# Comparison of Ampicillin and Hetacillin Pharmacokinetics in Man

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Abstract ☐ The pharmacokinetics of oral and intravenous doses of ampicillin were studied when ampicillin was given directly and as its inactive precursor, hetacillin. The two compounds were assayed separately in plasma using an electrophoresis–bioautography method. Plasma concentration and urinary excretion rates of ampicillin after direct intravenous injection and after hetacillin administration were computer fitted using a two-compartment open model. Hetacillin hydrolyzed rapidly and completely to ampicillin with an *in vivo* half-life of 11 ± 2 min. in eight human subjects. No differences were found in the distribution rate and volume constants, elimination rates and clearance parameters, and urinary recovery of ampicillin when it was given either as intravenous ampicillin or intravenous hetacillin. The major effect of intravenous hetacillin is that early plasma, urine, and peripheral compartment levels of ampicillin resemble those from a rapid absorption process, with a peak plasma concentration found at about 0.5 hr. Limited bioavailability studies in eight fasting subjects showed 32% absorption from ampicillin capsules, while 38 and 42% absorption values from hetacillin capsules were found in four fasting and four nonfasting subjects, respectively. The primary rationale for clinical use of ampicillin precursors is, in general, the improvement of the limited intestinal absorption of the antibiotic as well as increasing the stability of ampicillin in aqueous solutions.

Keyphrases ☐ Ampicillin pharmacokinetics—oral and intravenous, compared to intravenous hetacillin, man ☐ Hetacillin pharmacokinetics—intravenous, compared to oral and intravenous ampicillin, man ☐ Pharmacokinetics—ampicillin compared to hetacillin, man ☐ Drug precursors—oral and intravenous ampicillin compared to intravenous hetacillin ☐ Absorption, drug—ampicillin and hetacillin, man ☐ Elimination, drug—ampicillin and hetacillin, man

Hetacillin is representative of an unusual and somewhat controversial class of chemotherapeutic agents. It is a condensation product of ampicillin and acetone, which is stable in the dry state but susceptible to rapid conversion to ampicillin (Scheme I) in aqueous solution (1, 2). A recent study employing a short-duration

hetacillin

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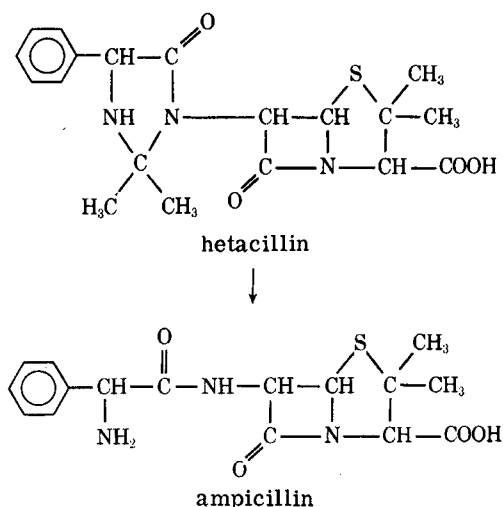
ampicillin

Scheme I

bacterial lysis technique showed that unchanged hetacillin has little or no bactericidal activity (3). However, because of its hydrolysis, microbiological assays that require prolonged incubation reveal hetacillin and ampicillin to be eventually equipotent on a molar basis (4). Hetacillin is thus an inactive precursor of ampicillin, but its slightly different physicochemical properties and the fact that it must hydrolyze to ampicillin can be expected to modify the pharmacokinetics and possibly the chemotherapeutic effect of ampicillin. In fact, apparent increases in blood levels and decreases in renal clearance of ampicillin have been reported after hetacillin administration (5–7). However, most of these studies did not involve separation of unchanged hetacillin from ampicillin in plasma specimens prior to microbiological assay and are, therefore, misleading.

The present investigation was performed to elucidate and compare the pharmacokinetic properties of ampicillin when administered directly and as hetacillin to human subjects. These studies are of general interest because hetacillin represents the first of several attempts to enhance the chemotherapeutic usefulness of ampicillin by the use of precursors of the antibiotic.

METHODS

Eight normal subjects, four males and four females, participated in the study; their ages and relevant descriptions are listed in Table I. Each volunteer received doses of intravenous ampicillin[1], intravenous hetacillin[1], and oral ampicillin[1], with the latter following an overnight fast. The female subjects also received oral doses of hetacillin[1] after fasting and immediately following a standard breakfast consisting of 30 g. cornflakes, 250 ml. whole milk, and 5 g. sucrose. The oral dosage forms were loosely packed commercial capsules, which were ingested with 100–200 ml. of tap water. The sequences of drugs and dosage forms were randomized for the female subjects, except that determination of the effect of food on hetacillin absorption was performed after completion of all other studies.

The dosages were determined by analysis of several capsules and vials of injectable material. The ampicillin and hetacillin capsules contained 550 and 483 mg. ampicillin equivalent, respectively. All intravenous solutions were prepared within 3 min. of injection, and the residual drug in the vial was analyzed to allow calculation of the actual dose administered. These doses are listed in Table I.

Urine specimens and, in most instances, blood samples were obtained following each dose of antibiotic. Blood was collected from either the cubital vein or forearm cephalic vein using an indwelling needle and cannula and heparinized vacuum tubes. Clotting in the tubing was prevented with a dilute solution of heparin in saline. Blood samples were drawn prior to drug administration and at seven intervals during the subsequent 8 hr. A blank urine specimen was obtained and hourly urine collections were made for 12 hr. followed by less frequent samples for an additional 12 hr. All specimens were refrigerated until drug analyses were performed on the day after drug administration. However, during the studies with hetacillin, the first four blood samples were rapidly centrifuged, diluted with the blank plasma from the same subject when appropriate, and quickly frozen to −60°, in which state hetacillin is

Table I—Drug Dosages and Physiological Characteristics of the Subjects

Subject	Sex	Age, Years	Surface Area[a], m.[2]	Intravenous Doses, mg.[b] Ampicillin	Intravenous Doses, mg.[b] Hetacillin	Plasma[c] Proteins, g. % Total	Plasma[c] Proteins, g. % Albumin	Creatinine[d] Clearances, ml./min.
1	F	26	1.73	647	533	7.0	3.1	104
2	F	23	1.65	608	550	7.2	3.2	94
3	F	26	1.82	639	532	7.2	2.9	128
4	F	23	1.57	633	533	6.9	2.8	101
5	M	28	2.09	570	554	7.2	2.8	174
6	M	43	2.01	570	538	7.1	2.9	127
7	M	28	1.92	570	558	6.6	3.0	92
8	M	24	2.03	570	561	6.9	3.4	109

[a] Determined from height and weight by the method of Dubois and Dubois (20). [b] Ampicillin equivalent. [c] Mean of two determinations. [d] Mean of four and two determinations for the F and M subjects, respectively.

stable. These specimens were subjected to electrophoresis to separate ampicillin and hetacillin immediately following collection of the 2.5-hr. sample.

