Carbenicillin Prodrugs: Kinetics of Intestinal Absorption Competing Degradation of the α-Esters of Carbenicillin and Prediction of Prodrug Absorbability from Quantitative Structure–Absorption Rate Relationship

AKIRA TSUJI *×, ETSUKO MIYAMOTO §, TETSUYA TERASAKI *, and TSUKINAKA YAMANA ‡

Received June 8, 1981, from the *Faculty of Pharmaceutical Sciences,‡ Hospital Pharmacy, Kanazawa University, Takara-machi, Kanazawa 920, Japan, and the §School of Pharmacy, Hokuriku University, Kanagawa-machi, Kanazawa 920-11, Japan. Accepted for publication July 17, 1981.

Abstract □ The intestinal absorption of α-esters of carbenicillin disodium, carbenicillin phenyl sodium, and carbenicillin indanyl sodium was investigated using the *in situ* rat intestinal recirculating method. In the *in situ* intestinal lumen at pH 7, two prodrugs were rapidly converted to poorly absorbable carbenicillin, possibly by the action of intestinal nonspecific esterase in competition with the slow absorption of prodrugs. At pH 5, the reduced action of esterase and the increased absorption rate after 3 hr resulted in 50 and 60% absorption of carbenicillin phenyl sodium and carbenicillin indanyl sodium, respectively. The absorption rate constants determined for both prodrugs were in good agreement with the prediction from the quantitative structure–absorption rate relationship derived from the two-compartment aqueous diffusion model.

Keyphrases □ Carbenicillin—prodrugs, kinetics of intestinal absorption, α-esters □ Prodrugs—carbenicillin, kinetics of intestinal absorption, α-esters □ Absorption, intestinal—carbenicillin prodrugs, kinetics

Carbenicillin (II) is very acid unstable and has low lipid solubility; thus, it is poorly absorbed by the GI tract after oral administration and its use is limited to parenteral administration. A series of α-carboxyl esters (I) of carbenicillin was synthesized in an attempt to overcome these disadvantages in the physicochemical properties of II and to increase oral bioavailability. These esters are designed to hydrolyze in the body to liberate II (1). The *in vitro* degradation kinetics of therapeutically useful derivatives of I, phenyl ester (carfecillin, I*a*) and indanyl ester (carindacillin, I*b*), in a wide pH range at 35° and ionic strength of 0.5 were reported previously (2). Bundgaard (3) showed the acid degradation kinetics of the same compounds at 60°. These kinetic data predicted that the β-lactam moiety of both prodrugs is six times more stable than that of II at pH 2.0 and the half-lives of the ester hydrolysis *in vitro* are 8.5 hr for I*a* and 17 hr for I*b* at pH 7.0 and 37° (2).

The present study was undertaken to evaluate the relative importance of parallel rate processes, absorption, and both chemical and enzymatic degradations (possibly proceeding at the absorption sites after oral administration of these esters) by utilizing the *in situ* rat intestinal recirculating method. The physicochemical properties of carbenicillin prodrugs were also evaluated in order to improve the GI absorption rate of the parent antibiotic by application of the structure–absorption rate relationship established previously for β-lactam antibiotics (4).

EXPERIMENTAL

Materials and Reagents—The materials, reagents, and equipment used in this study were, unless otherwise stated, the same as those used previously (2, 4). All isotonic buffer solutions were prepared with the highest reagent grade chemicals available.

Intestinal Absorption Experiments—Male albino rats (Wistar strain) weighing ~200 g, were fasted over a 20-hr period prior to the experiments, but water was given freely. The rats were anesthetized with urethan, 1.3 g/kg ip.

The intestinal recirculating absorption procedure was reported previously (4). The *in situ* loop absorption method was essentially the same as that of Perrier and Gibaldi (5) except for the use of the duodenum and for the ligation of the bile duct.

Each antibiotic solution was prepared with an isotonic buffer of pH 5 or 7 to make a final concentration of 1–4 mg/ml. Aliquots (0.1 ml) of the samples were withdrawn at appropriate intervals, diluted with distilled water, filtered through a 0.45-μm filter[1] to remove any solid materials, and analyzed.

Analytical Procedure—Samples were analyzed by UV spectrometry developed previously to quantify I and II simultaneously in a solution (2).

