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GI Absorption of β-Lactam Antibiotics I: Kinetic Assessment of Competing Absorption and Degradation in GI Tract

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Abstract ☐ An equation was derived for the simultaneous assessment of rate constants for absorption and nonenzymatic degradation of unstable drugs in *in situ* absorption experiments. The equation was substantiated by using a variety of β-lactam antibiotics in the recirculation technique through the rat small intestine. Plots of the apparent first-order rate constant for the disappearance of the drug from the gut lumen *versus* the reciprocal of the volume of recirculating solution yielded a straight line with a slope equal to the intrinsic absorption rate constant and with an intercept equal to the nonenzymatic degradation rate constant in the GI lumen. The kinetic method for evaluation of the absorption rate constant also was developed for a more complex situation in the GI lumen involving absorption, nonenzymatic degradation, and enzymatic metabolism. The proposed method was confirmed with carbenicillin indanyl, which was metabolized rapidly to carbenicillin by the action of nonspecific esterase in the intestine. In the absence of information on Michaelis–Menten kinetic parameters, the present method is advantageous for evaluation of the intrinsic absorption rate of all unstable drugs.

Keyphrases ☐ β-Lactam antibiotics—kinetics of GI absorption and nonenzymatic degradation in rats ☐ Absorption, GI—β-lactam antibiotics, kinetics in rats ☐ Degradation, nonenzymatic GI—β-lactam antibiotics, kinetics in rats ☐ Antibiotics, β-lactam—kinetics of GI absorption and nonenzymatic degradation in rats

Penicillins are susceptible, depending on the nature of the side chain, to acid degradation and attack by β-lactamase in the GI tract (1). Since such degradations and absorption processes compete at the absorption site, the GI absorption of penicillins is difficult to study. A great deal of information is now available concerning the serum levels after oral administration of a variety of penicillins to experimental animals and humans (1, 2). It is, however, not always easy to discuss the absorption rate and mechanism from serum level data only because of the complex kinetic processes for the degradation in the GI tract and distribution in and elimination from the body (3).

In situ animal techniques are suitable for the quantitative determination of absorption rates and the clarification of the transport mechanism of a drug from the GI walls. A single perfusion method (4), a recirculation method (4, 5), and the method of Doluisio *et al.* (6) were used to study drug absorption. The present investigation was undertaken to establish a kinetic method for the evaluation of chemically and enzymatically unstable drugs such as β-lactam antibiotics by use of the recirculation technique. This technique has advantages of accurate pH control and suitable change in the perfusion volume. In the accompanying paper (7), the techniques developed were applied to study the *in situ* absorption of penicillins.

EXPERIMENTAL

Materials—The following β-lactam antibiotics were used as supplied: carbenicillin indanyl sodium[1] (704 μg/mg as carbenicillin free acid), propicillin potassium[2] (993 μg/mg), penicillin V potassium[3] (1490 units/mg), penicillin G potassium[3] (1600 units/mg), carbenicillin disodium[1] (799 μg/mg), and cephalothin sodium[4] (930 μg/mg).

All other chemicals were analytical reagent grade. Imidazole was recrystallized from benzene, followed by a thorough washing with ether (8).

Test Animals—Male albino Wistar rats, 180 ± 45 g, were fasted 20 hr prior to the experiment. Water was given freely..

Rat *In Situ* Intestinal Absorption, Degradation, and Metabolism—The recirculation method was based on those of Schanker *et al.* (4) and Koizumi *et al.* (5). The rats were anesthetized with urethan, 1.3 g/kg ip, approximately 1 hr prior to surgery. A midline abdominal incision was made, and the small intestine was exposed. The glass tubings connected to silicone tubing were cannulated into both ends of the small intestine. The double ligations were made with a 000 silk thread, with care being taken not to interfere with blood flow. The bile duct was ligated to avoid any flow of fluid into the intestinal lumen during the absorption experiments. The intestine was replaced in the abdomen, the incision was closed with metal clips, and the cannula was connected to a microtube pump[5].