Assays—All samples were analyzed for ampicillin using a fluorometric method described previously (8) as well as the agar-well diffusion assay of Bennett *et al.* (9), with *Bacillus subtilis* as the microorganism. Both assays involved use of reference standards prepared with blank plasma from the individual subject as well as nonproteinaceous standards. Hetacillin was separated from ampicillin using duplicate electrophoretic agar gels buffered with tris-maleate at pH 5.6 (10). After electrophoresis at approximately 240 v. and 60 ma. for 45 min., the gels were overlayed with 1.5% nutrient agar containing *B. subtilis* spores. Following overnight incubation, the relative fractions of hetacillin and ampicillin were determined by comparison of the zone sizes with a graph of the mean inhibition diameter plotted *versus* the logarithm of ampicillin concentration of standards. All measurements were made with analytical grade sodium ampicillin[1] (potency 865 mcg./mg.) as the reference material.

One set of urine and plasma samples obtained after each dose of drug was analyzed for endogenous creatinine, using the alkaline picrate method (11) to calculate creatinine clearances. Plasma

Figure 1—*Plasma concentrations* (●,○) *and urinary excretion rates* (■,□) *of ampicillin as a function of time after intravenous administration of 570 mg. to Subject 5. Closed symbols are results of microbiological assay, and open symbols are measurements by fluorometry. The solid lines represent a least-squares fit of the microbiological data according to Eqs. 1 and 2.*

samples collected after each intravenous dose of antibiotic also were analyzed to determine total protein by the biuret method (12) and the albumin fraction using cellulose acetate electrophoresis.

Protein Binding—The degree of protein binding of ampicillin and α-aminobenzylpenicilloic acid was determined at 23° using a centrifugal ultrafiltration procedure described previously (13). Fractional binding of both compounds to 4 g.% human serum albumin was measured with the fluorometric assay procedure (8).

Dissolution Tests—Dissolution rates of ampicillin and hetacillin from capsules were determined by a method similar to that of Levy and Hayes (14). The dissolution medium was 1000 ml. of 0.1 N HCl maintained at 37°. The capsule was restrained near the bottom of the beaker by means of an aluminum screen. The concentration of ampicillin or hetacillin in Millipore-filtered dissolution medium was measured at 5–10-min. intervals by spectrophotometric analysis at 258 nm.

RESULTS

Ampicillin Disposition—Typical plasma concentration and urinary excretion rate data obtained after intravenous injection of ampicillin are shown in Fig. 1. The data obtained by microbiological assay represent unchanged ampicillin, while the fluorometric procedure also measures a metabolite in plasma (8). The two assays produced essentially identical results when ampicillin was analyzed in urine, which indicates that little of the apparent metabolite is excreted by the kidneys. Similar assay differences which reflect the presence of the metabolite were found whether ampicillin or hetacillin was administered. The urinary recovery of unchanged ampicillin averaged 90 ± 7.5% after intravenous administration, which indicates that only a minor portion of the dose was eliminated by metabolism and/or biliary excretion.

The decline in plasma concentrations (C_p) and urinary excretion rates (ER) of unchanged ampicillin as a function of time (t) after intravenous injection is curvilinear, as shown in Fig. 1. The experimental data were fit to the biexponential equations:

$$C_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \qquad \text{(Eq. 1)}$$

and:

$$ER = Cl_R(A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}) \qquad \text{(Eq. 2)}$$

where A and B represent zero-time intercepts on the ordinate, α and β are disposition slope constants, and Cl_R is the renal clearance. The data were fit to these equations simultaneously by digital computer least-squares iteration using a time-share adaptation of the program NONLIN (15). The data employed were the microbiologically assayed plasma concentrations of ampicillin and the average of the microbiological and fluorometric assay results for the urinary excretion rates. After the plasma concentration data were included twice to compensate for their fewer number but greater intrinsic reliability, all data values were weighted numerically equal. Within the computer program, this involves multiplying the squared deviations: $(Y_{\text{observed}} - Y_{\text{calculated}})^2$ by a weight factor, which was calculated for individual data values as: weight =
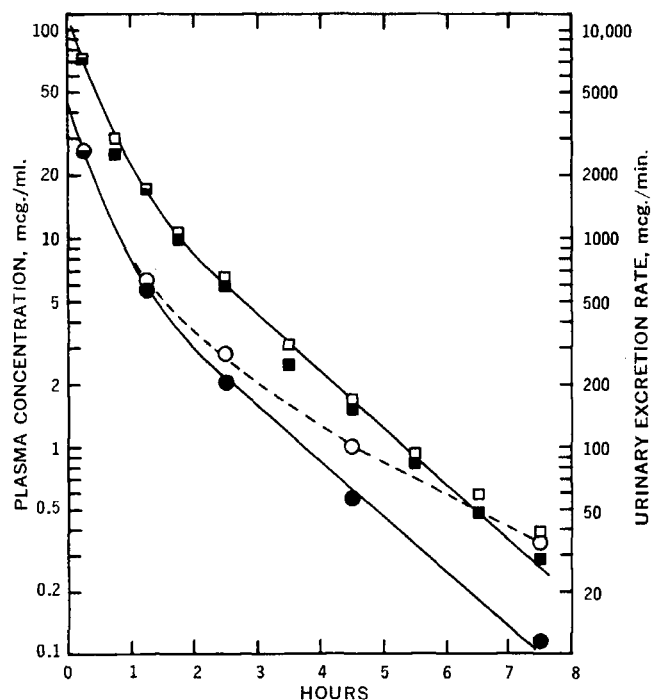
Table II—Biexponential Least-Squares Regression Parameters of Total Plasma Antibiotic after Intravenous Injection of Ampicillin and Hetacillin
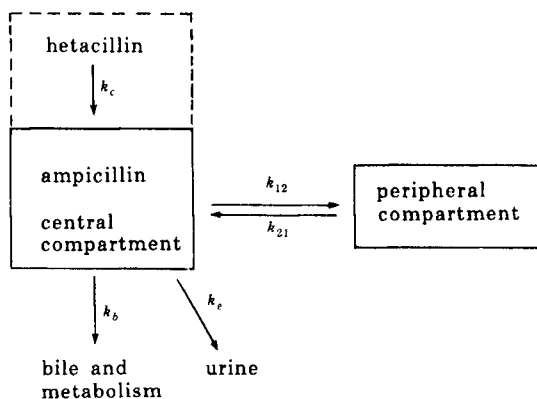
Parameter[a]	Ampicillin (SD)	Ampicillin with Hetacillin (SD)[b]
A, mcg./ml.	33.7 (7.9)	35.4 (9.1)
α, hr.$^{-1}$	2.30 (0.60)	1.81 (0.35)
B, mcg./ml.	4.04 (1.78)	7.66 (4.56)
β, hr.$^{-1}$	0.541 (0.048)	0.501 (0.090)
Cl_R, ml./min.	341 (91)	224 (50)
area, mcg. hr./ml.	22.7 (5.2)	34.7 (10.4)

