GI Absorption of β-Lactam Antibiotics II: Deviation from pH-Partition Hypothesis in Penicillin Absorption through *In Situ* and *In Vitro* Lipoidal Barriers

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Abstract □ The absorption of propicillin from the rat stomach and small intestine *in situ* was examined as a function of recirculating solution pH. The *in vitro* interphase transport from an aqueous buffer of various pH values to the octanol phase was also studied for several penicillins by the use of a two-phase rolling cell. The rate–pH profiles obtained from both *in situ* and *in vitro* experiments deviated significantly from the dissociation curves. The degrees of the shifts were approximately 2 pH units for the *in situ* intestinal absorption of propicillin and *in vitro* transport of propicillin and cloxacillin, approximately 1.5 pH units for the *in vitro* transport of penicillin V, and 0.8 pH unit for the *in situ* stomach absorption of propicillin. These discrepancies from the classical pH-partition hypothesis can be interpreted by the permeation through the lipoidal barrier of the undissociated species of penicillins transported through the aqueous diffusion layer adjacent to the lipoidal surface. All *in situ* and *in vitro* experiments tend to support this theory.

Keyphrases □ β-Lactam antibiotics—propicillin, GI absorption, deviation from pH-partition hypothesis, rats □ Propicillin—GI absorption, deviation from pH-partition hypothesis, rats □ Absorption, GI—propicillin, deviation from pH-partition hypothesis, rats □ pH-partition hypothesis—propicillin, GI absorption, rats □ Antibiotics, β-lactam—propicillin, GI absorption, deviation from pH-partition hypothesis, rats

The mucosal surface of the GI tract acts as a lipoidal barrier to drug absorption. To characterize absorption of foreign compounds through such a barrier, Schanker *et al.* (1) proposed the pH-partition hypothesis that the degree of absorption of weakly acidic and basic drugs depends on their lipid solubility and degree of ionization.

Penicillins are weakly acidic antibiotics with pKa values close to 2.7–2.8 (2, 3) and exhibit a wide range of lipid solubility, depending on the nature of the side chain (3–5). Much attention has been focused on the relative serum concentrations after oral administration, and GI absorption was evaluated from comparative serum level and urinary recovery data (6–10). Although some basic studies on the absorption mechanism for β-lactam antibiotics recently appeared (11–14), it is not clearly understood yet whether the absorption of penicillins obeys the pH-partition hypothesis or another specialized mechanism.

Previously, a novel kinetic method to determine simultaneously the rate constants of both absorption and degradation in the GI tract was reported (15). Based on this kinetic approach, the present investigation was performed to elucidate the pH dependency of the absorption rate and its mechanism for the undissociated penicillin from the rat GI tract. To evaluate the fundamental importance of diffusion transport of penicillins through a lipoidal barrier of the GI tract, the *in vitro* two-phase transport kinetics of penicillins also were studied.

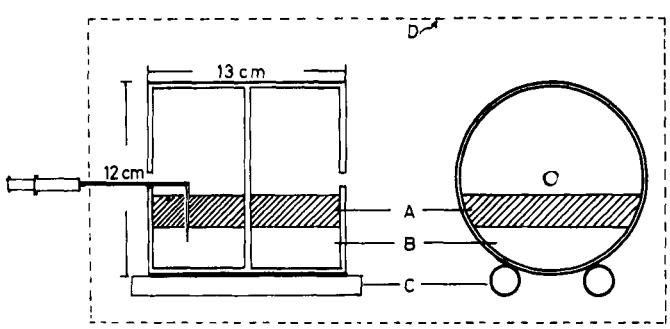

Figure 1—*Apparatus for rolling cell used for the* in vitro *two-phase transport study. Key: A, lipoidal phase; B, aqueous phase; C, rollers; and D, oven.*

EXPERIMENTAL

Materials—Dicloxacillin sodium[1] (900 μg/mg), cloxacillin sodium[1] (907 μg/mg), and oxacillin sodium[2] (840 μg/mg) were used as supplied. Other penicillins were the same as those used previously (15).

All other chemicals were reagent grade and were used without further purification, except octanol, cyclohexane, and imidazole. Octanol was purified according to a literature method (16), and cyclohexane was used after a single distillation. Imidazole was purified by double recrystallization from benzene, followed by a thorough washing with ether (17).

***In Situ* Recirculation Procedure**—Male albino Wistar rats, 165 ± 17 g, were fasted for 20 hr prior to the experiments. Water was allowed freely. The rats were anesthetized with urethan, 1.3 g/kg ip, approximately 1 hr prior to surgery.

Intestinal Absorption Procedure—The procedure for studying drug disappearance from the intestine (duodenum to ileum; ~100 cm in length) was described previously (15). A solution of propicillin (1 mg/ml) dissolved in an isotonic buffer of the desired pH (18) was perfused at the rate of 10 ml/min, unless otherwise stated. The recirculating solution was maintained at 37°, and its pH was kept constant at the desired value during the absorption experiments with a pH-stat[3]. Aliquots, 0.2 ml, of the sample solution were taken at suitable time intervals and analyzed. The apparent first-order rate constants, k_{app}, were determined by least-squares analysis.

Stomach Absorption Procedure—In a similar manner to that for the intestinal absorption experiments, the stomach was exposed, cannulated at the cardiac and duodenal ends, and washed from the cardiac end with approximately 100 ml of the perfusion solution (19). The perfusion solution was expelled with air, and the penicillin solution of various volumes (4.5–50 ml) was recirculated with a pump at 10 ml/min.