The small intestine was washed with 100 (at pH 4.0) or 50 (at pH 7.0) ml of a perfusion solution (6) maintained at 37°. Then the desired volume of the perfusion solution containing the antibiotic was recirculated from the duodenum to the ileum (~100 cm in length) at pH 4.0 or to the jejunum (~30 cm in length) at pH 7.0. The perfusion solution consisted of 1–2 mg/ml, unless otherwise stated, of a β-lactam antibiotic together with the following compounds per liter at pH 4.0: hydrochloric acid, 3.8 ml; citric acid, 12.6 g; sodium hydroxide, 1.8 g; and sodium chloride, 2.1 g (5). At pH 7.0, the compounds used were: dibasic sodium phosphate, 14.3 g; monobasic potassium phosphate, 3.6 g; and sodium chloride, 4.3 g (5).

The solution was maintained at 37° and perfused with a pump at 10 ml/min at pH 4.0 or at 2 ml/min at pH 7.0. The perfusion solution pH was kept constant at the desired value during the absorption experiments by a pH-stat[6]. The samples (0.1–0.2 ml, unless otherwise stated) were taken at suitable time intervals and analyzed.

At pH 4.0, the volume changes were almost negligible. For carbenicillin indanyl sodium, a 0.025-mg/ml drug solution was used because of its low

[1] Taito Pfizer Co., Tokyo, Japan.
[2] Takeda Chemical Industries, Osaka, Japan.
[3] Meiji Seika Kaisha, Tokyo, Japan.
[4] Shionogi and Co., Osaka, Japan.
[5] Tokyo Rika Kikai Co. or Mitsumi Scientific Industries, Tokyo, Japan.
[6] pH-Stat titrator assembly consisting of TTT2 titrator and ABU12b autoburet, Radiometer, Copenhagen, Denmark.

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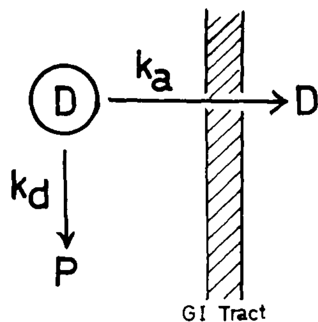


Scheme I—Simultaneous absorption and nonenzymatic degradation. Key: D, *intact drug;* P, *product;* k_a, *absorption rate constant; and* k_d, *degradation rate constant.*

solubility. Aliquots (5 ml) were taken, 5 ml of drug-free perfusion solution was added to maintain a constant solution volume, and the arithmetical correction of concentration resulting from the dilution was made. At pH 7.0, a sample was taken at each time period, the intestine was emptied, and the perfusion solution corresponding to the reduction in volume, except for sampling, was added to the recirculating drug solution. The volume added was only 1.5 ml.

Analysis—The samples were diluted appropriately, filtered with a 0.45-μm filter[7], and then analyzed.

Propicillin, penicillin V, and penicillin G were determined by the method of Bundgaard and Ilver (8). Carbenicillin indanyl was determined according to a spectrophotometric method developed for the simultaneous determination of carbenicillin indanyl and carbenicillin[8], based on the method reported by Bundgaard and Ilver (8). Cephalothin was determined by high-pressure liquid chromatography (HPLC)[9]. The eluant was 0.25 *M* acetate buffer (9), and the pressure was 100 kg/cm^2. The sample was injected in a 10-μl portion and detected at 254 nm[10].

RESULTS AND DISCUSSION

Case I: Simultaneous Absorption and Nonenzymatic Degradation in GI Tract—*Theoretical*—Scheme I represents the simultaneous absorption of a drug from the GI tract and nonenzymatic degradation of the drug in the perfusion solution. If the absorption kinetics obey Fick's law, if the overall transfer of a drug from the gut lumen to blood is irreversible, if drug accumulation in the gut membrane is negligible, and if first-order degradation proceeds, then the total disappearance of the amount of a drug in the perfusion solution can be expressed as:

$$-\frac{dA}{dt} = k_a(C - C_b) + k_d A \qquad \text{(Eq. 1)}$$

where A represents the amount of drug; C and C_b represent the drug concentrations in the perfusion solution and blood, respectively; k_a is the intrinsic absorption rate constant having a unit of volume × time^{-1}; and k_d is the competitive first-order rate constant having a unit of time^{-1}. For nonenzymatic and parallel degradation to produce n kinds of products, k_d can be expressed as:

$$k_d = \sum_i^n (k_d)_i \qquad \text{(Eq. 2)}$$

The parameter A can be expressed by the drug concentration, C, and the volume of the drug solution, V, as follows:

$$-\frac{d(VC)}{dt} = k_a(C - C_b) + k_d(VC) \qquad \text{(Eq. 3)}$$

Under the experimental conditions of the constant volume of the drug solution and $C \gg C_b$, Eq. 3 becomes:

$$-\frac{dC}{dt} = \left(\frac{k_a}{V} + k_d\right)C \qquad \text{(Eq. 4)}$$

[7] Sartorius-Membranfilter GmbH, 34 Göttingen, Germany.
[8] Details of the analytical method and the stability kinetics of the carbenicillin prodrug will be published; A. Tsuji, E. Miyamoto, T. Terasaki, and T. Yamana.
[9] Model 830, Shimadzu-DuPont, Kyoto, Japan. SAX on Zipax, 2.1 mm i.d. × 0.5 m, DuPont.
[10] UV 202 recording double-beam spectrophotometer, Shimadzu, Kyoto, Japan.

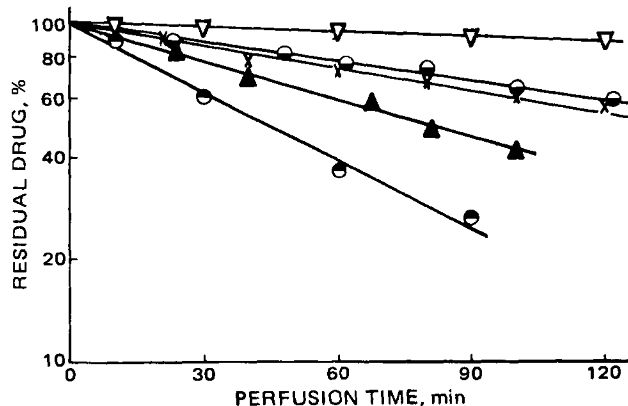


Figure 1—*First-order plots of percent β-lactam antibiotics remaining in the* in situ *rat intestinal recirculating experiments at pH 4.0 and at a perfusion flow rate of 10 ml/min. The pH of the drug solution was maintained constant by a pH-stat. Key (volume of the perfusion solution):* ⊖, *carbenicillin indanyl, 80 ml;* ▲, *propicillin, 70 ml;* ×, *penicillin G, 50 ml;* ⊖, *penicillin V, 60 ml; and* ▽, *cephalothin, 50 ml.*

By integration:

$$\log\left(\frac{C}{C_0}\right) = -\frac{k_{app}}{2.303}t \qquad \text{(Eq. 5)}$$

where C_0 is the concentration at $t = 0$ and k_{app} is:

$$k_{app} = k_a\frac{1}{V} + k_d \qquad \text{(Eq. 6)}$$

Equation 5 predicts that the semilogarithmic plots of C/C_0 *versus* time show a straight line to give the apparent first-order rate constant, k_{app}.

Method 1—When the quantitative determination of all degradation products at time t after the start of the recirculating experiments yields the values of $\Sigma(C_d)_i$, the apparent first-order rate constant for the absorption, $(k_a)_{app}$, can be calculated easily according to:

$$(k_a)_{app} = k_a\frac{1}{V} \qquad \text{(Eq. 7a)}$$

Table I—Apparent Rate Constants, k_{app}, of β-Lactam Antibiotics for Various Volumes of Perfusion Solution from the *In Situ* Rat Small Intestine at pH 4.0 [a], 37°, and a Flow Rate of 10 ml/min

Compound	Perfusion Volume, ml	$k_{app} \times 10^3$, min^{-1}
Carbenicillin indanyl	80	15.27
	200	6.05
	400	3.80
Propicillin	47	11.55
	50	11.11
	70	8.44
	80	6.55
	100	5.42
	200	3.00
	400	1.52
	1000	0.85
Penicillin V	37	7.22
	50	5.40
	60	4.22
	70	4.22
	100	2.80
	200	1.67
Penicillin G	50	4.98
	60	5.30
	80	4.57
	100	4.53
	145	3.97
	200	4.07
Cephalothin	50	0.90
	70	0.72

[a] The pH of the perfusion solution was maintained constant with a pH-stat. No significant volume changes were observed during the absorption experiments.