[a] According to Eqs. 1 and 2 with A, B, Cl_R, and area normalized for a 500-mg. dose of ampicillin and 1.73 m.^{2} body surface area. [b] For curve-fitting purposes only.

($Y_{mean}/Y_{observed}$). The use of Eqs. 1 and 2 together permits all of the experimental data to be used simultaneously in calculation of the least-squares parameters. The averages of the latter values, as well as the plasma level area, are listed in Table II.

Hetacillin Disposition—Plasma concentration and urinary excretion rate data obtained after intravenous injection of both ampicillin and hetacillin are shown in Fig. 2. The disappearance of unchanged hetacillin was extremely rapid in both subjects, and the half-life of hetacillin averaged about 11 min. in the eight subjects. Hetacillin could be detected only in the first two plasma samples due to the limited sensitivity of the bioautography procedure. Figure 2 also shows the plasma concentrations of total antibiotic (ampicillin + hetacillin) after injection of both compounds. If hetacillin is not separated from ampicillin prior to microbiological or fluorometric analysis, the plasma antibiotic levels after hetacillin injection appear to exceed those obtained after ampicillin. For the purpose of estimating the total plasma area and obtaining a general impression of the behavior of both compounds after hetacillin injection, the total antibiotic data were also fit with Eqs. 1 and 2. These results are shown in Table II along with the intravenous ampicillin data for comparative purposes. It can be noted that the apparent β-values are very similar after administration of both ampicillin and hetacillin, but the apparent renal clearances are much less after hetacillin. The latter is due to the elevation of ampicillin plasma levels by the inclusion of hetacillin and suggests that little unchanged hetacillin appears in the urine.

Pharmacokinetic Analysis—The general pharmacokinetic model used to describe the distribution and elimination behavior of ampicillin after intravenous injection and with hydrolysis from hetacillin is shown in Scheme II. The rate constant for hydrolysis of hetacillin to ampicillin is k_c. The distribution rate constants of ampicillin are k_{12} and k_{21}, and elimination of ampicillin (k_{el}) occurs by renal (k_e) and biliary metabolic (k_b) pathways. These distribution and elimination constants for ampicillin were calculated from the least-squares
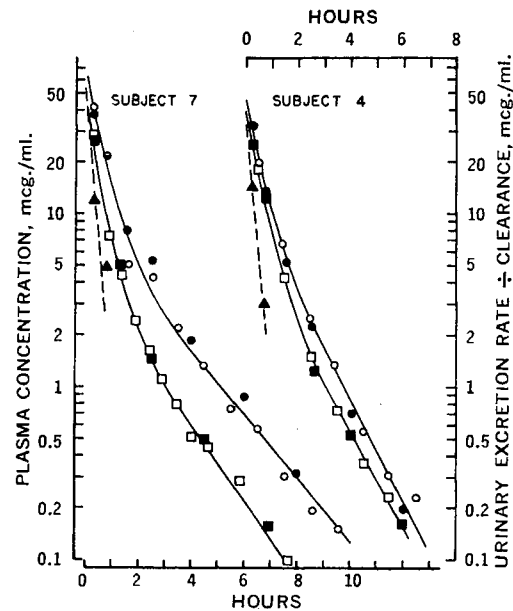
Figure 2—*Plasma concentrations of total apparent ampicillin as a function of time after intravenous administration of ampicillin (■,□) and hetacillin (●,○) to Subjects 7 and 4. Triangles and dashed lines show disappearance of unchanged hetacillin. The data are normalized for a 500-mg. dose of ampicillin. Solid symbols are actual plasma concentrations, and open symbols are urinary excretion rates divided by the least-squares renal clearance. Solid lines were obtained by computer least-squares regression according to Eqs. 1 and 2.*

parameters of Eqs. 1 and 2 using methods described previously (16–19), except that k_e and k_b were calculated from:

$$k_e = f_e \cdot k_{el} \qquad \text{(Eq. 3)}$$

and:

$$k_b = (1 - f_e) \cdot k_{el} \qquad \text{(Eq. 4)}$$

where f_e is the fraction of the intravenous dose (D_o) excreted in the urine unchanged. Other conventional parameters of a two-compartment open model (16–19) that were also calculated included the central compartment volume (V_c), steady-state distribution volume (V_{DSS}), and body clearance (Cl_B). The data obtained after hetacillin injection were quantitated with a more direct approach. A general solution to the model shown in Scheme II was generated using the methods recently proposed by Benet (18). The expression used to describe the time course of unchanged hetacillin (C_H) in the plasma was:

$$C_H = \frac{D_o}{V_c} \cdot e^{-k_c \cdot t} \qquad \text{(Eq. 5)}$$

Simultaneously, the time course of ampicillin concentrations (C_A) in plasma can be described by:

$$C_A = \frac{k_c \cdot (k_{21} - \alpha) \cdot D_o}{(\beta - \alpha) \cdot (k_c - \alpha)} \cdot e^{-\alpha \cdot t} + \frac{k_c \cdot (k_{21} - \beta) \cdot D_o}{(\alpha - \beta) \cdot (k_c - \beta)} \cdot e^{-\beta \cdot t} +$$