Propicillin sodium was dissolved in an isotonic buffer (18) for a final concentration of 0.5–1 mg/ml, depending on the solubility. The solution was maintained at 37°, and the solution pH was kept constant at the desired value for 3 hr with a pH-stat. After 3 hr, the remaining penicillin solution was collected, the stomach was washed thoroughly with the isotonic buffer, and the solutions were combined to make the desired volume. No significant volume change was observed, and the apparent disappearance first-order rate constant of propicillin from the perfusion solution was calculated from:

[1] Meiji Seika Kaisha, Tokyo, Japan.
[2] Banyu Pharmaceutical Co., Tokyo, Japan.
[3] pH-Stat titrator, assembly consisting of TTT2 titrator ABU12b autoburet, Radiometer, Copenhagen, Denmark.

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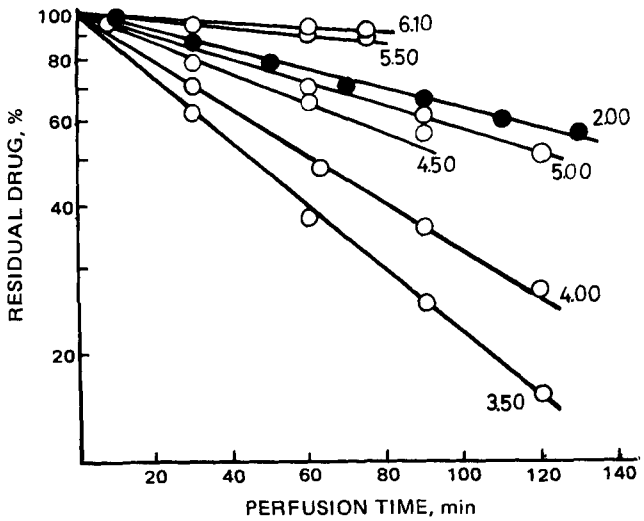

Figure 2—*Typical first-order plots of the percentage of propicillin remaining in the* in situ *rat GI recirculating perfusion experiments at various pH values and 37°. The 50-ml drug solution was perfused at the flow rate of 10 ml/min, and the pH of the solution was maintained constant with a pH-stat. Key: ●, experimental points from the stomach; and ○, experimental points from the intestine.*

$$k_{app} = -\frac{1}{3}\ln\frac{C^f}{C^0} \qquad \text{(Eq. 1)}$$

where C^0 and C^f are the concentrations of propicillin at initial and final stages, respectively.

Analytical Method—The samples were diluted with distilled water to produce a final concentration of 1×10^{-5}–1×10^{-4} *M*. They were then passed through a 0.45-μm filter[4] to remove any solid materials and assayed spectrophotometrically[5] at 325 nm by the method of Bundgaard and Ilver (17). No materials in the samples from the *in situ* experiments below pH 6 interfered significantly. This condition was confirmed by the use of recirculating solutions without the antibiotics.

Two-Phase Transport Procedure by Rolling Cell—A rolling cell (Fig. 1), similar to that described by Robertson and Madsen (20), was used for the *in vitro* transport study of penicillin. The apparatus consisted of a glass cell slowly rotated on rollers in an oven thermostated at a constant temperature of 32 ± 1°, unless otherwise stated. The cell was divided into two parts of an identical volume by a central partition.

All aqueous buffer solutions and lipid solutions were mutually saturated before the diffusion experiments. The buffers were citrate, acetate, and phosphate systems, depending on the pH range; their ionic strength was adjusted to 0.15 with potassium chloride. The pH of the aqueous phase was measured[6] before use and at the end of the experiments, and no significant change was observed. All experimental conditions, pH of the aqueous phase, temperature, and diffusion period, were chosen so that the degradation of the β-lactam moiety of penicillins may occur within 2% during the experiments.

The cell was rotated at a constant speed, followed by the addition of 25 ml of penicillin stock solution. This solution was prepared with the buffer used for the transport study to make a final concentration of 5×10^{-5}–2×10^{-4} *M*, according to the penicillin solubilities. At various time intervals, 3-ml samples were removed from the aqueous phase and 3 ml of aqueous buffer, preheated to the temperature studied, was added successively to maintain the constant surface area to volume ratio. All additions and withdrawals were made through a stainless steel tube inserted into the aqueous phase, which was connected with a 5-ml injector to the exterior of the oven (Fig. 1).

To measure the infinite concentration, C^∞, the same two-phase experiment was conducted in the other part of the cell without sampling for 20–120 min, depending on the rate of both the transfer and the degradation. The final sample aliquot then was taken from the aqueous phase.

All samples from the aqueous phase were centrifuged at 3000 rpm; after appropriate dilution with distilled water, if necessary, they were analyzed by UV spectrophotometry at 260 nm and/or by the method of Bundgaard and Ilver (17). The concentration of penicillins in the aqueous solution was determined from the previously prepared Beer's law plot for the respective antibiotics and was corrected for the dilution arising from each sampling.

[4] Sartorius-Membranfilter GmbH, 34 Göttingen, Germany.
[5] UV-200S double-beam spectrophotometer, Shimadzu, Kyoto, Japan.
[6] PHM 26 meter, Radiometer, Copenhagen, Denmark.