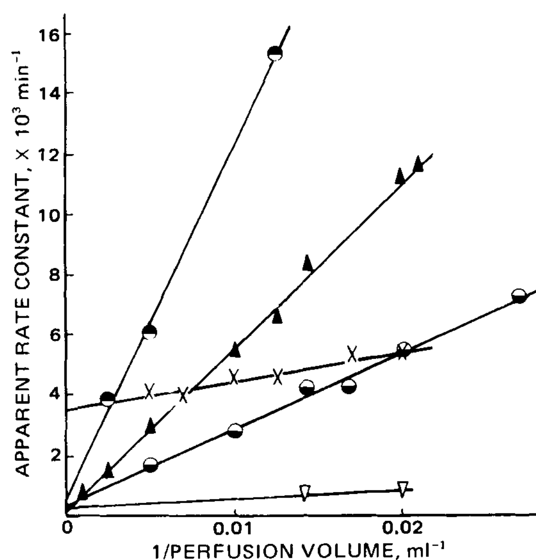


Figure 2—*Effect of the volume of the perfusion solution on the absorption of β-lactam antibiotics from the* in situ *rat small intestine at pH 4.0 and 37°. Key:* ●, *carbenicillin indanyl;* ▲, *propicillin;* ×, *penicillin G;* ◒, *penicillin V; and* ▽, *cephalothin.*

$$(k_a)_{\text{app}} = k_{\text{app}}\left[1 - \frac{\sum(C_d)_i}{C_0\,[1 - \exp(-k_{\text{app}}t)]}\right] \quad \text{(Eq. 7}b\text{)}$$

Method 2—When the quantitative analysis of the degradation products is difficult and/or impossible for the test drug, the following kinetic technique should be suitable for the simultaneous assessment of k_a and k_d.

Equation 6 predicts that plots of k_{app} *versus* the reciprocal of the volume of the drug solution, $1/V$, give a straight line under constant experimental conditions, except for the change of the perfusion volume. The absorption rate constant, k_a, can be obtained from the slope; the total degradation rate constant, k_d, can be obtained from the intercept value.

Application of Theory to β-Lactam Antibiotics—In an acidic environment, penicillins are easily degraded by intramolecular attack of the side-chain amide on the β-lactam moiety, depending on the nature of the side chain, to produce the corresponding penicillenic, penillic, and penicilloic acids (1, 10). Although the β-lactam moiety of cephalosporins is relatively acid stable, the initial degradation product corresponding to penicilloic acid is not stable and is readily fragmented in aqueous solution (1, 10). Additionally, the ester bond of acetylcephalosporins is easily hydrolyzed in acidic solutions to produce deacetylcephalosporin and its lactone (1, 11, 12). Because of these circumstances, quantitative determination of the acid degradation products resulting from β-lactam antibiotics seems to be experimentally difficult or impossible. Therefore, the absorption rates of β-lactam antibiotics may be suitably evaluated with the kinetic technique of Method 2.

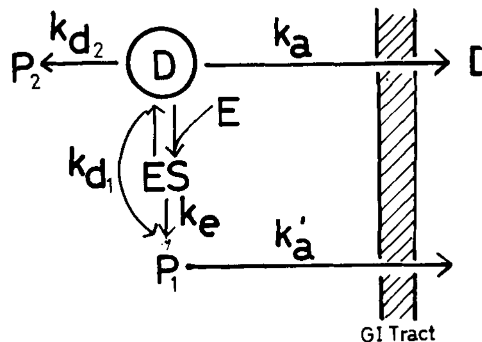
Figure 1 illustrates the disappearance of β-lactam antibiotics from the *in situ* rat intestinal lumen when the pH of the lumen solution was continuously controlled to pH 4.0 by a pH-stat. Disappearance followed first-order kinetics as predicted from Eq. 5. The rate constants, k_{app}, determined from the slopes by the least-squares treatment are listed in Table I; plots of k_{app} *versus* $1/V$ are illustrated in Fig. 2. For all β-lactam antibiotics, good straight lines with various slopes and intercepts were obtained, in agreement with Eq. 6.