$$\frac{k_c \cdot (k_{21} - k_c)}{(\alpha - k_c) \cdot (\beta - k_c)} \cdot e^{-k_c \cdot t} \qquad \text{(Eq. 6)}$$

where:

$$\alpha = \frac{1}{2} \cdot (b + \sqrt{b^2 - 4 \cdot k_{el} \cdot k_{21}}) \qquad \text{(Eq. 7)}$$

and:

$$\beta = \frac{1}{2} \cdot (b - \sqrt{b^2 - 4 \cdot k_{el} \cdot k_{21}}) \qquad \text{(Eq. 8)}$$
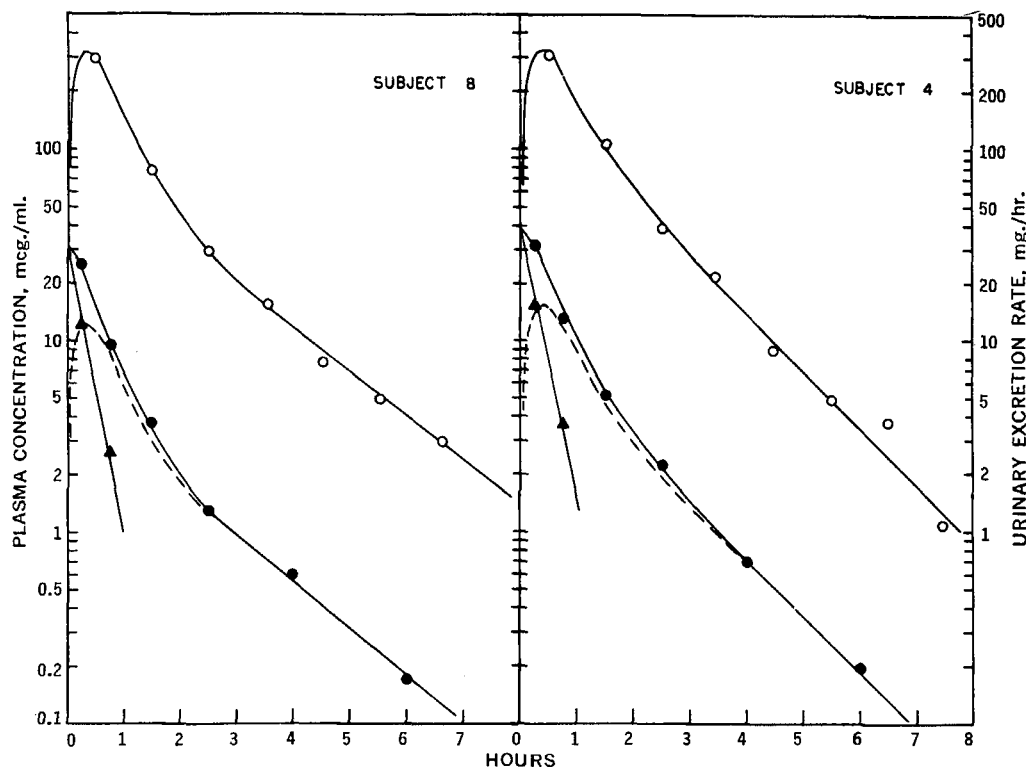
where $b = k_{12} + k_{21} + k_{el}$.

Scheme II—*Multiple-compartment pharmacokinetic model used to characterize hetacillin conversion to ampicillin (k_c), ampicillin distribution (k_{12}, k_{21}), and ampicillin renal (k_e) and extrarenal (k_b) elimination*

Figure 3—*Plasma concentrations of hetacillin* (▲), *ampicillin* (- - -), *and total antibiotic* (●) *as a function of time after intravenous administration of hetacillin to Subjects 4 and 8. The open circles are urinary excretion rates, and data are normalized for a 500-mg. dose. All lines were obtained by computer least-squares regression with the following constants:*

Subject	V_c, l.	k_o, hr.$^{-1}$	k_{12}, hr.$^{-1}$	k_{21}, hr.$^{-1}$	k_{el}, hr.$^{-1}$	Cl_R, l./hr.
4	13.3	3.18	0.441	1.00	1.81	21.4
8	16.5	3.23	0.405	0.704	2.05	26.5

The type of data fit by computer least-square iteration is shown in Fig. 3. Plasma levels of unchanged hetacillin were described with Eq. 5, while plasma levels of total antibiotic (C_T) were equal to:

$$C_T = C_H + C_A \qquad \text{(Eq. 9)}$$

where C_H and C_A were used as defined by Eqs. 5 and 6, respectively. Also, urinary excretion rates (ER_A) of unchanged ampicillin were quantitated with the enlargement of the expression:

$$ER_A = Cl_R \cdot C_A \qquad \text{(Eq. 10)}$$

where Eq. 6 was substituted into Eq. 10 for C_A. In essence, three detailed equations were used simultaneously to characterize hetacillin hydrolysis to ampicillin, ampicillin distribution, and ampicillin elimination, where the experimental data were C_H, C_T, and ER_A. However, the model was simplified by setting the distribution volume of hetacillin identical to that of ampicillin so that V_c was used as the same parameter for both compounds. The one-compartment assumption for hetacillin distribution was made because the hydrolysis of hetacillin to ampicillin was so rapid that hetacillin distribution rates could not be adequately characterized, because initial graphical estimation of V_c for both compounds yielded identical values, and because of the limited number of data points for hetacillin. The six least-squares constants thus generated from the hetacillin-ampicillin data were: V_c, k_o, k_{12}, k_{21}, k_{el}, and Cl_R. The excellent fit of the experimental data from two subjects using this approach is demonstrated by the results shown in Fig. 3. After these least-squares constants were obtained, the plasma levels of ampicillin were calculated for each subject using Eq. 6. The maximum level of ampicillin was attained at about 30 min. At a later time, ampicillin levels essentially converged with those of total antibiotic when most of the hetacillin was converted to ampicillin.

The computer-derived distribution and elimination constants of ampicillin that were obtained after injection of hetacillin are

listed in Table III along with the parameters obtained after intravenous ampicillin. The plasma level area of ampicillin formed from hetacillin (area$_A$) was calculated from the integral of Eq. 6 or:

$$\text{area}_A = \int_0^\infty C_A \cdot dt \qquad \text{(Eq. 11)}$$

The method of paired comparisons was used to evaluate statistically the effect of the two methods of ampicillin administration on its distribution and elimination parameters. As shown by the mean data values for the eight subjects in Table III, the use of hetacillin produced essentially no change in the distribution volumes, clearances, distribution rate constants, renal excretion fractions, and plasma level areas of ampicillin.

