Table VIII—Particle-Size Results for Triamcinolone

Sample	Geometric Volume Mean Diameter, μm	Geometric *SD*
A	9.5	1.7
B	8.5	1.8
C	9.2	1.7
D	10.3	1.8
E	10.8	1.8
F	9.3	1.9
G	8.3	1.8
H	8.7	1.8

sonable measure of the particle size for triamcinolone by shaking the sample vial gently to disperse the powder. The results presented for triamcinolone indicate that the method of sampling and dispersion is as important to the particle-size measurement as the parameters relating directly to the measurement. In fact, it was found previously that the error in counting particles by the electrozone method is less than the error due to sampling (3).

CONCLUSIONS

Experience has shown that, for pharmaceutical powders, the volume or mass basis (identical to volume if the measured particles have equal densities) is the most useful way of representing particle diameters for milled and micronized powders. These powders usually have log-normal particle distributions (7) that can be described completely by the geometric median or mean diameter and the geometric standard deviation. In addition, milled and micronized materials are described adequately by a spherical diameter; therefore, the automated electrozone system described in this paper is excellent for quality control purposes.

The electrolytic sensing zone method does have some limitations. The particles must be relatively insoluble in a solvent that has a moderate dielectric constant. Unusually shaped particles (*i.e.*, long rods or platelets) are converted to a mean diameter that is considerably less than the longest dimension of the actual particle. For example, a sphere of 4-μm diameter would have approximately the same volume as a cylinder of diameter 2.5 μm and length 7.5 μm. Thus, the method is relatively insensitive to particle shape. However, the advantages clearly outnumber the disadvantages. The method is highly sensitive, fast, and generally applicable. Sample preparation and data reduction are simple. The operator-fatigue factor is much less than for microscopy.

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Simultaneous Partitioning and Hydrolysis Kinetics of Amoxicillin and Ampicillin

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Abstract □ The kinetics of ampicillin and amoxicillin partitioning with simultaneous acid-catalyzed hydrolysis were studied in a stirred transfer cell containing isobutanol as the extract and aqueous hydrochloric acid (0.1–0.5 *N*) as the raffinate at 37°. Biexponential data for the concentration in both the raffinate (C_1) and the extract (C_2) as a function of time were analyzed simultaneously by nonlinear regression to estimate the apparent first-order rate constant for transfer from hydrochloric acid to isobutanol (k'_{12}), the reverse transfer constant (k'_{21}), and the hydrolysis rate constant (k). Agreement between k values determined in the presence of simultaneous partitioning and those determined in the absence of partitioning (k_{app}) verified the nonlinear estimates. Apparent partition coefficients, which represent the values that would be obtained in the absence of hydrolysis ($K'_D = C_1^\infty/C_2^\infty$), were estimated from $K'_D = k'_{12}/k'_{21}$. During terminal monoexponential loss, where $C_1 \simeq Y'e^{-\beta t}$ and $C_2 \simeq Z'e^{-\beta t}$, the kinetically controlled C_2/C_1 ratio (r) is described by $[k'_{12}/(k'_{21} - \beta)]$, which decreases with decreasing k values until r approaches K'_D. The difference between the terminal concentration ratio, r, and its corresponding partition coefficient, K'_D, is a measure of the degree to which kinetic processes control distribution. Both ampicillin and amoxicillin showed kinetic control of the distribution ratios in 0.5 *N* HCl, where the hydrolysis rate constant was significant relative to the distribution rate constants. Ampicillin had $r \simeq 1.74$ and $K'_D \simeq 0.92$; amoxicillin had $r \simeq 0.95$ and $K'_D \simeq 0.65$. As the $(k'_{12} + k'_{21})/k$ ratio increased, the r values approached K'_D so that in 0.1 *N* HCl, $r \approx K'_D = 0.33$ for amoxicillin and $r \approx 0.6$ and $K'_D \approx 0.56$ for ampicillin. In general, amoxicillin distribution rate constants ($k'_{12} + k'_{21}$) were roughly twice those of ampicillin, whereas ampicillin K'_D and r values were nearly double those of amoxicillin. Thus, the kinetic and thermodynamic rank orders are opposite. This result may have implications in drug design *via* molecular modification.