Table I—Percentage Residue of Carfecillin (Ia) and Carbenicillin (II) in the *In Situ* Rat Small Intestinal Loop [a]

Rat No.	Residual Ia, %	Residual II, %	Disappearance of Total Antibiotics, %
1	38.6	38.2	22.2
2	0.0	80.4	19.6
3	28.8	47.6	23.6
Mean	22.5	55.4	21.8
SD	20.1	22.2	2.0

[a] All experiments were over 1 hr. I*a* was dissolved in isotonic phosphate buffer (pH 7) and injected in a volume of 1 ml (1 mg/ml) into a 5-cm intestinal loop (duodenum).

RESULTS AND DISCUSSION

Disappearance of Prodrugs from *In Situ* Rat Intestinal Loop—It was suggested that I*a* and I*b* are both well absorbed by the GI tract and can be converted rapidly to II during absorption through the mucous membrane and/or in the blood by a nonspecific esterase (1).

The percentage of the residual I*a* and II produced after 1 hr in the *in situ* rat intestinal loop at pH 7 is shown in Table I. From previous kinetic data (2), the rate constant for the ester hydrolysis of I*a* was evaluated at 1.5×10^{-3} min^{-1} for the isotonic and 0.066 *M* phosphate buffer solution (pH 7.0) used in this study. The 55.4 ± 22.2% of *in situ* formation of II at the end of 1 hr was about six times greater than that predicted from the chemical hydrolysis rate of the phenyl ester. Because the percentage disappearance of the total antibiotics (I*a* + II) was 21.8 ± 2.0%, the result suggests that the prodrug I*a* may be absorbed as well as converted to II by the chemical hydrolysis and enzymatic action of nonspecific esterase in the intestinal fluid and/or mucosal surface of the intestine.

Kinetics of Absorption and Metabolism of Prodrugs by the *In Situ* Rat Intestine—To clarify the GI absorption of I from its kinetics, the intestinal absorption experiments were performed using the recirculating perfusion technique. The pH of the perfusion solution was maintained

[1] Sartorius-Membranfilter, GmbH, 34 Göttingen, West Germany.