Integral Coefficients—The use of integral coefficients to summarize the interaction of the major factors that control body levels of drugs, including protein binding, was recently introduced (19). An integral coefficient represents the fractional dose–time area for nonprotein-bound drug located in each pharmacokinetic compartment. For ampicillin, the integral coefficients for the central (D_{1a}) and peripheral (D_2) compartments are defined as:

$$D_{1a} = \frac{\int_0^\infty X_1^* \cdot dt}{D_o} = \frac{1}{k_{el}} \cdot \left(1 - \frac{V_p \cdot F_b}{V_c}\right) \qquad \text{(Eq. 12)}$$

and:

$$D_2 = \frac{\int_0^\infty X_2 \cdot dt}{D_o} = \frac{k_{12}}{k_{21} \cdot k_{el}} \qquad \text{(Eq. 13)}$$

where X_1^* is the amount of free drug in Compartment 1, V_p is the actual plasma volume, and F_b is the mean fractional binding of the

Table III—Distribution and Elimination Parameters of the Two-Compartment Open Model for Ampicillin

Parameter[a]	Intravenous Ampicillin (SD)	Ampicillin from Hetacillin (SD)
Distribution volumes, 1.		
V_c	12.0 (1.9)	12.5 (2.8)
$V_D{}^{ss}$	17.9 (1.5)	19.3 (2.9)
Clearances, ml./min.		
Cl_R	341 (91)	296 (91)
Cl_B	335 (56)	350 (102)
Rate constants, hr.$^{-1}$		
k_{12}	0.384 (0.185)	0.419 (0.180)
k_{21}	0.733 (0.163)	0.728 (0.161)
k_{el}	1.73 (0.49)	1.68 (0.30)
k_e	1.55 (0.47)	1.58 (0.31)
k_b	0.17 (0.12)	0.10 (0.13)
Slow $t_{1/2}$, hr.	1.29 (0.11)	1.34 (0.20)
Fraction (f_e) excreted in urine	0.899 (0.075)	0.939 (0.076)
Plasma level area, mcg. hr. ml.$^{-1}$	22.7 (5.2)	22.9 (7.6)
Integral coefficients, hr.		
D_{1a}	0.581 (0.133)	0.581 (0.118)
D_2	0.297 (0.081)	0.355 (0.102)
D_T	0.878 (0.151)	0.921 (0.141)

[a] V_c, $V_D{}^{ss}$, Cl_B, Cl_R, and area are normalized for 1.73 m.^{2} body surface area.

drug to plasma proteins. The plasma volume was estimated by the method of Dagher (20), using the age and body weight of each subject. Over the concentration range of 1–10 mcg./ml., the fractional binding of ampicillin to 4 g. % human serum albumin was 0.29 ± 0.01, which yielded an association constant (k) of 704 1./mole, assuming one ampicillin binding site per protein molecule. This constant, along with the plasma albumin concentration (P) of each subject, was used to estimate F_b according to (13):

$$F_b = \frac{k \cdot P}{1 + k \cdot P} \qquad \text{(Eq. 14)}$$

A summary of the central and peripheral compartment integral coefficients for the two studies in each of the eight subjects is shown in Fig. 4. The total integral coefficient (D_T), which is equal to:

$$D_T = D_{1a} + D_2 \qquad \text{(Eq. 15)}$$

is a model-independent parameter which best reflects the overall fractional dose–time area between subjects and among various drugs (19). As shown in Table III and Fig. 4, these were essentially identical for ampicillin (0.878 hr.) and ampicillin formed from hetacillin (0.921 hr.). These values are also very similar to the total integral coefficient previously generated for ampicillin (1.01 ± 0.07 hr.) based on data of Jusko *et al.* (19) and Dittert *et al.* (21). The peripheral compartment integral contributes, on the average, about one-third of the whole body integral. There was greater variation in both D_{1a} and D_2 among subjects than within each subject for the studies with ampicillin and hetacillin. This was primarily due to the intrasubject variation in ampicillin elimination which, as can be noted from Eqs. 12 and 13, is the major factor controlling the value of both integral coefficients.

Rate of Hetacillin Hydrolysis—The least-squares rate constant for the conversion of hetacillin to ampicillin (k_c) was relatively constant among the eight subjects, with an arithmetic mean of 3.83 hr.$^{-1}$ and a standard deviation of 0.78 hr.$^{-1}$. The range of half-lives for this process was 8–13 min., with an average of 11.2 min. The narrow range of k_c values is consistent with a chemical, rather than biochemical, mechanism of hetacillin hydrolysis (1, 2). The listed rate constants for hetacillin degradation are probably slight overestimates because of hydrolysis occurring during the centrifugation process after collection of the blood samples. The blood samples were cooled during centrifugation to minimize such an effect.

Absorption Properties—The time course of plasma concentrations of ampicillin in a representative subject after administration of ampicillin and hetacillin during fasting (both compounds) and following ingestion of food (hetacillin) is shown in Fig. 5. Electro-
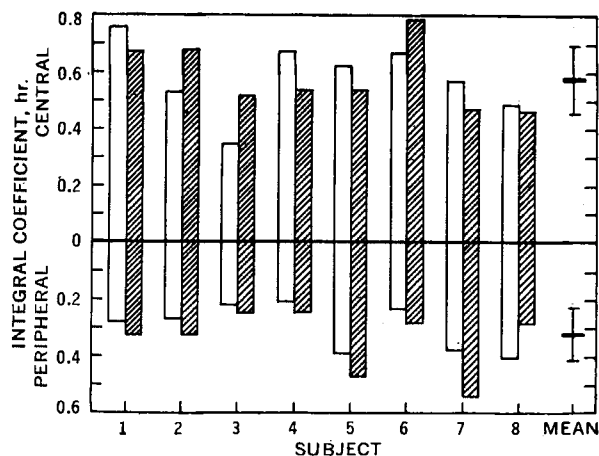