The values of k_a and k_d estimated according to Eq. 6 are given in Table II. The k_d values were in relatively good agreement with those determined *in vitro* under the same conditions (Table II), suggesting that drug degradation in recirculating solution through the rat small intestine depends only on the bulk pH of the perfusion solution. The more lipophilic β-lactam antibiotics exhibited larger absorption rate constants. The absorption mechanism and the relationship between the absorption rate and the structure of β-lactam antibiotics are discussed in detail elsewhere (7).

Table II—Absorption and Degradation Rate Constants Determined from the *In Situ* and *In Vitro* Experiments at pH 4.0 and 37°

Compound	k_a [a], ml min⁻¹	k_d, × 10³ [b] min⁻¹	k_d', × 10³ [c] min⁻¹
Carbenicillin indanyl	1.17	0.60	0.25
Propicillin	0.54	0.23	0.28
Penicillin V	0.25	0.36	0.28
Penicillin G	0.08	3.62	4.00
Cephalothin	0.02	0.28	0.43

[a] Evaluated from the slope in Fig. 2. [b] Evaluated from the intercepts in Fig. 2. [c] Pseudo-first-order rate constant for the *in vitro* degradation in isotonic buffer solution of pH 4.0 at 37°.



Scheme II—Simultaneous absorption and enzymatic degradation. Key: D, *intact drug;* E, *specific or nonspecific enzyme;* ES, *substrate–enzyme complex;* P, *product;* k_a *and* k_a', *absorption rate constants of intact drug and products, respectively;* $(k_d)_1$ *and* $(k_d)_2$, *degradation rate constants of nonenzymatic reaction; and* k_e, *degradation rate constant of* ES *complex.*

Case II: Simultaneous Absorption and Both Enzymatic and Nonenzymatic Degradations in GI Tract—*Theoretical*—Drug-metabolizing enzymes existing in intestinal secretions and microflora (13) may influence the kinetics of disappearance of a drug during *in situ* absorption experiments. Scheme II shows the processes involving the competing first-order absorption with rate constant k_a, first-order nonenzymatic degradation with rate constants $(k_d)_1$ and $(k_d)_2$ to yield the corresponding products P_1 and P_2, and enzymatic degradation obeying Michaelis–Menten kinetics to produce the metabolite P_1, which is also absorbed with the rate constant k_a'.

The differential equations of a drug, D, and a metabolite, P_1, can be expressed as Eqs. 8 and 9, respectively, according to Scheme II:

$$\frac{dC}{dt} = -\left[k_a\frac{1}{V} + (k_d)_1 + (k_d)_2\right]C - \frac{k_e(A_e/V)C}{K_m + C} \quad \text{(Eq. 8)}$$

$$\frac{d(C_p)_1}{dt} = (k_d)_1C - k_a'\frac{1}{V}(C_p)_1 + \frac{k_e(A_e/V)C}{K_m + C} \quad \text{(Eq. 9)}$$

where K_m is the Michaelis–Menten constant, A_e is the amount of enzyme, k_e is the degradation rate constant for the drug–enzyme complex, and $(C_p)_1$ is the concentration of the metabolite P_1.

Method 3—When a nonspecific enzyme, such as esterase, participates in the drug metabolism, the K_m value is unknown, and/or the amount of enzyme cannot be assumed to be constant during the experiments, the following kinetic approach is the most covenient for determining the absorption rate constant of a drug.