0022-3549/82/0400-0403$01.00/0

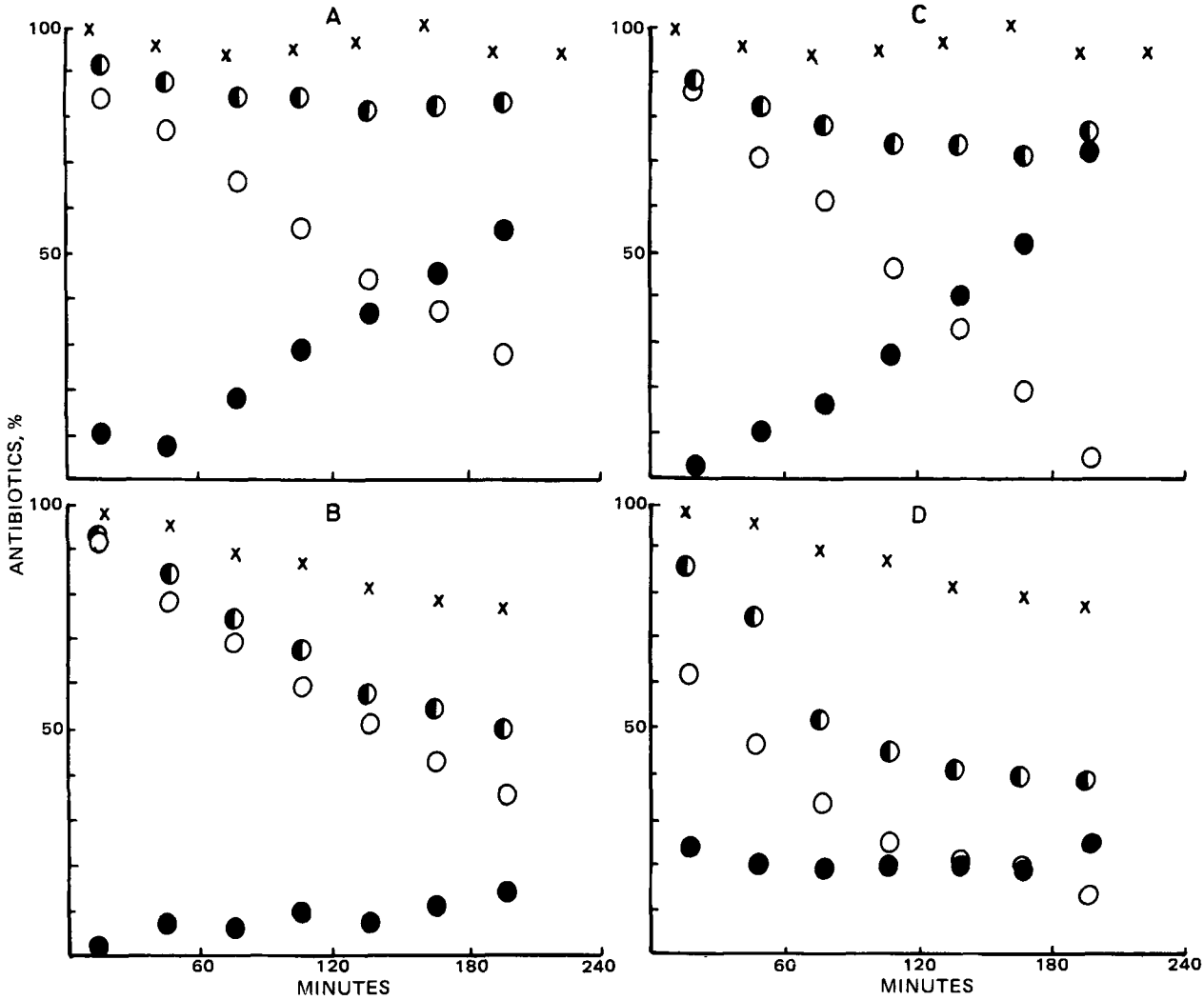


Figure 1—*Time course of absorption and metabolism of carfecillin (Ia) (A and B) and carindacillin (Ib) (C and D) during* in situ *recirculation through the rat small intestine at pH 7.0 (A and C) and pH 5.0 (B and D). Perfusion volume, 15 ml; initial concentration, 4 mg/ml (A and B), 2 mg/ml (C), and 1 mg/ml (D); flow rate, 2 ml/min. Key:* ◐, *total antibiotics;* ○, *prodrug (I); and* ●, *carbenicillin (II). The symbol* (x) *shows the experimental points for II under the same conditions.*

at a constant of pH 5.0 or 7.0 by use of a pH-stat during the absorption experiments.

Results of the lumen content analysis are shown in Fig. 1, indicating that disappearance of I at pH 7.0 proceeds to a similar extent for Ia and Ib, accompanying an increase of II up to ~50–60% after 3 hr in 15-ml of lumen perfusion solution (Figs. 1A and 1C). Interestingly the rates for total loss of I and the formation of II depend markedly on the volume of the perfusion solution. The loss of total antibiotic from 15-ml lumen solution was about 20% for Ib after 3 hr. The disappearance of II was only ~6% after 3 hr under the same condition (Fig. 1A and 1C). The decrease in the total antibiotic may be attributed mostly to the absorption of intact I. However, in the experiments with 40–50 ml of lumen solution, about 35–40% of I loss corresponded to the formation of II; the absorption of the antibiotics was negligible.

At pH 5, on the other hand, absorption was 50% for Ia and 60% for Ib but only 20% for the parent antibiotic II after 3 hr for the 15-ml solution (Figs. 1B and 1D). Under this condition, a relatively small amount (10–20%) of II was produced, probably due to the reduced action of nonspecific esterase.

From these results, the kinetic model for the absorption and metabolism of I may be derived as reported previously (6). I is absorbed by the intestine in an intact form but is partially degraded by two possible processes as shown in Scheme I. One process is a nonenzymatic degradation of β-lactam moiety to produce the penicilloic acid (III) of I and the other is both a nonenzymatic and enzymatic hydrolysis of ester linkage to form the poorly absorbable II. Compound II is also degraded to its penicilloic acid (IV) similarly to form III from I along with the slow absorption by the intestine.