Figure 4—*Integral coefficients of ampicillin in the central and peripheral compartments after administration of ampicillin (□) and hetacillin (▨) to eight normal subjects. Standard deviations are shown as vertical lines with the mean values.*

phoresis of the early plasma samples revealed no detectable hetacillin when it was given. The rapid absorption and apparent monoexponential decline of plasma levels of ampicillin made it difficult to characterize the data using a two-compartment open model, even by applying computer least-squares techniques. The plasma concentration (C_p) and urinary excretion rate (ER) data were, therefore, analyzed semiempirically by appropriate use of a zero-order input function for a one-compartment open model (18) where:

$$C_p = \frac{R_a}{\delta} \cdot (e^{\delta \cdot t_0} - e^{\delta \cdot t_d}) \cdot e^{-\delta \cdot t} \qquad \text{(Eq. 16)}$$

and:

$$ER = C_p \cdot Cl_R \qquad \text{(Eq. 17)}$$

with the conditions that $t_o = t$ at $t < t_o$ and $t_d = t$ at $t_d < t$ and where R_a is a relative absorption rate constant that includes a volume of
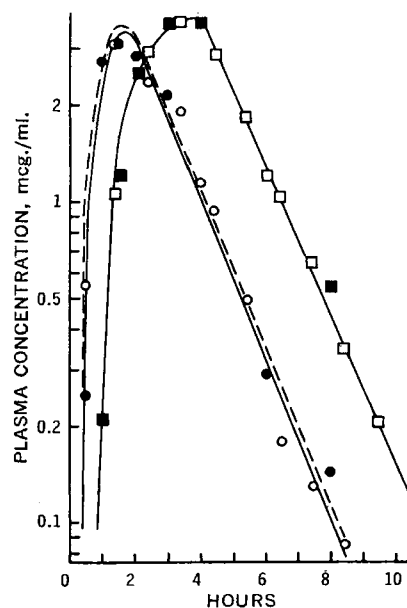

Figure 5—*Plasma concentrations of ampicillin in Subject 1 as a function of time after oral administration of ampicillin after fasting (●,○), hetacillin with food (■,□), and hetacillin after fasting (- - -, no symbols). Ampicillin data are corrected to the 483-mg. dose of hetacillin. Solid symbols are actual plasma concentrations, and open symbols are urinary excretion rates divided by the least-squares renal clearance. All lines were obtained by computer least-squares regression according to Eqs. 16 and 17.*

Table IV—Comparison of Ampicillin and Hetacillin Absorption Parameters[a] after Ingestion of Oral Capsules

Parameter[b]	Ampicillin Fasting (SD)	Hetacillin Fasting (SD)	Hetacillin With Food (SD)
Dose[c], mg.	550	483	483
Onset of absorption (t_o), hr.	0.32 (0.08)	0.65 (0.09)	0.64 (0.12)
Duration of absorption (t_d), hr.	1.70 (0.89)	1.74 (0.59)	2.22 (0.66)
Relative absorption rate (R_a), mg./hr. l.	4.05 (1.97)	3.45 (0.77)	2.99 (0.51)
Disposition half-life, $0.693/\delta$, hr.	1.08 (0.13)	1.08 (0.15)	1.12 (0.20)
Renal clearance, ml./min.	298 (71)	337 (59)	328 (115)
Plasma level area, mcg. hr./ml.	8.73 (1.43)	8.89 (1.79)	10.53 (3.71)

[a] Least-squares parameters calculated with Eqs. 16 and 17. [b] Mean and standard deviation for four female subjects. [c] Ampicillin equivalent.

distribution, δ is the disposition rate constant, t_d is the duration of the absorption process, and t_o is the lag time before absorption starts. This function is useful primarily for curve fitting, comparison of crossover data, and numerical estimation of plasma level areas (trapezoidal rule). The plasma concentration and urinary excretion data were used simultaneously with the weighting method described previously to obtain least-squares estimates of the parameters: R_a, δ, t_d, t_o, and Cl_R.

As shown in Fig. 5, an excellent fit of the experimental data was obtained by using Eqs. 16 and 17. The average values of the least-squares parameters are listed in Table IV for the four female subjects. The t_o values show that the onset of absorption of hetacillin is slightly delayed relative to ampicillin. When food is coadministered with hetacillin, the onset of absorption is unaffected, but the duration of absorption is increased by about 0.5 hr. Partially offsetting this effect, food also produces an apparent decrease in the relative absorption rate of hetacillin. The apparent disposition half-life of oral ampicillin is slightly less than that found after intravenous ampicillin or hetacillin. As will be considered further, the plasma level areas listed in Table IV indicate that ampicillin is somewhat better absorbed when administered as hetacillin than as ampicillin.

Bioavailability—Since both plasma level areas and urinary excretion data were obtained, the bioavailability of ampicillin was calculated using two methods. The urinary recovery ratios after oral (po) and parenteral (iv) doses were used to calculate the fractional absorption (F_U) according to:

$$F_U = \frac{\% \text{ recovery}_{po}}{\% \text{ recovery}_{iv}} \quad \text{(Eq. 18)}$$

The plasma level areas were similarly used to calculate bioavailability (F_A) from:

$$F_A = \frac{\text{area}_{po} \cdot \text{dose}_{iv}}{\text{area}_{iv} \cdot \text{dose}_{po}} \quad \text{(Eq. 19)}$$

In both equations, the average results from the studies with intravenous ampicillin and ampicillin formed from hetacillin were used
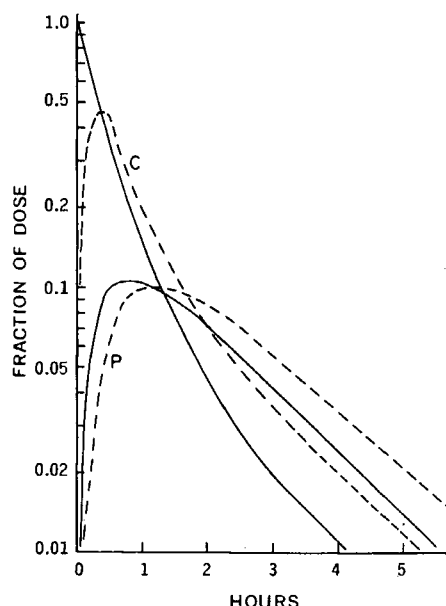

Figure 6—*Mean fractional dosage levels (semilogarithmic scale) of ampicillin in the central (C) and peripheral (P) compartments as a function of time after intravenous administration of ampicillin (———) and hetacillin (- - - -). The linear areas under the respective curves are the approximate integral coefficients.*

as denominator data for each subject. Numerator data were obtained by the measurement of total antibiotic in plasma or urine after oral doses of the two compounds. The bioavailability estimates are summarized in Table V for all studies performed. In general, there is good agreement between the F_U and F_A values, with an average difference of 1.7% absorption. The fact that the F_A values do not exceed the F_U values is in agreement with the electrophoretic absence of hetacillin in the plasma. If hetacillin itself was absorbed unchanged, the use of total antibiotic measurements would elevate the F_A estimate relative to the F_U value due to the addition of hetacillin plasma concentrations to those of ampicillin.