Keyphrases □ Amoxicillin—kinetics of simultaneous partitioning and hydrolysis □ Ampicillin—kinetics of simultaneous partitioning and hydrolysis □ Hydrolysis kinetics—amoxicillin and ampicillin, simultaneous partitioning □ Partitioning kinetics—amoxicillin and ampicillin, simultaneous hydrolysis □ Kinetics—amoxicillin and ampicillin, simultaneous partitioning and hydrolysis

A stirred transfer cell can be used to determine the rate constants for distribution between partially miscible phases with simultaneous degradation in the aqueous phase (1). This technique is potentially useful for comparing the partitioning of closely related drugs under conditions where they are not stable. The method allows calculation of the apparent partition coefficient, K'_D, that would be observed if the drug were stable. It also allows estimation of the concentration ratio, r, of the two phases at a time sufficiently long for r to approach a constant value. Thus, the calculated equilibrium value, K'_D, and the kinetic value, r, can be compared. If these values vary

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Table I—Apparent First-Order Rate Constants (10^3 k in minutes^{-1}) for the Transfer and Hydrolysis (k'_{12}, k'_{21}, and k) of Ampicillin in an Isobutanol–Aqueous Hydrochloric Acid Stirred Transfer Cell at 37° [a]

Revolutions per Minute	[HCl][b]	k'_{12}	k'_{21}	k	K'_D	r
75	0.5	7.57	8.03	10.5	0.94	1.78
	0.3	6.66	7.46	5.78	0.89	1.34
	0.2	6.09	7.35	3.53	0.83	1.08
	0.1	4.69	8.28	1.53	0.57	0.64
120	0.5	8.71	9.05	10.7	0.96	1.71
	0.3	7.46	8.29	6.09	0.90	1.32
	0.3[c]	8.14	8.68	5.56	0.94	1.30
	0.2	6.41	7.80	3.60	0.82	1.06
	0.1	5.00	9.04	1.51	0.55	0.62

[a] The apparent partition coefficient ($K'_D = k'_{12}/k'_{21}$) is compared to the distribution ratio according to Eq. 5, where $r = [AMP]_i/[AMP]_w = k'_{12}/(k'_{21} - \beta)$. [b] Concentration prior to equilibration with isobutanol. [c] Initial ampicillin concentration of 2.4×10^{-3} M. The other concentrations were 4.0×10^{-3} M.

widely, *e.g.*, $r > K'_D$, then the phase ratio is controlled by the rate constants. This observation may be important for molecular modification.

BACKGROUND

Ampicillin and amoxicillin were studied because of their molecular similarity and the dramatic increase in the oral absorption of amoxicillin compared to ampicillin (2). The kinetics of acid-catalyzed hydrolysis and partitioning into isobutanol are represented in Scheme I:

$$C_{1p} \xleftarrow{k} C_1 \underset{k'_{21}}{\overset{k'_{12}}{\rightleftharpoons}} C_2$$

Scheme I

where k represents the apparent first-order hydrolysis constant; the distribution constants, k'_{12} and k'_{21}, were described previously (1). The penicillin concentration in the raffinate is represented by C_1 and that in the extract is represented by C_2. The hydrolysis product concentration in the raffinate is represented by C_{1p}. According to theory (1), the concentration in the raffinate (aqueous hydrochloric acid) is described by:

$$C_1 = X'e^{-\alpha t} + Y'e^{-\beta t} \qquad \text{(Eq. 1)}$$

where $X' = C_1^0(\alpha - k'_{21})/(\alpha - \beta)$, $Y' = C_1^0(k'_{21} - \beta)/(\alpha - \beta)$, C_1^0 is the initial concentration in the raffinate, and α and β are as defined previously (1). The concentration in the extract (isobutanol) is described by:

$$C_2 = Z'(e^{-\beta t} - e^{-\alpha t}) \qquad \text{(Eq. 2)}$$

where $Z' = C_1^0 k'_{12}/(\alpha - \beta)$. In agreement with theory, both penicillins illustrate kinetic control of the phase ratios when hydrolysis is significant.