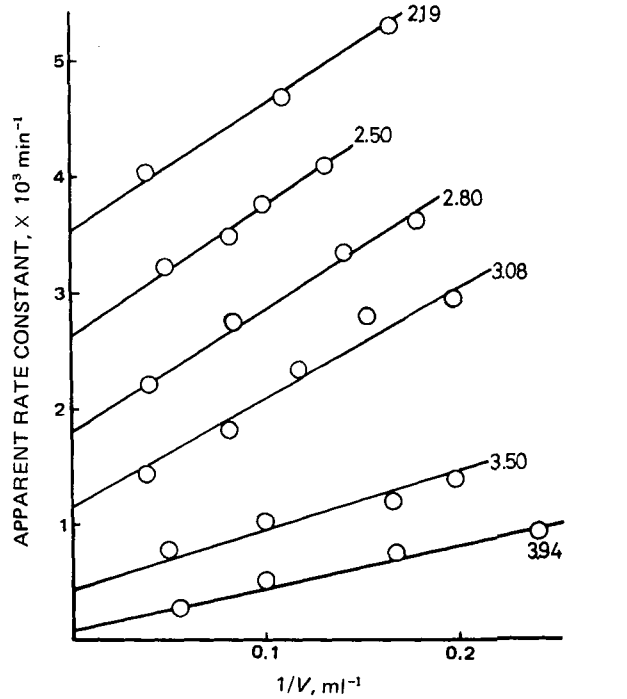

Figure 3—*Effect of the volume of the perfusion solution on absorption rate constants of propicillin from the* in situ *rat stomach at various pH values and 37°. The drug solution was perfused at the flow rate of 10 ml/min, and the pH of the solution was maintained constant with a pH-stat.*

RESULTS

Absorption of Propicillin from Rat Stomach and Intestine *In Situ*—*Rates of Nonenzymatic Degradation and Absorption*—Semilogarithmic plots, showing the disappearance of propicillin from the perfusion solution through the rat stomach and intestine, are shown in Fig. 2. The total disappearance followed first-order kinetics, as observed previously for other semisynthetic β-lactam antibiotics (15).

The apparent first-order rate constants, k_{app}, can be expressed as (15):

$$k_{app} = k_a\frac{1}{V} + k_d \qquad \text{(Eq. 2)}$$

where k_a is the first-order absorption rate constant with units of milliliters time^{-1}, k_d is the first-order nonenzymatic degradation rate constant with units of time^{-1}, and V is the volume of the recirculating drug solution. According to Eq. 2, a plot of k_{app} *versus* $1/V$ provides a straight line with

Table I—Absorption Rate Constant of Propicillin from the *In Situ* Rat GI Tract at Various pH Values and 37°

Stomach		Intestine	
pH[a]	$k_a \times 10^3$, ml min^{-1} [b]	pH[a]	$k_a \times 10^2$, ml min^{-1} [c]
2.19	11.0	3.30	68.2
2.50	11.2	3.50	63.2
2.80	10.5	3.50	75.8
3.08	9.33	4.00	53.8[d]
3.50	5.17	4.50	35.8
3.94	3.42	5.00	28.0
		5.50	8.32
		6.10	4.82

[a] The pH of the perfusion solution was maintained constant with a pH-stat. [b] Obtained from the slope in Fig. 3 according to Eq. 2. [c] Calculated according to Eq. 2. [d] Values determined previously (15).

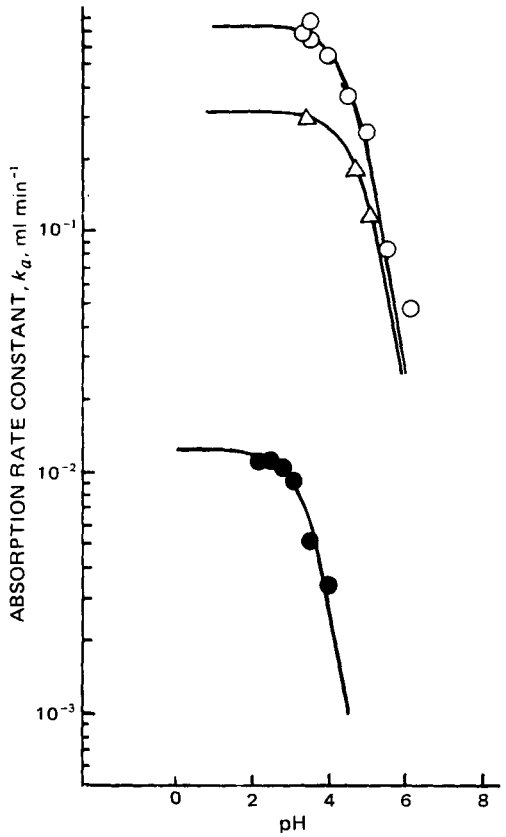

Figure 4—*Plots of the* in situ *rat GI absorption rate constants,* k_a, *of propicillin* versus *the pH of the perfusion solution at 37°. Key:* ●, *experimental points from the stomach at the flow rate of 10 ml/min;* ○, *experimental points from the intestine at the flow rate of 10 ml/min; and* △, *experimental points from the intestine at the flow rate of 10 ml/min for 2.5 min and with perfusion stopped for 10 min. The solid lines were generated from Eq. 17 and the parameters listed in Table VII.*

an intercept equal to k_d and a slope equal to k_a if the absorption process obeys Fick's law. This equation was substantiated previously in the *in situ* rat intestinal absorption experiments of various β-lactam antibiotics at pH 4.0 (15).

For the stomach recirculation experiments with propicillin at various pH values, plots according to Eq. 2 are shown in Fig. 3. Good straight lines were obtained with different slopes and intercepts at every pH of the propicillin solution. The k_d values determined from the intercepts were almost equal to those determined *in vitro* at the same pH, suggesting that the nonenzymatic degradation of penicillin in perfusion solution through the rat stomach depends only on the bulk pH values, as observed previously in the experiment on rat small intestinal absorption (15). Table I summarizes the absorption rate constants, k_a, determined according to Eq. 2 for the gastric and intestinal absorption of propicillin.