If it is assumed (not being influenced by the volume change of the perfusion solution) that the absorption kinetics obey Fick's law, that enzymatic ester hydrolysis is described by Michaelis–Menten kinetics, and that all β-lactam cleavages obey apparent first-order kinetics, then the rate of each species can be written:

$$\frac{d[\mathrm{I}]}{dt} = -[k_a/V + (k_d)_1 + (k_d)_2][\mathrm{I}] - \frac{k_e(A_e/V)\,[\mathrm{I}]}{K_m + [\mathrm{I}]} \qquad \text{(Eq. 1)}$$

$$\frac{d[\mathrm{II}]}{dt} = (k_d)_1[\mathrm{I}] - [k_a'/V + (k_d)_3]\,[\mathrm{II}] + \frac{k_e(A_e/V)\,[\mathrm{I}]}{K_m + [\mathrm{I}]} \qquad \text{(Eq. 2)}$$

where [I] and [II] represent the concentration of I and II, respectively, at time t, K_m is the Michaelis constant, A_e represents the amount of enzyme, k_e is the degradation rate constant for the formation of II from the drug–enzyme complex, $(k_d)_1$ is the first-order rate constant for the nonenzymatic ester hydrolysis to produce II, $(k_d)_2$ and $(k_d)_3$ are the first-order rate constants for the nonenzymatic β-lactam cleavage reactions of I and II, respectively, k_a and k_a' are the absorption clearance (ml/time) of I and II, and V is the volume of perfusion solution. From Eqs. 1 and 2, Eqs. 3 and 4 can easily be derived (6):

$$k_{app} = k_a/V + (k_d)_2 \qquad \text{(Eq. 3)}$$

$$k_{app} = \frac{[\mathrm{I}]_{t_1} - [\mathrm{I}]_{t_2} + [\mathrm{II}]_{t_1} - [\mathrm{II}]_{t_2} - [k_a'/V + (k_d)_3]\int_{t_1}^{t_2}[\mathrm{II}]dt}{\int_{t_1}^{t_2}[\mathrm{I}]dt} \qquad \text{(Eq. 4)}$$

where subscripts t_1 and t_2 represent the sampling times. The values of

Table II—Absorption Clearance Determined at pH 5 and 7 in the *In Situ* Absorption Experiments through the Rat Small Intestine and the Related Physicochemical Parameters for Carbenicillin Prodrugs (I) and Carbenicillin (II)

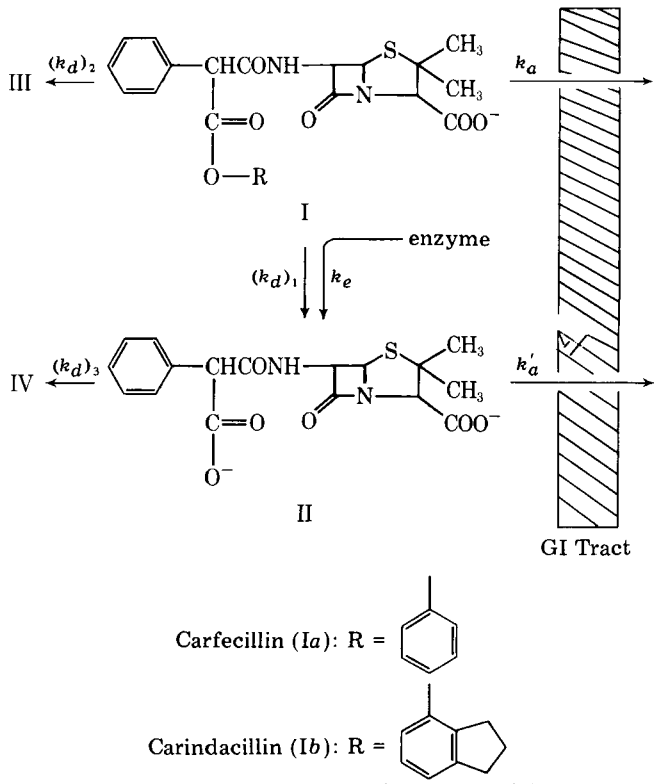
	Ia		Ib		II	
	pH		pH		pH	
	5	7	5	7	5	7
Molecular weight[a]		454.5		494.6		378.4
pKa[b]		2.91		2.94		3.06[c]
Log P_u (octanol–water)[b]		2.96		3.77		1.95[d]
P_{app}[e]	7.35	0.0741	50.8	0.512	0.207	0.00549
$10^2 k_u$ (theor)[f], ml/min	3.68	0.05	12.17	0.29	0.37	0.00
$10^2 k_{theor}$[g], ml/min	4.83	1.20	13.32	1.44	1.52	1.15
$10^2 k_{obs}$[h], ml/min	6.6	1.7	13.5	2.3	1.5	0.45[i]