Table II—Apparent First-Order Rate Constants (10^3 k in minutes^{-1}) for the Transfer and Hydrolysis (k'_{12}, k'_{21}, and k) of Amoxicillin [a] in an Isobutanol–Aqueous Hydrochloric Acid Stirred Transfer Cell at 37° [b]

Revolutions per Minute	[HCl][c]	k'_{12}	k'_{21}	k	K'_D	r
75	0.5	10.7	16.4	11.1	0.65	0.99
	0.3	9.17	16.3	5.37	0.56	0.70
	0.2	7.39	16.6	3.04	0.44	0.51
	0.1	6.22	18.8	1.03	0.33	0.34
120	0.5	14.2	21.4	10.9	0.66	0.91
	0.3	10.4	18.9	5.71	0.55	0.67
	0.2	10.3	23.2	3.16	0.44	0.49
	0.1	6.87	20.9	1.03	0.33	0.34

[a] The apparent partition coefficient ($K'_D = k'_{12}/k'_{21}$) is compared to the distribution ratio according to Eq. 5, where $r = [AMOX]_i/[AMOX]_w = k'_{12}/(k'_{21} - \beta)$. [b] Initial concentration of 4.4×10^{-2} M. [c] Concentration prior to equilibration with isobutanol.

Table III—Apparent First-Order Rate Constants (k_{app} at 37°) for Hydrolysis in Aqueous Hydrochloric Acid Saturated with Isobutanol

[HCl][a], N	10^3 k_{app}, min^{-1} Ampicillin[b]	10^3 k_{app}, min^{-1} Amoxicillin[b]
0.1	1.32	1.25
0.2	2.82	3.21
0.3	4.89	4.83
0.4	8.44	7.89
0.5	10.5	10.9
0.7	18.1	18.0

[a] Concentration prior to equilibration with isobutanol. [b] Initial concentration of $4.4–4.5 \times 10^{-2}$ M.

EXPERIMENTAL

Analytical Methods—Amoxicillin trihydrate[1] (85.0% amoxicillin) and ampicillin trihydrate[1] (83.7% ampicillin) were 97% pure after accounting for the water of hydration when they were assayed by the acid degradation method. All other materials were analytical grade.

Both penicillins were assayed by a literature method that involves reaction with hydroxylamine and subsequent complexation with a ferric reagent (3). Absorbance was measured at 486 nm against appropriate blanks that were modified to accommodate aqueous acid or isobutanol samples.

Kinetics of Simultaneous Partitioning and Hydrolysis of Ampicillin and Amoxicillin—Aqueous hydrochloric acid (0.1–0.5 N) and isobutanol were equilibrated by shaking at 37°. The phases then were separated and kept at 37°. A stirred transfer cell containing 100 ml of each of the previously equilibrated phases was used to study the kinetics of simultaneous partitioning and hydrolysis at 37°. This system was described previously (1).

Samples of ampicillin or amoxicillin were introduced into the aqueous phase. Aliquots (0.25 or 0.5 ml) were withdrawn simultaneously from both phases as a function of time and assayed. The phase volume ratio thus was kept constant. The number of samples withdrawn was limited to provide a maximum decrease in the total volume of ~5%. Experimental conditions are listed in Tables I and II.

Control Experiments for Kinetics of Degradation in Separated Phases—Experimental controls were designed to take into account both the miscibility of phases and the partitioning of hydrochloric acid into isobutanol during the equilibration step. Aqueous hydrochloric acid (0.1–0.7 N) and isobutanol were equilibrated by shaking at 37°. The phases were separated, and the aqueous acid was kept at 37°. A penicillin sample then was dissolved in the acid to provide an initial concentration of $\sim 4.5 \times 10^{-2}$ M. Aliquots were assayed as a function of time. Experimental conditions are listed in Table III. Apparent first-order rate constants, k_{app}, were obtained from the negative slopes of linear plots of ln [concentration] *versus* time.