Table II—Degree of the pH Shift of the *In Situ* Absorption Rate- and *In Vitro* Transfer Rate-pH Profiles for Propicillin, Cloxacillin, and Penicillin V under Various Hydrodynamic Conditions

Penicillin	Rotating Speed, rpm, or GI Tract	pKa[a]	pKa^{app} [b]	Degree of pH Shift[c]
		In Vitro		
Propicillin	10	2.76	5.08 ± 0.07	2.32
Propicillin	20	2.76	5.02 ± 0.11	2.26
Propicillin	38	2.76	4.89 ± 0.05	2.13
Cloxacillin	20	2.78	4.72 ± 0.04	1.94
Penicillin V	10	2.79	4.35 ± 0.12	1.56
Penicillin V	38	2.79	4.00 ± 0.18	1.21
		In Situ		
Propicillin	Stomach[d]	2.76	3.49 ± 0.08	0.73
Propicillin	Intestine[d]	2.76	4.61 ± 0.10	1.85
Propicillin	Intestine[e]	2.76	4.87 ± 0.03	2.11

[a] Reference 3. [b] pKa^{app} ± *SD*, computed from the data in Fig. 4 and Figs. 8–10 according to Eq. 17 by nonlinear regression analysis[7]. [c] pKa^{app} − pKa. [d] Perfused at 10 ml/min. [e] Perfused at 10 ml/min for 2.5 min and statically for 10 min (see text).

pH–Absorption Rate Profile of Propicillin—In Fig. 4, the absorption rate constants, k_a, from the rat stomach and small intestine are plotted against the bulk pH of the perfusion solution. Intestinal absorption rates of propicillin were about 100 times faster than the gastric absorption rates at every pH, undoubtedly because of the relative surface areas and physiological organization of the two sites (19, 21).

From these pH–rate profiles, it is apparent that the major absorbable species of propicillin is its undissociated form rather than the ionized one at both absorption sites. If the pH–partition hypothesis is valid for propicillin absorption, the theory described by Eq. 3 gives, for the apparent pKa^{app} values (Table II) to be best fit[7] with the data for the perfusion flow rate of 10 ml/min (Fig. 4), 4.61 and 3.49 for the intestinal and gastric absorption, respectively:

$$k_a = k_u \left(\frac{a_H}{a_H + K_a^{app}}\right) \qquad \text{(Eq. 3)}$$

where a_H is the bulk hydrogen-ion activity of the drug solution, K_a^{app} is the apparent dissociation constant of the drug, and k_u is the absorption rate constant for the undissociated drug. The pKa^{app} values thus computed, however, significantly differ from the true pKa = 2.76 (3) of propicillin itself. This discrepancy is not well interpreted by the classical pH–partition theory.

***In Vitro* Transport of Penicillins in Water–Oil Two-Phase Model**—*Kinetics of Interphase Transfer*—When the transfer of a drug from the aqueous phase to the lipid phase obeys Fick's law of diffusion, Eq. 4 can be derived by assuming that a rapid partition equilibrium is always present at the interphase:

$$-\frac{dC_I}{dt} = k_{app}\left(C_I - \frac{C_{II}}{P_{app}}\right) \qquad \text{(Eq. 4)}$$

where C_I and C_{II} represent the concentrations of the drug in the aqueous phase (Compartment I) and in the oil phase (Compartment II), respectively; P_{app} is the apparent partition coefficient; and k_{app} is the apparent first-order transfer rate constant. The total quantity of the drug in two phases is given as:

$$Q_0 = Q_I + Q_{II} \qquad \text{(Eq. 5)}$$

Therefore:

$$V_I C_0 = V_I C_I + V_{II} C_{II} \qquad \text{(Eq. 6)}$$

where Q_I and Q_{II} represent the quantities of the drug in the aqueous and oil phases, respectively; Q_0 and C_0 are the quantity and concentration in the aqueous phase at time zero, respectively; and V_I and V_{II} are the volumes of the respective phases.

Combination of Eqs. 4–6 leads to:

$$-\frac{dC_I}{dt} = k_{app}[(F+1)C_I - FC_0] \qquad \text{(Eq. 7)}$$

where:

$$F = \frac{V_I}{P_{app} V_{II}} \qquad \text{(Eq. 8)}$$

Integration of Eq. 7 yields:

$$\log(C_I - C_I^\infty) = \log(C_0 - C_I^\infty) - \frac{k_{obs}}{2.303} t \qquad \text{(Eq. 9)}$$

where:

$$C_I^\infty = \frac{F}{1+F} C_0 \qquad \text{(Eq. 10)}$$

$$k_{obs} = k_{app} \frac{C_0}{C_0 - C_I^\infty} \qquad \text{(Eq. 11)}$$

where C_I^∞ represents the concentration of the drug in the aqueous phase at infinite time.

According to Eq. 9, it is predicted that a plot of the logarithm of $C_I - C_I^\infty$ *versus* time provides a straight line with a slope of $-k_{obs}/2.303$. Typical plots for propicillin, cloxacillin, and penicillin V are shown in Fig.

[7] A nonlinear regression analysis by the Fortran IV computer program written by the authors. The digital computer, FACOM 230-35, was used at the Data Processing Center, Kanazawa University.

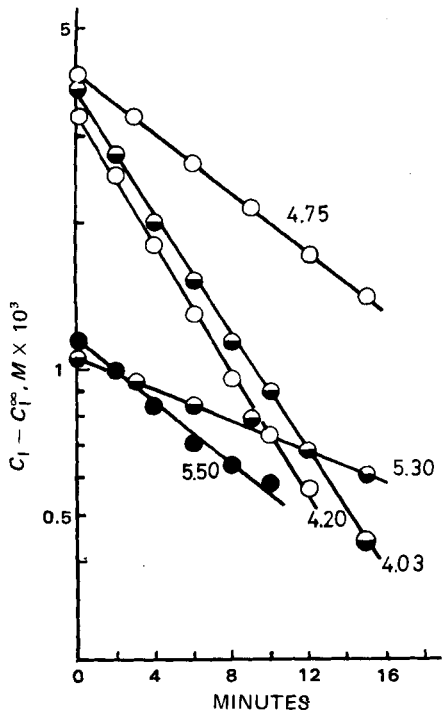

Figure 5—*Typical plots of* ($C_l - C_l^\infty$) versus *time for the two-phase transport of penicillins at various pH values and 32 ± 1°. Key:* ●, *cloxacillin, pH 5.50 (20 rpm);* ○, *propicillin, pH 4.20 (20 rpm) and pH 4.75 (10 rpm); and* ◒, *penicillin V, pH 5.30 (10 rpm) and pH 4.03 (38 rpm). The aqueous phase was acetate and citrate buffers of 0.15 ionic strength, and the oil phase was octanol.*

5, giving good straight lines in accordance with the prediction. The observed first-order rate constants, k_{obs}, can be converted to the apparent first-order transfer rate constant, k_{app}, with Eq. 11.