Summation of Eqs. 8 and 9 leads to:

$$\frac{dC}{dt} + \frac{d(C_p)_1}{dt} = -[(k_a)_{\text{app}} + (k_d)_2]C - (k_a')_{\text{app}}(C_p)_1 \quad \text{(Eq. 10)}$$

where $(k_a)_{\text{app}}$ and $(k_a')_{\text{app}}$ represent the apparent absorption rate constants of the drug and its metabolite P_1, respectively, depending on the perfusion volume as shown in Eq. 7b. Integration of Eq. 10 from $t = 0$ to $t = t$ yields:

$$\int_0^t \frac{dC}{dt}\,dt + \int_0^t \frac{d(C_p)_1}{dt}\,dt = -[(k_a)_{\text{app}} + (k_d)_2]\int_0^t C\,dt - (k_a')_{\text{app}}\int_0^t (C_p)_1\,dt \quad \text{(Eq. 11)}$$

Since $C = C_0$ and $(C_p)_1 = 0$ at $t = 0$, Eq. 11 gives:

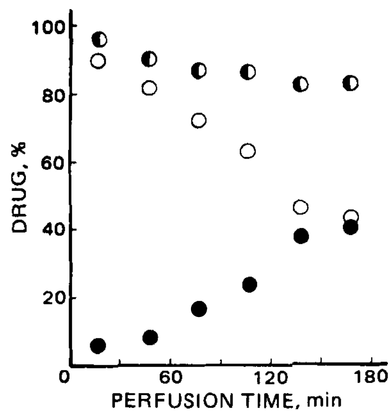


Figure 3—*Time course of absorption and metabolism of carbenicillin indanyl during the* in situ *recirculating experiment in a rat at pH 7.0. The following conditions were used: perfusion volume, 25 ml; initial drug concentration, 2 mg/ml; and flow rate, 2 ml/min. The pH of the drug solution was maintained constant with a pH-stat. Key:* ◐, *total antibiotics;* ○, *carbenicillin indanyl; and* ●, *carbenicillin.*

$$(k_a)_{app} + (k_d)_2 = k_{app}$$

$$= \frac{C_0 - C - (C_p)_1 - (k_a')_{app} \int_0^t (C_p)_1\, dt}{\int_0^t C\, dt} \quad \text{(Eq. 12)}$$

If the quantitative determination of the concentrations of both the drug and its metabolite at time t is possible, the apparent first-order rate constant, k_{app}, can be calculated from Eq. 12, where $\int_0^t C\, dt$ and $\int_0^t (C_p)_1\, dt$ are the areas under the respective concentration of the drug and the metabolite *versus* time curves from time zero to t. The values of $(k_a')_{app}$ can be obtained from another absorption experiment of the metabolite under the same conditions with the same volume of recirculating perfusion solution, V. Therefore, by plotting the k_{app} values determined according to Eq. 12 *versus* $1/V$, the absorption rate constant, k_a, and the nonenzymatic degradation rate constant, $(k_d)_2$, of the test drug can be obtained from the slope and the intercept, respectively.

Application of Method 3 to β-Lactam Antibiotics and Prodrug—Carbenicillin indanyl, which is the carbenicillin α-ester of 5-indanol, is a carbenicillin prodrug. It has been used orally with a sufficient therapeutic effect (14–17). Figure 3 illustrates the time course of the prodrug and the parent drug (carbenicillin, P_1) during recirculation of carbenicillin indanyl through the rat small intestine at pH 7.0. This graph indicates the slow absorption of the prodrug and rapid cleavage of the α-ester bond to yield carbenicillin. Since the ester was relatively stable with a half-life of ~17 hr at pH 7.0 and 37° *in vitro*[8], the rapid formation of carbenicillin is virtually the result of the action of a nonspecific intestinal esterase. Therefore, the behavior of carbenicillin indanyl in the perfusion solution essentially follows Scheme II. Under this condition, the absorption of carbenicillin itself was negligible [*i.e.*, $(k_a')_{app} \simeq 0$)]. The apparent rate constant, k_{app}, could be calculated from the data of Fig. 3 according to Eq. 12.