Degradation in the isobutanol phase was shown to be insignificant by the following technique. Experiments were initiated as described for the simultaneous partitioning and hydrolysis with the 0.5 N HCl system. After the isobutanol phase had achieved a peak penicillin concentration, the phases were separated; the isobutanol phase was maintained at 37°. Aliquots were assayed as a function of time for 24 hr. The apparent first-order rate constants were negligible relative to those for hydrolysis in the aqueous controls. The rate constant for amoxicillin was 1% of the control, and that for ampicillin was 2% of the control. Therefore, Scheme I is adequate to describe the simultaneous partitioning and hydrolysis experiments.

RESULTS AND DISCUSSION

Kinetics of Hydrolysis of Amoxicillin and Ampicillin—In aqueous solutions, aminopenicillins can undergo hydrolysis with simultaneous polymerization. Bundgaard (4, 5) demonstrated both ampicillin and amoxicillin dimerization *via* nucleophilic attack by the free side-chain amino group in one molecule on the β-lactam of a second molecule. The percentage of total degradation attributed to dimerization ranged from 98% at pH 8.10 (0.673 M) to 13% at pH 9.10 (0.054 M) for ampicillin and from 93% at pH 8.6 (0.477 M) to 9% at pH 10 (0.024 M) for amoxicillin. Under the conditions in Table III, both penicillins are in the cationic form (AH^+) since, in both cases, the pKa (35°) of the carboxylic acid is ~2.6

[1] Beecham Pharmaceuticals, Sussex, England.

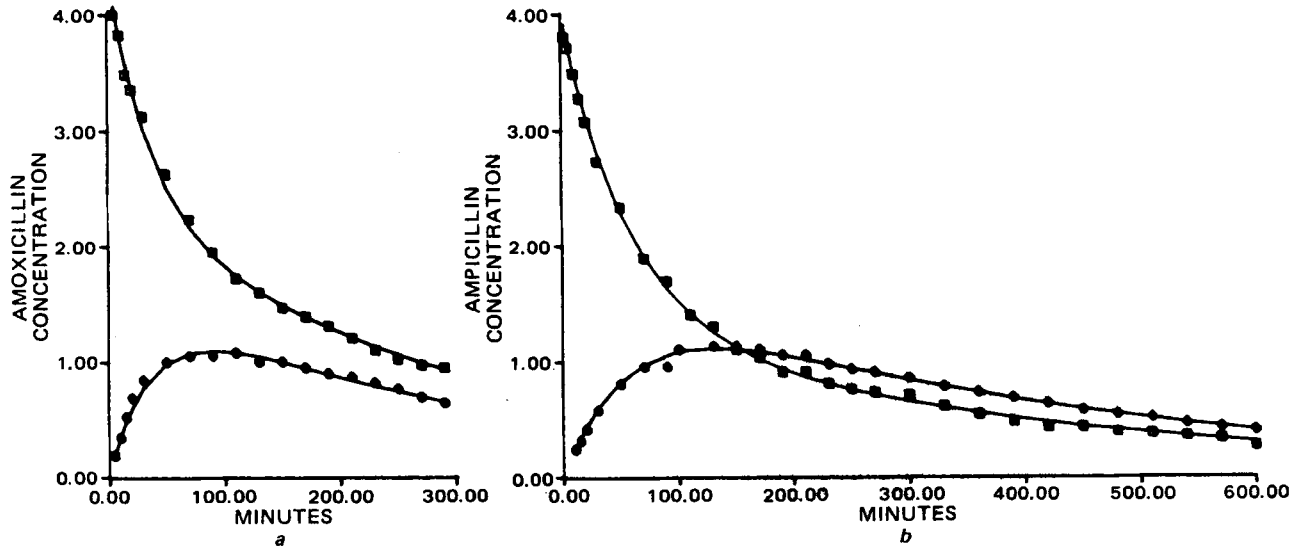

Figure 1—*Data representing the simultaneous determination of* C_1 (□) *and* C_2 (○) *in Scheme I using a stirred transfer cell containing 0.3* N *HCl and isobutanol (preequilibrated) at 37° at 75 rpm. The curves were obtained by simultaneous nonlinear regression based on Eqs. 1 and 2. The resulting estimates for the rate constants are given in Tables I and II.*

and that of the side-chain protonated amine is ~7.2 (4, 5). The lack of unprotonated amine to act as the nucleophile together with the dilute solutions (~0.04 *M*) makes self-association unlikely. Hou and Poole (6) reported simple first-order hydrolysis in the presence of 0.04–0.20 *N* HCl at 35°.