Factors Affecting Apparent Intestinal Transfer Rates—The diffusion rate constant, k_{app}, can generally be represented as:

$$k_{app} = \Phi \frac{DS}{V_I L_I} \qquad \text{(Eq. 12)}$$

where D is the diffusion coefficient of the drug, L_I is the aqueous diffusion layer thickness, S is the geometrical surface area of the interphase, and Φ represents a factor affected by the degree of ionization of diffusion molecule, solvent composition, and hydrodynamic situations. If Eq. 12 is valid for the interphase transfer of penicillins, k_{app} should be affected by the change in L_I, V_I, and S.

Table III demonstrates the effect of rotating speed on the cloxacillin transport rate from the aqueous phase, pH 4.0 acetate buffer, to the lipoidal phase, octanol–cyclohexane (1:1 v/v). The enhanced transport rates with an increasing rotating rate may exclusively be the result of a decrease in the diffusion layer thickness, L_I, since D, V_I, and S remain constant.

Equation 12 predicts that k_{app} depends on the change in the surface of the aqueous phase to volume ratio. As shown in Fig. 6 for the transfer of cloxacillin at 30 rpm from the aqueous pH 4.0 acetate buffer into octanol–cyclohexane (1:1 v/v), a plot of k_{app} *versus* S/V_I yields a straight line through the origin in accordance with the theory (Eq. 12).

The temperature effect on the transfer rate of penicillins from the

Table III—Effect of Rotating Rate on the Transfer Rate Constant of Cloxacillin at 33° from the Aqueous Phase [a] to the Lipoidal Phase [b]

Rotating Rate, rpm	Transfer Rate Constant, k_{app}, min^{-1}
9	0.05
20	0.10
30	0.12
38	0.17

[a] Acetate buffer (0.02 *M*) of 0.15 ionic strength and pH 4.0. [b] Octanol–cyclohexane (1:1 v/v).

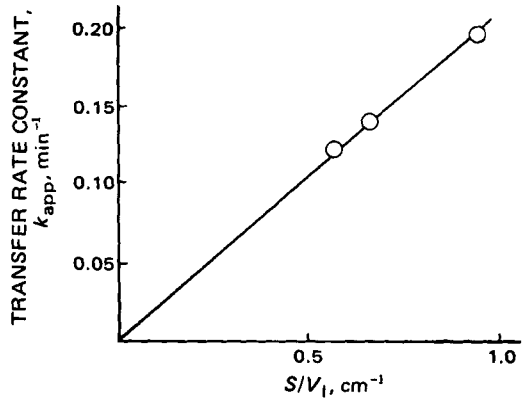

Figure 6—*Effect of the surface area to volume ratio on the transfer rate constant,* k_{app}, *for the* in vitro *two-phase transport of cloxacillin at 30 rpm at pH 4.0 and 33°. The aqueous phase was 0.02* M *acetate buffer of 0.15 ionic strength, and the oil phase was octanol–cyclohexane (1:1 v/v).*

aqueous phase into the octanol phase was examined for various penicillins. The Arrhenius equation predicts:

$$k_{app} = k_0 e^{-\Delta E_a/RT} \qquad \text{(Eq. 13)}$$

where k_0 represents the transfer rate constant independent of temperature and ΔE_a is the activation energy for the interphase transfer. Arrhenius plots (Fig. 7) yielded good straight lines for all penicillins studied. The activation enthalpies, $\Delta H^{\neq}$ ($= \Delta E_a - RT$), at 37° (Table IV) ranged from 1 to 4 kcal/mole and were similar to those obtained for the interphase transport of sulfonamides (22). Such small activation enthalpies may reflect those of diffusion coefficients (23).

All results obtained suggest strongly that *in vitro* interphase transport of penicillins in the two-phase rolling cell follows the rate-limiting diffusion in accordance with Eq. 12.

pH Dependency of Apparent Interphase Transfer Rates—Since quasi-steady-state and perfect sink conditions can be achieved in a system where the drug concentration transferred into the oil phase may be sufficiently diluted, experiments in such a transport system are expected to be sufficient for theoretical consideration of the diffusion kinetics. Rate constants, k_{app}, determined under sink conditions, which could be obtained by the dilution of the oil phase with fresh octanol through a recirculating pump, were considered to be approximately equal to those determined in the system with an equal volume phase of 100 ml (Table V).

For experimental convenience, therefore, k_{app} values were all determined as a function of pH over the 3.0–6.0 range in the two-phase system of 100 ml each of aqueous buffer and octanol under various hydrodynamic conditions, being regarded approximately as quasi-steady-state rate constants.