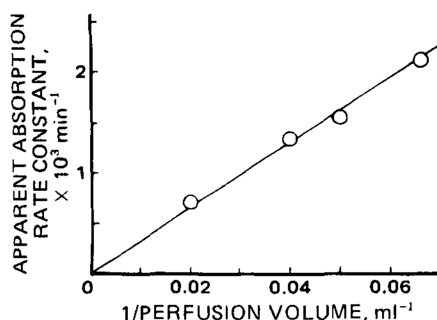


Figure 4—*Effect of the volume of the perfusion solution on the absorption rate of carbenicillin indanyl from the* in situ *rat small intestine at pH 7.0. The pH of the drug solution was maintained constant with a pH-stat.*

Figure 4 shows the plot of k_{app} *versus* $1/V$, yielding a good straight line with a slope equal to the absorption rate constant, k_a, of carbenicillin indanyl and almost through the origin. This result suggests that a nonenzymatic degradation process of carbenicillin indanyl other than that producing carbenicillin is essentially negligible. At pH 7.0 at 35°, the nonenzymatic β-lactam degradation of both carbenicillin indanyl and carbenicillin was only 1% during 2 hr[8].

Method 3 can also be utilized for the metabolism of penicillins by β-lactamase to produce the corresponding penicilloic acid, if the metabolite can be determined by a suitable analytical method.

SUMMARY AND CONCLUSIONS

A kinetic method was developed, allowing the simultaneous determination of rate constants for both absorption and nonenzymatic degradation of unstable drugs only by changing the volume of the drug solution from *in situ* absorption experiments. The method was experimentally confirmed for several β-lactam antibiotics by utilizing an *in situ* recirculation technique on the rat small intestine. The present kinetic approach provides vital information for the intrinsic GI absorption process of some drugs whose absorption experiments have been difficult because of their lability in the GI lumen.

For the more complex situation *in situ*, involving enzymatic metabolism of drugs, a method was developed for the accurate assessment of the absorption rate constant and was substantiated by a carbenicillin prodrug, which was metabolized rapidly to carbenicillin by the action of nonspecific intestinal esterase.

The present methods, although based on simple kinetic considerations, may be widely applicable to evaluate the intrinsic absorption rate from *in situ* absorption experiments for all drugs enzymatically or nonenzymatically unstable.

REFERENCES

(1) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **60,** 503 (1971).

(2) G. N. Rolinson and R. Sutherland, in "Advances in Pharmacology and Chemotherapy," vol. 11, G. Garrattini, A. Goldin, F. Hawking, and I. J. Kopin, Eds., Academic, New York, N.Y., 1973, p. 151.

(3) R. E. Notari, J. L. Deyound, and R. H. Reuning, *J. Pharm. Sci.*, **61,** 135 (1972).

(4) L. S. Schanker, D. J. Tocco, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **123,** 81 (1958).

(5) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12,** 412 (1964).

(6) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58,** 1196 (1969).

(7) A. Tsuji, E. Miyamoto, N. Hashimoto, and T. Yamana, *ibid.*, **67,** 1705 (1978).

(8) H. Bundgaard and K. Ilver, *J. Pharm. Pharmacol.*, **24,** 790 (1972).

(9) R. P. Buhs, T. E. Maxim, N. Allen, T. A. Jacob, and F. J. Wolf, *J. Chromatogr.*, **99,** 609 (1974).

(10) M. A. Schwartz, *J. Pharm. Sci.*, **58,** 643 (1969).

(11) T. Yamana and A. Tsuji, *ibid.*, **65,** 1563 (1976).

(12) J. Konecny, E. Felber, and J. Gruner, *J. Antibiot.*, **26,** 135 (1973).

(13) R. R. Schline, *Pharmacol. Rev.*, **25,** 451 (1973).

(14) J. F. Wallace, E. Atlas, D. M. Bear, N. K. Brown, H. Clark, and M. Turck, *Antimicrob. Agents Chemother., 1970,* **1971,** 223.

(15) K. Butler, A. R. English, A. K. Knirsch, and J. J. Korst, *Del. Med. J.*, **43,** 366 (1971).

(16) A. R. English, J. A. Retsema, V. A. Ray, and J. E. Lynch, *Antimicrob. Agents Chemother.*, **1,** 185 (1972).

(17) D. C. Hobbs, *ibid.*, **2,** 272 (1972).

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