The precise degradation mechanism is not critical to this study since identical conditions were employed for both the simultaneous partitioning and hydrolysis kinetics (Tables I and II) and the controls (Table III). Furthermore, it is not necessary to determine the exact content of the two phases since hydrolysis in the aqueous phase is carried out as a control in which phase separation represents the sole difference between the experiments. At 37°, the solubility of isobutanol in water is ~7.4% (w/w) and that of water in isobutanol is ~18% (w/w) (7). Miscibility of the solvents, while significant, was kept constant throughout all experiments by preequilibration. Therefore, the reported rate constants are associated with water–isobutanol–hydrochloric acid mixtures. This technique provides a simple control experiment without requiring an exact definition of the phases.

Kinetics of Simultaneous Partitioning and Hydrolysis of Ampicillin and Amoxicillin—A previous report (1) demonstrated that the apparent partition coefficient, K'_D, defined by:

$$K'_D = C_2^\infty / C_1^\infty \qquad \text{(Eq. 3)}$$

where, at constant pH, C_2^∞ is the equilibrium concentration in the extract and C_1^∞ is the equilibrium concentration in the raffinate, may be calculated from:

$$K'_D = k'_{12}/k'_{21} \qquad \text{(Eq. 4)}$$

where k'_{12} and k'_{21} are the observed first-order rate constants for transfer from the raffinate to the extract and from the extract to the raffinate, respectively. Data for both phases (such as those shown in Fig. 1) were analyzed simultaneously using nonlinear regression (8) for the best estimates of k'_{12}, k'_{21}, and k based on Eqs. 1 and 2, which are derived from Scheme I.

According to theory, the rate constants for hydrolysis (k in Scheme I) should agree with those determined without partitioning under the same aqueous conditions (1). Thus, a plot of the k values *versus* the k_{app} values (Table II) should be linear with a slope of unity and an intercept of zero. Figure 2 shows good agreement with this prediction. Figure 2*a*, for ampicillin, has a slope of 0.97 and an intercept of 0.6×10^{-3}. Figure 2*b*, for amoxicillin, has a slope of 1.0 and an intercept of -0.05×10^{-3}. In both cases, the correlation coefficient exceeds 0.996. The hydrolysis rate constant also should be independent of the stirring rate. Tables I and II show good agreement between the k values determined with different stirring rates.