For comparison between *in situ* and *in vitro* absorption and transport,

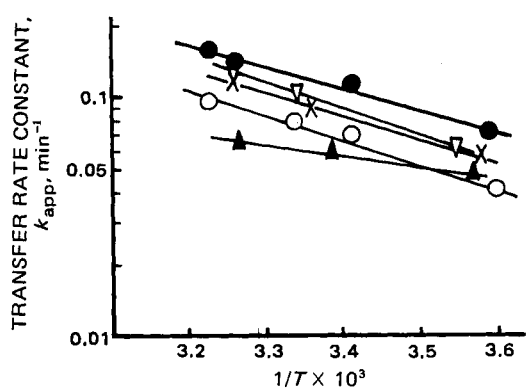

Figure 7—*Arrhenius plots for the* in vitro *two-phase transport rate constants of penicillins at 20 rpm. The aqueous phase was 0.15* M *acetate buffer of pH 4.20, except for dicloxacillin at pH 4.70, and of 0.15 ionic strength. The oil phase was octanol. Key:* ▲, *dicloxacillin;* ▽, *cloxacillin;* ×, *oxacillin;* ●, *propicillin; and* ○, *penicillin V.*

Table IV—Activation Enthalpy at 37° for the Transfer Rate at 20 rpm of Penicillins from the Aqueous Phase [a] to the Lipoidal Phase [b]

Penicillin	$\Delta H^{\neq}$ [c], kcal/mole
Dicloxacillin	1.4
Cloxacillin	4.4
Oxacillin	3.7
Propicillin	3.5
Penicillin V	4.0

[a] Acetate buffer (0.15 *M*) of 0.15 ionic strength. [b] Octanol. [c] $\Delta H^{\neq} = \Delta Ea - RT$ at pH 4.20, except dicloxacillin at pH 4.70.

Table V—Effect of Dilution of the Lipoidal Phase to Examine Sink Conditions for the Transfer Rate Constant of Propicillin at 10 rpm at 31°

pH	Aqueous Phase[a], ml	Lipoidal Phase[b], ml	Transfer Rate Constant, $k_{app} \times 10^2$ min^{-1}
5.27	100	600[c]	2.60
5.25	100	350[d]	2.59
5.23	100	100	3.38

[a] Citrate buffer (0.03 *M*) of 0.15 ionic strength. [b] Octanol. [c] The oil phase of 100 ml was exchanged continuously with 500 ml of fresh octanol through a circulation pump. [d] The oil phase of 100 ml was exchanged continuously with 250 ml of fresh octanol through a circulation pump.

Table VI—Effect of the Initial Concentration for the Transfer Rate Constant of Propicillin from the Aqueous Phase [a] to the Lipoidal Phase [b] at 20 rpm and 31°

pH	Initial Concentration, *M*	Transfer Rate Constant, k_{app}, min^{-1}
4.20	1×10^{-2}	0.154
4.20	5×10^{-3}	0.140
4.10	1×10^{-3}	0.150
4.10	5×10^{-4}	0.152
4.10	1×10^{-4}	0.153

[a] Acetate buffer (0.15 *M*) of 0.15 ionic strength. [b] Octanol.

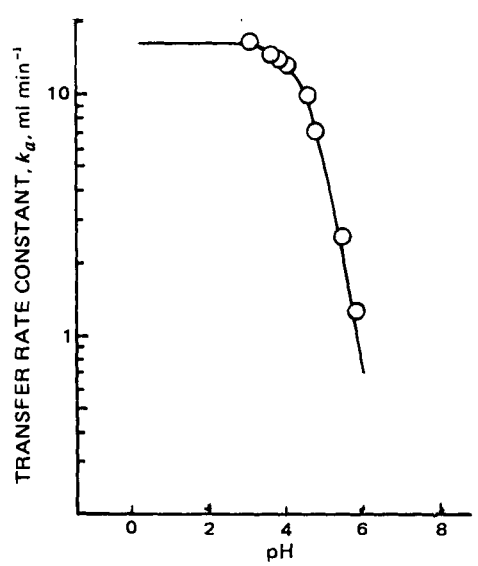

Figure 8—*Plots of the transfer rate constant,* k_a, *of cloxacillin* versus *the pH of the aqueous phase in* in vitro *two-phase transfer experiments at 20 rpm and 33°. The points are experimental values, and the solid line was generated from Eq. 17 and the parameters listed in Table VII.*

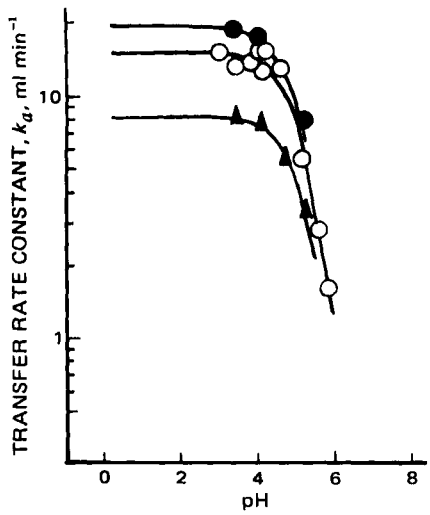

Figure 9—*Plots of the transfer rate constant,* k_a, *of propicillin at various rotating rates* versus *the pH of the aqueous phase in* in vitro *two-phase transfer experiments at 31°. Key:* ▲, *10 rpm;* ○, *20 rpm; and* ●, *38 rpm. The points are experimental values, and the solid lines were generated from Eq. 17 and the parameters listed in Table VII.*