The average bioavailability estimates from the crossover studies in the female subjects can be used for comparison of the relative absorption of ampicillin and hetacillin. These data suggest that ampicillin is better absorbed when given as hetacillin (38%) than as ampicillin (29%). Coadministration of food with hetacillin appears to enhance ampicillin absorption slightly (42%). The individual data values are somewhat variable, and Subject 4 showed essentially constant absorption of ampicillin in the three studies. This tended to offset the increases in absorption found with the other three female subjects. No difference can be seen in the limited data reflecting bioavailability in male versus female subjects, and overall the eight subjects absorbed an average of 32 ± 8% of the dose of ampicillin.

Dissolution Rates—The small differences in absorption from ampicillin and hetacillin capsules may be caused partly by dissolution rate differences. The time for 50% dissolution of ampicillin in 0.1 N HCl was 13.4 ± 0.4 min., while that of hetacillin was 19.3 ± 2.0 min. (five and three determinations, respectively). This may account for the slight delay in onset of hetacillin absorption compared to that of ampicillin (Table IV).

Table V—Bioavailability of Ampicillin and Hetacillin from Gelatin Capsules[a]

Subject	Ampicillin: Fasting F_U	Ampicillin: Fasting F_A	Hetacillin: Fasting F_U	Hetacillin: Fasting F_A	Hetacillin: With Food F_U	Hetacillin: With Food F_A	Subject	Ampicillin: Fasting F_U
1	0.320	0.303	0.396	0.318	0.516	0.476	5	0.281
2	0.235	0.274	0.468	0.442	0.485	0.510	6	0.210
3	0.306	0.317	0.433	0.382	0.518	0.394	7	0.405
4	0.306	0.285	0.299	0.289	0.195	0.281	8	0.462
Mean	0.293		0.378		0.422		5–8	0.340
							1–8	0.316

[a] Calculation methods: F_U = urinary recovery ratio (Eq. 18), and F_A = plasma area ratio (Eq. 19).

DISCUSSION

Ampicillin Elimination—The elimination of ampicillin from the body primarily involves renal excretion, since about 90% of an intravenous dose of ampicillin or hetacillin appears in the urine unchanged. A similar value was found previously by Dittert *et al.* (21) in normal subjects. The remaining portion of the dose is eliminated by metabolism and biliary excretion. The occurrence of biliary excretion of ampicillin in man was confirmed by Mortimer *et al.* (22), who detected the antibiotic in the bile of patients. Although the fraction of the dose excreted in the bile was not determined, enterohepatic cycling of a small amount of ampicillin is thus possible.

The susceptibility of ampicillin to *in vivo* metabolism or degradation was demonstrated in the rat by Kind *et al.* (23) using ³⁵S-labeled ampicillin, but the apparent metabolite of ampicillin in man has not been identified. It was previously suggested on the basis of indirect evidence (8) that α-aminobenzylpenicilloic acid could be the degradation product. β-Lactamase enzymes are normally present in bacteria of the GI tract (24), and at least three other penicillins are known to yield their penicilloic acid derivatives in urine (25). However, the time course of plasma metabolite levels shown in Fig. 1 indicates that the apparent metabolite is eliminated more slowly than is ampicillin. Since the degree of human serum albumin (4 g. %) binding of α-aminobenzylpenicilloic acid was found to be only 9 ± 2%, slow elimination caused by strong protein binding is ruled out. Additional studies (unpublished) in rats have shown that ampicillin and α-aminobenzylpenicilloic acid have very similar elimination half-lives, and it therefore appears unlikely that the latter is the metabolite. Studies of the transformation product of ampicillin in the rat were carried out recently by Nishida *et al.* (26). In agreement with the results of the present study, the transformation product was found in greatest relative concentration in the serum of the rat and was retained in the body longer than ampicillin. Further identification of this metabolite would be of interest since its slow elimination may lead to accumulation during multiple dosing.

Hetacillin Elimination—Since hetacillin itself is not bactericidal, an essential consideration in its clinical use is whether all of the dose is converted to ampicillin *in vivo*. Because of its rapid rate of hydrolysis, it was not feasible to assay unchanged hetacillin in the urine. However, the evidence indicates that most, if not all, of the hetacillin is converted to ampicillin in the central compartment. Extrarenal elimination of appreciable hetacillin is ruled out by the urinary recovery of the same fraction of the dose whether ampicillin or hetacillin is injected (Table III). Negligible renal excretion of hetacillin is suggested by the renal clearances of total antibiotic (ampicillin + hetacillin), which are substantially less than renal clearances of ampicillin alone (Table II). The strongest evidence is the excellent fit of the urinary excretion rate data to the pharmacokinetic model when it was assumed that renal excretion of only ampicillin occurs (Scheme II and Fig. 3). If renal excretion of both compounds occurred, then it would not be possible to fit both the early and the late urinary excretion rate data with the devised pharmacokinetic model.

The rate at which hetacillin converts to ampicillin is somewhat greater *in vivo* than has been found *in vitro*. A conversion half-life for hetacillin hydrolysis *in vitro* has been found to be about 25 min. both in pH 7 buffered solution (1) and in plasma (2) at 37°. At 4°, the half-life in plasma increases to more than 4 hr. and thus freezing freshly drawn plasma samples stabilizes hetacillin somewhat (2). The *in vivo* half-life estimates for hetacillin conversion found in the present study (average of 11.2 min.) are more rapid than, but consistent with, the previously determined *in vitro* values. This suggests that the *in vivo* mechanism for hetacillin conversion to ampicillin does not necessarily require the presence of drug-metabolizing enzymes.

Effect of Hetacillin on Ampicillin Pharmacokinetics—The distribution and elimination parameters of ampicillin formed from hetacillin were essentially identical to those for intravenous ampicillin (Table III). The integral coefficients of the antibiotic were not changed (Fig. 4), and all of the dose of hetacillin appeared to be converted *in vivo* to ampicillin. Based on these characteristics, the use of equimolar parenteral doses of ampicillin and hetacillin should be chemotherapeutically equivalent (19). This, in fact, appears to be the case since clinical studies with the two compounds have produced essentially identical results (3, 27).