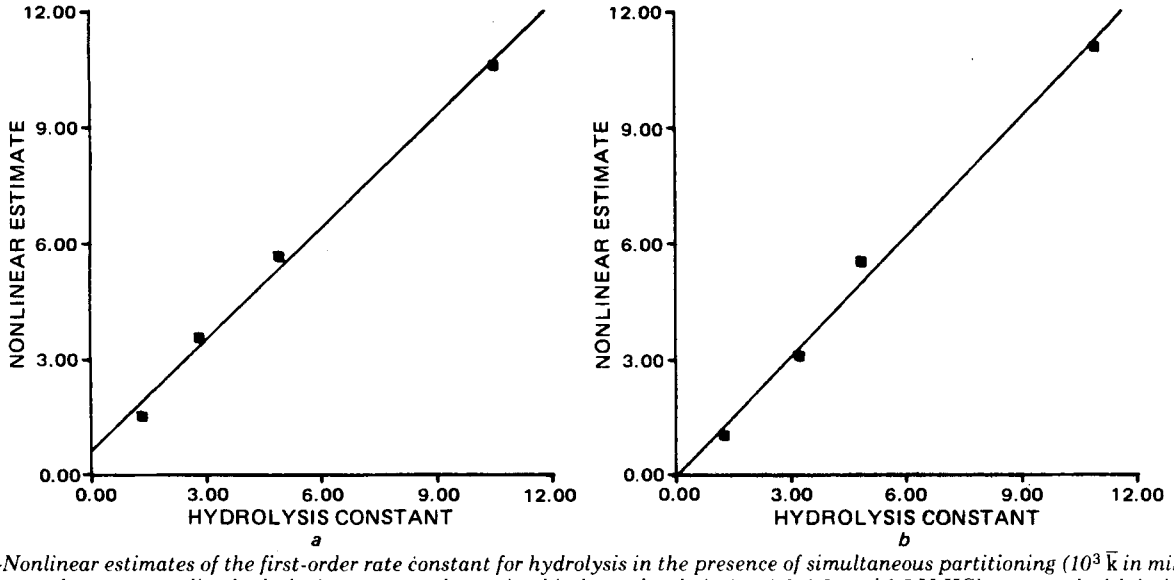

Figure 2—*Nonlinear estimates of the first-order rate constant for hydrolysis in the presence of simultaneous partitioning (10^3 $\bar{k}$ in min^{-1}, Tables I and II)* versus *the corresponding hydrolysis constants determined independently in 0.1, 0.2, 0.3, and 0.5* N *HCl saturated with isobutanol (10^3 k_{app} in min^{-1}; Table III) for ampicillin* (a) *and amoxicillin* (b). *In each case, the correlation coefficient of the regression line exceeds 0.996 and the slope is ~1.*

The apparent partition coefficient that would be obtained for the penicillin if it were stable in aqueous hydrochloric acid may be calculated from Eq. 4. Estimates for k'_{12}, k'_{21}, and K'_D are given in Tables I and II. Contrary to the values for hydrolysis, k, the distribution constants increase with increased stirring rate, as expected. However, the partition coefficient, K'_D, should be independent of the stirring rate. Comparison of the K'_D values at a fixed hydrochloric acid concentration but with different stirring rates shows good agreement between the estimates.

There also is evidence for an increase in K'_D with increasing hydrochloric acid concentration. Since the pKa values are ~2.6 (carboxylic acid) and ~7.2 (protonated amine), both penicillins exist in the cationic form (AH^+) under these conditions. [The pKa of the phenolic hydroxyl group on amoxicillin is 9.5 at 35° (5)]. Therefore, it is not likely that the increase in K'_D is due to any pH effect on the ionic species. Examination of the values for k'_{12} and k'_{21} reveals that k'_{21} is relatively independent of the hydrochloric acid concentration but that k'_{12} increases with increased hydrochloric acid concentration. Therefore, the observed increase is due to an effect in the aqueous phase since the rate constant for transfer from isobutanol to aqueous hydrochloric acid is relatively constant. This should not be due to the increase in ionic strength in going from 0.1 to 0.5 N HCl. Since the aqueous solutes are in the form of cations (AH^+), increased ionic strength would be expected to decrease the activity coefficient, thus decreasing the apparent K'_D value since the latter is based on concentration measurements.

A second and more likely possibility is the increased rate of diffusion of the neutral ion-pair (AH^+, Cl^-), as reported previously for bromide on the dextromethorphanium ion (9). This effect appears to be very likely based on the work of Hurwitz and Carney (10), who enhanced the apparent partition coefficients for ampicillin using several ion-pair or adduct-forming additives. At pH 7, quaternary compounds were the most effective, but the results also were dependent on the anion. For example, the chloroform–water K'_D values using tetraheptylammonium with various anions were: acetate, 4.78; hydroxide, 1.74; chloride, 1.80; bromide, 0.46; and iodide, 0.016. At pH 3, the anions trichloroacetate (added as the carboxylic acid, pKa = 0.70) and picric acid (as the phenol, pKa = 0.38) increased the K'_D value in octanol by ~30 and ~250 times, respectively. Thus, chloride was effective as the counteranion at pH 7. At pH 3, where ampicillin exists in the AH^+ form, K'_D was increased by the addition of anions without the quaternary cations. These observations support the suggestion that the increase in k'_{12} values with increased hydrochloric acid may be due to the formation of the neutral ion-pair (AH^+, Cl^-).