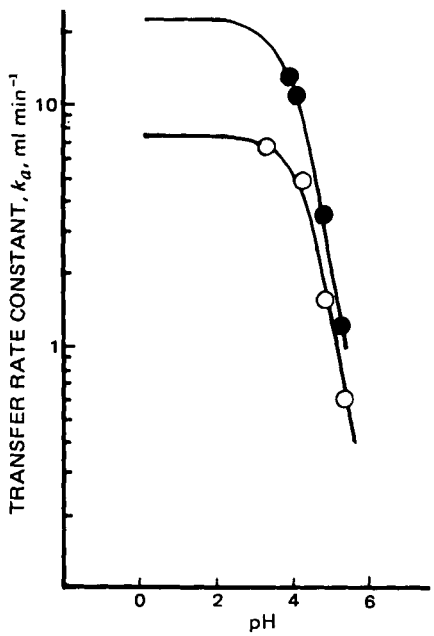

Figure 10—*Plots of the transfer rate constant,* k_a, *of penicillin V at various rotating rates* versus *the pH of the aqueous phase in* in vitro *two-phase transfer experiments at 31°. Key:* ○, *10 rpm; and* ●, *38 rpm. The points are experimental values, and the solid lines were generated from Eq. 17 and the parameters listed in Table VII.*

the first-order interphase transfer rate constants, k_a, can be defined as:

$$k_{app} = k_a \frac{1}{V} \qquad \text{(Eq. 14)}$$

These results are illustrated in Figs. 8–10 as plots of log k_a for cloxacillin, propicillin, and penicillin V *versus* the bulk pH of the aqueous phase. The k_a–pH profiles showed apparently rightward shifts relative to the corresponding dissociation curves. The best fitting pKa^{app} values were computed according to Eq. 3 in a manner similar to that already described (Table II). These values differed by 1–2 pKa units from the respective true pKa values (3).

In the present *in vitro* two-phase experiments, the extent of the penicillin degradation in the aqueous phase was within 2% during transport kinetics. No significant change in the rates was observed by the change in the initial concentration of penicillins (Table VI). Below pH 5, trans-

port of ionized species of penicillin can be regarded as almost negligible, considering that the partition ratios of undissociated to ionized species between water and octanol are approximately 10^3 for the respective penicillins (3).

From these findings, the deviation of Eq. 3 from the pH–partition theory can be concluded to be virtually due to the diffusion kinetics of undissociated penicillin species.

DISCUSSION

Discrepancies from the pH–partition hypothesis occur in the GI absorption of some drugs (19, 24–35). The contribution of physiological factors such as the requirement of a higher acid pH, *i.e.*, virtual pH at the membrane surface (24, 27, 28), or drug binding to the intestinal mucosal surface (25, 26, 29) was proposed as being responsible for deviation from this hypothesis. The two possibilities cannot explain the shifts of pH–rate curves of penicillins observed for *in vitro* interphase transport, where the presence of complicated physiological factors can be completely excluded. Therefore, there is no reason to apply both the virtual pH and binding hypotheses for the harmonious interpretation of both *in situ* and *in vitro* data observed for penicillins.

In situ kinetic evaluation of the absorption of ionic species of the drug was successful in the interpretation of deviation from the pH–partition equation for intestinal absorption of sulfaethidole and barbital (31), salicylic acid (30), and carbenoxolone (26). Ionized penicillin can be absorbed by the rat intestine to a considerable extent in neutral and alkaline pH regions[8], but this contribution to the total intestinal absorption rates had no significant effect on the pH–intestinal absorption curve. For a similar deviation from the pH–partition hypothesis in the *in vitro* experiments for propicillin, cloxacillin, and penicillin V, contribution of the transport rate of the ionic species was also negligible in the water–octanol system, as already discussed.

Therefore, attribution of both *in situ* and *in vitro* deviations observed for penicillins to the hydrodynamic diffusion theory involving the aqueous diffusion layer seems to be the most reasonable explanation.

A two-compartment diffusion model related to drug transport and absorption was developed (33–35). The first compartment (mucosal side; aqueous phase) consists of a bulk aqueous drug solution phase and a diffusion layer of thickness L_I, and it is in series with the second compartment consisting of a lipid phase (membrane) of thickness L_{II}. If there is a perfect sink on the second side after the lipid barrier and only the nonionized drug species permeates through the lipid membrane, the diffusion model yields the apparent quasi-steady-state first-order absorption rate constant, k_{app}, for acidic drugs (33):

$$k_{app} = \frac{SD_{aq}}{V_I L_I}\left[\frac{1}{\left[1 + \frac{K_a}{(a_H)s}\right]T + 1}\right] \qquad \text{(Eq. 15)}$$

where:

$$T = \frac{L_{II} D_{aq}}{L_I D_{lip} P_u R} \qquad \text{(Eq. 16)}$$

where D_{aq} and D_{lip} are the diffusion coefficients of the drug in water and lipid, respectively; P_u is the partition coefficient of the undissociated drug between the lipid and aqueous solution; $(a_H)s$ is the hydrogen-ion activity at the surface of the lipoid phase (membrane); and R is the ratio of the true interfacial area to the geometrical area. In a well-buffered solution, as in the present *in situ* and *in vitro* experiments, the pH at the lipoidal surface would not differ substantially from that in the bulk of the solution (36). Hence, $(a_H)s = a_H$.

Rearrangement of Eq. 15 yields:

$$k_a = k_u' \frac{a_H}{\left(1 + \frac{1}{T}\right)a_H + K_a} \qquad \text{(Eq. 17)}$$

where:

$$k_u' = \frac{SD_{lip} P_u R}{L_{II}} \qquad \text{(Eq. 18)}$$

where k_a is equal to $k_{app} V_I$ and the first-order rate constant is independent of the volume of the bulk drug solution. If T is large, the T^{-1} term in Eq. 17 becomes negligible compared to 1 and Eq. 17 can be reduced to an equation similar to Eq. 3, predicted by the pH–partition hypothesis.

[8] To be published.