[a] As free acid. [b] Determined at 37° and ionic strength 0.15, from Ref. 8. [c] Determined at 37° and ionic strength 0.5, from Ref. 9. [d] Reference 13. [e] Defined by Eq. 7. [f] Calculated from the first term of the right side in Eq. 5. [g] Calculated from Eq. 5 where k_i = 0.0115 ml/min. [h] Observed value. [i] Total disappearance including possible degradation.

the integrals, which should be equal to the areas under the respective concentration–time curves of I and II, were calculated by the trapezoidal rule.

As shown in Fig. 2, the plots of k_{app} *versus* $1/V$ for both prodrugs gave reasonably straight lines[2]. Absorption clearances at pH 7.0 calculated from the slopes were 0.017 and 0.023 ml/min for I*a* and I*b*, respectively. Despite the 50-fold difference at maximum in the oil–water partition coefficient of the ionized species of I and the other β-lactam antibiotics (7, 8), the absorption clearance of I was very close to that (average 0.012 ml/min) of other monobasic penicillins (4). This supports the fact that the intestinal absorption rate of ionic species of monobasic β-lactam antibiotics is almost independent of their lipophilicity (4).

At pH 5, the absorption clearances were calculated to be 0.066 and 0.135 ml/min for I*a* and I*b*, respectively, according to Eqs. 3 and 4. *In vitro* degradation rate constants of $(k_d)_2 = 9.7 \times 10^{-5}$ min^{-1} and 8.9×10^{-5} min^{-1} for I*a* and I*b* and $(k_d)_3 = 4.3 \times 10^{-4}$ min^{-1}, which were evaluated at 37° and pH 5.0 from the kinetic data (2, 3, 9, 10) were used in the calculations. The absorption clearance of II, k'_a, was determined to be 0.015 ml/min (Fig. 1). The prodrug absorbabilities were ~4 times larger for I*a* and 9 times larger for I*b* than that of the parent drug, II. The increased absorption rate at pH 5 with the increased lipophilicity of I may be due to the enhanced lipoidal membrane transport of undissociated species of I across the aqueous diffusion layer adjacent to the mucosal surface (11).



Scheme I—Pathways of simultaneous absorption and degradation of carbenicillin prodrug (I) in the GI.

Prediction of Penicillin Prodrug Absorbability from Quantitative Structure–Absorption Rate Relationship—A previous report (4) established that the theoretical absorption clearance, k_{theor}, for monobasic β-lactam antibiotics was generalized by the sum of the absorption clearances, $k_u + k_i$, for the undissociated and ionized species of the drug as:

$$k_{theor}\ (\text{ml/min}) = \frac{\alpha}{\sqrt{MW}}\left(\frac{f_u P_u}{\beta + f_u P_u}\right) + k_i \qquad \text{(Eq. 5)}$$

where MW is the molecular weight of the undissociated form of antibiotics, P_u is the partition coefficient of the undissociated drug between oil (*e.g.*, octanol) and water, α, and β are constants, and f_u is the fraction of undissociated species as a function of pH expressed as:

$$f_u = \frac{a_H^+}{K_a + a_H^+} \qquad \text{(Eq. 6)}$$

where a_H^+ is the hydrogen-ion activity of perfusion solution and K_a is the dissociation constant of drug. Thus, $f_u P_u$ represents the apparent partition coefficient, P_{app}, as a function of the solution pH:

$$P_{app} = f_u P_u \qquad \text{(Eq. 7)}$$

The first term in Eq. 5 was derived for k_u from the kinetic model of membrane permeation across the lipoidal barrier for the undissociated penicillin species transported through the aqueous diffusion layer adjacent to the GI membrane surface (11). With the parameters[3] α (4.62), β (35.9), and k_i (0.0115) evaluated from the absorption rates (three phenoxy derivatives and four isoxazole derivatives of penicillins) in the same experiments (4), the theoretical absorption clearances of I*a*, I*b*, and II were calculated according to Eqs. 5 and 6 as a function of lumen solution pH[4]. The results are shown in Fig. 3.

