivity to alkaline degradation of these antibiotics may be attributed mainly to a suppression of the usual amido resonance resulting from the nonplanarity in the β-lactam nitrogen atom (37, 38) and to the intramolecular participation of the neighboring acylamino groups (39, 40). The latter contribution to penicillin reactivity was ruled out by other researchers (1, 41, 42), because changing the substituent of the acylamino group in penicillin only influenced the reaction rates slightly with amines, oxyanion compounds, and hydroxide ion (1, 4, 5, 41, 42).

A similar possibility for intramolecular participation of acylamino groups can also be excluded in the alkaline degradation of cephalosporins, because no significant variations of k_{OH} for the series of Compounds I and II with various acylamino substituents were observed (Table III and Fig. 13). The relatively low reactivities observed for 6-aminopenicillanic acid, 7-aminocephalosporanic acid, and 7-aminodeacetoxycephalosporanic acid (Table III and Fig. 15) as compared with *N*-acylated compounds of the corresponding nucleus may result from the inductive effect of the 6- or 7-amino (electron-donating) group.

The sensitivity of cephalosporins to degrade varied greatly with different 3-methylene substituents. In the series of 7-acylated cephalosporins, cephaloridine was the most reactive; cephalothin, cephaloglycin, and cefazolin exhibited the intermediate reactivity; and cephalexin and cephradine were the most resistant to the hydroxide-ion-catalyzed degradation. The reactivity variation among these cephalosporins was about 20-fold. The significant influence of the substituents at the 3-methylene position upon the chemical reactivity of the β-lactam may be attributed to the long-range inductive effect on the electrophilicity of the β-lactam carbonyl carbon atom toward hydroxide-ion attack (43) and/or the leavability of the 3-methylene moiety, R_2, which may lower the energy of the transition state (44).

The order (cephaloridine > cephalothin = cefazolin = cephaloglycin > cephradine = cephalexin) of the alkaline degradation rates of therapeutically useful cephalosporins parallels their antibacterial activities against Gram-negative bacteria. The present kinetic data support the suggestions of several investigators (38, 43, 45) that the biological activity of penicillins and cephalosporins is related to the chemical reactivity of the β-lactam carbon–nitrogen bond as well as to the physicochemical properties such as lipophilic character of the antibiotic molecules.

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Systems Approach to Vaginal Delivery of Drugs II: *In Situ* Vaginal Absorption of Unbranched Aliphatic Alcohols

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Abstract □ The absorption of unbranched aliphatic alcohols in the rabbit vagina was studied using a perfusion method, and the absorption rates were found to be first order with respect to the drug concentration in the vagina from methanol to octanol. A physical model involving an aqueous diffusion layer in series with a membrane consisting of aqueous pores and lipoidal pathways was used for analyzing the data. The physically based parameters in the model were determined. An effective diffusion layer thickness ("unstirred layer") of around 0.035 cm was found. The increase in the permeability coefficient for the lipoidal pathway per methylene group was around 2.5 for this homologous series.

Keyphrases □ Drug delivery, vaginal—*in situ* absorption of unbranched aliphatic alcohols, rabbits □ Absorption, vaginal—unbranched aliphatic alcohols, rabbits □ Alcohols, unbranched aliphatic—vaginal absorption, rabbits □ Permeability—unbranched aliphatic alcohols, vaginal absorption, rabbits

The objectives of this investigation were: (*a*) to develop suitable methodology in an appropriate animal system and to obtain firm, baseline data on vaginal absorption; (*b*) to delineate the general barrier properties of the vaginal mucosa; and (*c*) to develop quantitative, integrated models describing both the release of drugs from vaginal devices and subsequent drug absorption.

A method was described previously (1) for evaluating drug absorption in the vagina, using the rabbit doe as a prototype animal. A rib-cage-type cell, which provides a closed absorptive compartment in the vaginal tract, was designed and surgically implanted in the rabbit. Drug absorption was determined by perfusing the drug solution through this system and following the time changes in drug concentration in the system. The study showed that the method generally affords good precision and should provide a sound basis for meeting the objectives of this research.

The present study employed this general perfusion technique and was concerned with the permeability behavior of a homologous series of unbranched aliphatic alcohols in the rabbit vaginal membrane. These compounds were expected to exhibit a systematic increase in the permeability coefficient with increasing carbon number, and a systems analysis of the resulting data was expected to define the operational barrier characteristics of the rabbit vaginal mucosa.

The method of data analysis essentially follows that delineated in previous studies on the human buccal membrane (2, 3), the rat intestinal membrane (4–7), and the silicone rubber membrane (8, 9).

An improved technique employing two simultaneously permeating species with different radiolabels (tritium and carbon-14) is introduced. By using one species as a control, the precision of the method is substantially improved. In addition, a GLC procedure was developed to investigate solutes that are not conveniently available with a radiolabel. Finally, a new cell with a magnetic stirrer was developed to manipulate the hydrodynamics within the absorption cell compartment independently of the hydrodynamics induced by the perfusate flow itself.