Table VII—Computed Parameters in Accordance with Aqueous Diffusion Layer Theory for pH–k_a Profiles of *In Situ* Rat Intestinal and Gastric Absorption of Propicillin and *In Vitro* Interphase Transport of Propicillin, Cloxacillin, and Penicillin V under Various Hydrodynamic Conditions

Penicillin	Rotating Speed, rpm, or GI Tract	k_u'[a], ml min^{-1}	$T \times 10^3$
		In Vitro	
Propicillin	10	1697 ± 250	4.79 ± 0.81
Propicillin	20	2748 ± 706	5.53 ± 1.57
Propicillin	38	2593 ± 288	7.52 ± 0.93
Cloxacillin	20	1397 ± 116	11.73 ± 1.14
Penicillin V	10	266 ± 67	28.66 ± 9.06
Penicillin V	38	367 ± 87	65.42 ± 3.15
		In Situ	
Propicillin	Stomach[b]	0.07 ± 0.01	229.70 ± 54.08
Propicillin	Intestine[b]	51.14 ± 12.31	128.70 ± 9.16
Propicillin	Intestine[c]	40.77 ± 2.39	69.80 ± 19.63

[a] Computed from the data in Figs. 4 and 8–10 according to Eq. 17 by nonlinear regression analysis. [b] Perfused at 10 ml/min. [c] Perfused at 10 ml/min and statically for 10 min (see text).

If T is not large or if it cannot be ignored, Eq. 17 predicts the rightward deviation from the pH–partition curve depending on the magnitude of T. This physical model was applied to various *in situ* absorption rates involving buccal, gastric, intestinal, and rectal absorption with satisfactory results (34, 35, 37).

If both *in situ* and *in vitro* deviations from the pH–partition hypothesis presently observed for penicillins were to be substantially attributed to the aqueous diffusion layer theory described by Eq. 17, rather than to the extraction theory (32) derived from the distribution model of the permeating substance between the barrier and bulk phase solution, a change in the T value should produce a change in the shift of pH–absorption profiles. The increasing thickness of the aqueous diffusion layer, L_I, should lower the T value and thereby not only decrease the absorption rate but also yield a rightward shift of the pH–rate curve from the dissociation curve.

Such a prediction was examined for both *in vitro* interphase transport and *in situ* absorption experiments. Figures 9 and 10 show that the effect of the rotating speed decreased the transfer rates and also produced a significant rightward shift of the profile. Figure 4 shows the *in situ* absorption rate–pH profiles of propicillin under two different hydrodynamic conditions; one was carried out at the flow rate of 10 ml/min for 2.5 min with a static situation for 10 min. When the drug solution was less agitated, the intestinal absorption rates were significantly decreased and the profile shifted rightward, in accordance with the prediction from Eq. 17. The best fit parameters[7] according to Eq. 17 for various pH–k_a data are summarized in Table VII.

All experimental evidence obtained in this study strongly suggested the importance of the aqueous diffusion layer adjacent to the lipid, both for the *in vitro* transport and the *in situ* absorption of penicillin molecules. The deviation of the *in situ* gastric and intestinal absorption rate–pH profiles by approximately 0.8 and 2 pH units, respectively, from the dissociation curve can be regarded as the result of the membrane transport of undissociated species of penicillin permeating through the barrier of the aqueous diffusion layer rather than other possibilities such as the virtual pH hypothesis and drug–mucosal binding theory, which can be easily ruled out in the *in vitro* simple diffusion experiment. The difference in the extent of the shifts observed between the gastric and intestinal absorption rate–pH profiles is probably due to the ratio of the aqueous diffusion layer permeability, D_{aq}/L_I, to the lipoidal membrane permeability, $D_{lip}P_u/L_{II}$, and/or the different ratio of the true interfacial area to geometrical area, R, at the respective absorption sites.

The results of the intestinal absorption behavior of various penicillins in the region of pH 4–9 will be presented in a subsequent paper, and the quantitative relationship between the rates and the structural properties will be discussed according to the aqueous diffusion layer theory.

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Dissolution Kinetics of Cholesterol in Simulated Bile II: Influence of Simulated Bile Composition

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Abstract □ Normal human gallbladder bile and gallbladder bile of patients undergoing chenodeoxycholic acid therapy were simulated by using appropriate combinations of taurine and glycine conjugates of cholic, chenodeoxycholic, and deoxycholic acids. Also, the total bile acid concentration and the total bile acid to lecithin ratio were varied over physiological ranges. Dissolution rates of cholesterol monohydrate pellets (model gallstone) in these solutions were 90–99% interfacially controlled. Even under conditions favorable for dissolution, *i.e.*, high bile acid concentration and high bile acid to lecithin ratio, the interfacial resistances were extremely large. These results are of the same order of magnitude as those found in the limited studies with actual gallbladder bile and suggest that the bile acids, lecithin, and the electrolytes are the primary determinants of the interfacial resistance for cholesterol dissolution. Furthermore, the kinetics of dissolution were always much faster with the chenodeoxycholic acid-rich compositions than with the corresponding normal compositions. This finding suggests, therefore, that in addition to desaturating bile with respect to cholesterol, the feeding of chenodeoxycholic acid further facilitates cholesterol gallstone dissolution by reducing the interfacial resistance of the process.

Keyphrases □ Cholesterol—dissolution kinetics in simulated bile, effect of bile composition □ Dissolution kinetics—cholesterol in simulated bile, effect of bile composition □ Bile composition—effect on dissolution kinetics of cholesterol in simulated bile

Recent studies (1–7) on the dissolution of human cholesterol gallstones and cholesterol monohydrate pellets in bile acid–lecithin solutions and in human gallbladder bile indicated that:

1. The dissolution of both cholesterol gallstones and cholesterol monohydrate pellets (model gallstones) was interfacially controlled rather than diffusion–solubility controlled, providing a possible explanation for the rather slow rate of stone dissolution *in vivo* (8).

2. The interfacial resistance to dissolution was a function of the composition of the simulated bile solution—*viz.*, the bile acid type and concentration, the bile acid to lecithin ratio, and the electrolyte type and concentration.

3. The magnitudes of the interfacial resistances in both the simulated bile solutions and the human gallbladder biles were indeed comparable.

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