In Fig. 3, the k_a–pH profiles for penicillin V, propicillin, and dicloxacillin (4) are redrawn by conversion of the first-order absorption rate constant to its clearance using the volume (9 ml) of the perfusion solution used. The predicted values are in fairly good agreement with the experimental data for carbenicillin prodrugs and the parent drug, as well as

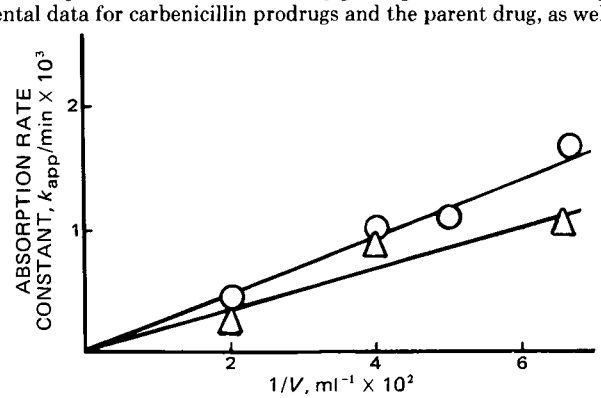


Figure 2—*Effect of the volume of perfusion solution on the absorption rates of carfecillin (Ia, △) and carindacillin (Ib, ○) from the* in situ *rat intestine at pH 7.0 under the same conditions as in Fig. 1.*

[2] For I*b*, k_{app} was reevaluated according to Eqs. 3 and 4 because the calculation in the previous paper (5) neglected the absorption rate of II.

[3] These values were evaluated from Eqs. 6, 12, 14 in Ref. 4 by changing the perfusion solution volume to 9 ml.

[4] The parameters based on these calculations are listed in Table II.

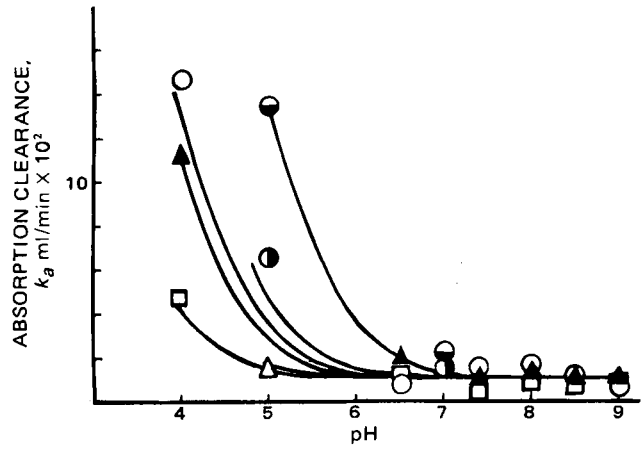


Figure 3—*Plots of the* in situ *rat intestinal absorption clearance,* k_{obs}, *of penicillins* versus *pH of the perfusion solution at 37°. The curves were generated from Eq. 5 with the corresponding parameters listed in Table II and from Ref. 4. Key:* ◒, *carindacillin (Ib);* ◑, *carfecillin (Ia);* ○, *dicloxacillin;* ▲, *propicillin;* □, *penicillin V; and* △, *carbenicillin (II). Data for dicloxacillin, propicillin, and penicillin V were taken from Ref. 4.*

for other antibiotics. The results indicate that the prodrugs, Ia and Ib, are sufficiently lipophilic to be absorbed rapidly from the GI tract by crossing the first barrier of the aqueous diffusion layer in front of the GI membrane surface and the second barrier of the lipid membrane.

The two prodrug chemical modifications of carbenicillin increase both the GI absorption rate and the acid-stability and exhibit sufficient chemical stability of the ester bond in the GI lumen. However, the results suggest that since the ester moieties of Ia and Ib are easily subject to the intestinal enzymatic metabolism, both ester prodrugs may liberate the poorly absorbable II as a result of nonspecific esterase action accompanied by absorption to reduce both bioavailabilities. After oral dosage in humans, 15–30% for Ia and 35–40% for Ib are recovered as II in urine compared with 75–100% after II intravenously (12). The relatively low recovery for I may be attributed to incomplete absorption and/or a first-pass effect. But II itself can not achieve a large urinary recovery after an oral dose.

REFERENCES

(1) J. P. Clayton, M. Cole, S. W. Elson, K. D. Hardy, L. W. Mizen, and R. Sutherland, *J. Med. Chem.*, **18**, 172 (1975).