Both penicillins illustrate the difference between the observed phase ratios, r, and the apparent partition coefficients, K'_D. This difference was most pronounced with 0.5 N HCl. When a constant r value was observed, the values agreed with those calculated from Eq. 5 (11):

$$r = k'_{12}/(k'_{21} - \beta) \qquad \text{(Eq. 5)}$$

For example, with 0.5 N HCl, where β is most significant relative to k'_{12} and k'_{21}, the ampicillin r value was 1.78, while K'_D was 0.94. This result illustrates how kinetic control can reverse the expected results based on partition coefficients. Here, the K'_D value predicts that the aqueous concentration should be greater than that in the isobutanol phase. However, the concentration in isobutanol was 1.78 times that of the aqueous phase. As the hydrochloric acid concentration decreased, r approached K'_D. This result is due to the decreasing significance of the competing hydrolysis process.

The observed rate constant for the partitioning between the phases is the sum of the forward and reverse constants, k'_{12} and k'_{21} (1). If this sum becomes large enough relative to the hydrolysis constant, k, then r approaches K'_D. This effect has been referred to as preequilibrium, and a first approximation that $(k'_{12} + k'_{21}) \geq 20k$ will provide $r \approx K'_D$ has been suggested (11). In both cases (Tables I and II), k decreased nearly 10-fold as the hydrochloric acid concentration was decreased from 0.5 to 0.1 N. At the same time, k'_{21} remained nearly constant, while k'_{12} only decreased to about 60% of its value using 0.5 N HCl. Therefore, the $(k'_{12} + k'_{21})/k$ ratio increased 10-fold (75 rpm) and eight-fold (120 rpm) for amoxicillin and six-fold (75 rpm) and 5.6-fold (120 rpm) for ampicillin. The r values for amoxicillin at 0.1 N HCl were the same as its K'_D values since the $(k'_{12} + k'_{21})/k$ ratios were 24 (75 rpm) and 27 (120 rpm).

Although the K'_D and r values were larger for ampicillin than for amoxicillin, the rate constants for distribution $(k'_{12} + k'_{21})$ were nearly twice as large for amoxicillin. Amoxicillin thus distributed more rapidly but showed a lower isobutanol concentration for any given C_1 value. With significant hydrolysis, neither drug distribution ratio (C_2/C_1) was reflected by the K'_D values.

These observations illustrate one potential limitation in the application of partition coefficients to biological systems. The use of partition coefficients to predict the biological activity of structurally nonspecific drugs has received much attention since its inception (Meyer–Overton law). These well-accepted concepts are included in most undergraduate textbooks on medicinal chemistry. Further quantitation of these principles uses linear free energy relationships of the type known to chemists as Hammett $\sigma\rho$ functions. Since K'_D values are thermodynamic parameters, they describe equilibrium ratios rather than distribution rates. For systems that do not achieve equilibrium, such as an intravenous injection of a rapidly metabolized drug, the apparent distribution ratio may be predicted better from kinetic parameters. Comparisons of the type reported here for ampicillin and amoxicillin may be useful in drug design *via* molecular modification.

Conclusion—Ampicillin and amoxicillin both illustrated a difference between the observed isobutanol–aqueous phase concentration ratio, r, and that predicted by the partition coefficient value, K'_D, when 0.5 N HCl was used. This difference diminished as the hydrochloric acid concentration (and, therefore, the hydrolysis rate) was decreased. Both the r and K'_D values were roughly twice as large for ampicillin compared to amoxicillin. However, the sums of the distribution rate constants for amoxicillin were larger than those for ampicillin. There was no significant difference between the hydrolysis rate constants for the compounds. The estimates for the hydrolysis rate constants in the presence of partitioning were in good agreement with those determined independently.

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