(2) A. Tsuji, E. Miyamoto, T. Terasaki, and T. Yamana, *J. Pharm. Sci.*, **68**, 1259 (1979).

(3) H. Bundgaard, *Arch. Pharm. Chemi, Sci. Ed.*, **7**, 95 (1979).

(4) A. Tsuji, E. Miyamoto, O. Kubo, and T. Yamana, *J. Pharm. Sci.*, **68**, 812 (1979).

(5) D. Perrier and M. Gibaldi, *ibid.*, **62**, 1486 (1973).

(6) A. Tsuji, E. Miyamoto, I. Kagami, H. Sakaguchi, and T. Yamana, *ibid.*, **67**, 1701 (1978).

(7) T. Yamana, A. Tsuji, E. Miyamoto, and O. Kubo, *ibid.*, **66**, 747 (1977).

(8) A. Tsuji, O. Kubo, E. Miyamoto, and T. Yamana, *ibid.*, **66**, 1675 (1977).

(9) T. Yamana, A. Tsuji, and Y. Mizukami, *Chem. Pharm. Bull.*, **22**, 1186 (1974).

(10) H. Zia, M. Tehrani, and R. Zargarbashi, *Can. J. Pharm. Sci.*, **9**, 112 (1974).

(11) A. Tsuji, E. Miyamoto, N. Hashimoto, and T. Yamana, *J. Pharm. Sci.*, **67**, 1705 (1978).

(12) T. Bergan, *Antibiot. Chemother.*, **25**, 1 (1978).

(13) A. E. Bird, *J. Pharm. Sci.*, **64**, 1671 (1975).

ACKNOWLEDGMENTS

Presented in part at the Pharmaceutical Society of Japan, 97th Annual Meeting, Tokyo, April 1977.

The authors thank Taito Pfizer, Co. and Beecham Yakuhin, Co. for the gift of the antibiotics.

A Dissolution Anomaly Involving Ticrynafen in Simulated Intestinal Fluid without Enzyme

ELISABETH S. RATTIE, JOSEPH G. BALDINUS, LOUIS J. RAVIN ˣ, INA B. SNOW, and MIRZA M. A. BEG

Received March 2, 1981, from the *Research and Development Division, Smith, Kline & French Laboratories, Philadelphia, PA 19101.* Accepted for publication July 20, 1981.

Abstract □ Data are presented showing that the anomalous dissolution behavior of ticrynafen in simulated intestinal fluid without enzyme is due to the presence of potassium ions in the dissolution medium. Solubility studies indicate that an insoluble 1:1 complex is formed between ticrynafen and its potassium salt. This complex apparently creates an insoluble barrier that prevents complete dissolution of ticrynafen. To determine whether this might also occur in clinical use, a three-way cross-over study in 12 subjects was done. Data from this investigation show that concomitant administration of ticrynafen tablets and potassium in the form of a commercial supplement does not adversely affect bioavailability.

Keyphrases □ Ticrynafen—dissolution anomaly in simulated intestinal fluid without enzyme, potassium ions □ Potassium ions—complex with ticrynafen, dissolution anomaly in simulated intestinal fluid without enzyme □ Dissolution—anomaly, ticrynafen in simulated intestinal fluid without enzyme

Considerable effort has been devoted to the development of *in vitro* dissolution test methods that attempt to characterize the *in vitro* dissolution rate-controlled absorption of drugs administered in solid dosage forms. Unfortunately, the lack of understanding surrounding the many variables that can influence the *in vivo* dissolution, and possibly the subsequent absorption, make predictions based on *in vitro* data alone extremely difficult. The nature of the dissolution media can sometimes influence *in vitro* dissolution behavior dramatically and be misleading with regard to *in vivo* performance. The present study shows how the presence of potassium ions in simulated intestinal fluid without enzyme retarded drug dissolution without affecting the *in vivo* performance of ticrynafen[1].

EXPERIMENTAL

Materials—Ticrynafen[2], potassium ticrynafen[2], and 500-mg ticrynafen tablets[1] were obtained. All other chemicals were reagent grade and were used without further purification.

[1] 'Selacryn', Smith, Kline & French Laboratories.
[2] Smith, Kline & French Laboratories.

0022-3549/82/0400-0406$01.00/0