The primary effect of parenteral injection of hetacillin, therefore, is to alter slightly the time course of the central and peripheral compartment levels of ampicillin. The average values of the constants (Table III) were used with appropriate equations (18) to obtain the fractional dose levels shown in Fig. 6 for each compartment after injection of the two compounds. It can be seen that intravenous injection of hetacillin is analogous to administration of ampicillin by a very rapid absorption process. Maximum levels of ampicillin in the central compartment are reached at about 30 min. after hetacillin injection. The time course of ampicillin in the peripheral compartment is similar after both compounds, except that occurrence of the peak level is delayed by about 30 min. after hetacillin is given. As shown previously by the individual values of the integral coefficients, the linear areas under the curves in the corresponding compartments do not differ significantly. The areas for the central compartment in the graph are slightly greater than the calculated D_{1a} values because graphical correction is not made for the small fraction of protein-bound ampicillin (Eq. 12).

Claims were made in earlier investigations (6, 7) that parenteral hetacillin produces an enhancement of ampicillin plasma levels by virtue of a decreased renal clearance. However, as shown by the data in Fig. 2 and Table II, this conclusion can be reached by misinterpretation of the results of nonspecifically assaying hetacillin along with ampicillin. When ampicillin and hetacillin are separated electrophoretically prior to the prolonged microbiological incubation, this technical artifact is resolved and the behavior of hetacillin and ampicillin formed from hetacillin can be described as shown in Fig. 3. Similar precaution must be made in pharmacokinetic evaluation of other ampicillin precursors when the commonly employed microbiological assay is involved.

Absorption of Ampicillin and Hetacillin—An average of 32% (ranging from 21 to 46%) of the dose of ampicillin was absorbed from the GI tract by the subjects of the present study. Although the data are limited due to the small number of subjects, there does not seem to be any difference in ampicillin absorption between the male and female subjects. This finding agrees with data of Poole (28) who found that in beagle dogs the amphoteric penicillins, unlike others such as dicloxacillin and nafcillin, do not exhibit sex differences in intestinal absorption.

The rapid rate and only partial absorption of ampicillin made pharmacokinetic characterization of its absorption properties difficult. The limited number of data points did not permit direct utilization of a two-compartment model, while the intrasubject variation in distribution parameters (Table III and Fig. 4) precluded accurate use of the methods of Loo and Riegelman (29). The day-to-day change in elimination also contributed to variation in plasma level areas, which probably caused the differences in bioavailability estimates when comparing the results from the two methods of calculation (Table V). The zero-order input function for a one-compartment model was, therefore, chosen primarily for curve-fitting purposes and to evaluate differences in the general time course of absorption. However, the intestinal absorption of many penicillins does not occur by simple diffusion (30) and the zero-order input function is thus partly consistent with a specialized absorption process.

The results of the present study suggest that ampicillin absorption is somewhat better when the antibiotic is administered as hetacillin. Of practical importance, the presence of food appeared to enhance absorption of the antibiotic in three of the four subjects. This finding agrees with the results of Sutherland and Robinson (4), who found that the 6-hr. urinary recovery of ampicillin after a 500-mg. dose of hetacillin increased from 32 to 48% of the dose in fasting and nonfasting subjects, respectively.

The literature concerning the comparative absorption properties of hetacillin and ampicillin has been conflicting. Sutherland and Robinson (4) as well as Modr and Dvoracek (7) found greater urinary recovery of the antibiotic after giving oral ampicillin in comparison with oral hetacillin. Another study by Magni *et al.* (1) showed plasma level areas and urinary recoveries of ampicillin to be almost twice as great after ampicillin capsules as compared to hetacillin capsules. On the other hand, two additional studies demonstrated similar absorption of the antibiotic from ampicillin and hetacillin capsules. Kirby and Kind (31) found essentially equal plasma level areas of ampicillin after oral doses of ampicillin or hetacillin, as did Smith and Hamilton-Miller (3). It thus appears that the comparison of the relative degree of absorption from ampicillin and hetacillin capsules depends on the particular investigation and the commercial products employed.

Several additional factors may account for differences in ampi-

cillin bioavailability among subjects and commercial products. Intersubject variability (32), the age of the subjects (33), the presence of liver disease (34), and the hydrate (35) and salt forms (36) of the antibiotic are several factors shown to affect ampicillin absorption. In general, the bioavailability of different pharmaceutical formulations of ampicillin should be considered on an individual basis and variation of between 20 and 50% absorption can be expected among subjects and commercial products.

Design and Use of Ampicillin Precursors—The rationale for designing precursors to ampicillin is partially for enhancement of GI absorption of the antibiotic. Since only 20–50% of an oral dose of ampicillin is absorbed from the GI tract, the potential for improvement of absorption of the antibiotic is considerable. Hetacillin appears to offer a small improvement in absorption of ampicillin when compared with the ampicillin product tested in the present study.

The parenteral administration of an inactive precursor is not an *in vivo* therapeutic advantage since a maximum of an equimolar amount of active product can be produced and, if the conversion rate is not sufficiently rapid, there exists the possibility that elimination of the precursor will result in loss of part of the intended dose of antibiotic. This appears to occur to a substantial degree with met-ampicillin, a condensation product of ampicillin and formaldehyde, which is converted to ampicillin during renal excretion (37). On the other hand, if the precursor has some degree of antibiotic activity, then its use could considerably enhance the effectiveness of the dose of drug. This is of greatest potential importance for antibiotics such as ampicillin which are excreted very rapidly. A major pharmaceutical or *in vitro* advantage to use of parenteral precursors is that, like hetacillin, they may provide greater chemical stability in infusion solutions which are used over extended time periods. For example, using the criterion of loss of 10% of microbiological activity as an end-point, ampicillin is "stable" for only 1 hr. while hetacillin can be used over a period of 6 hr. in infusion solutions (38).

The assay procedure must be carefully considered in pharmacological evaluation of a precursor of an antibiotic. The nonspecific type of microbiological procedure which is commonly used for most antibiotics requires several hours of incubation of the drug with the microorganism (9). This can result in hydrolysis of the inactive precursor to active compound, and the assay procedure will reveal bactericidal activity *in vitro* that exceeds that actually present *in vivo*. Since hetacillin appears to be completely converted to ampicillin in the GI tract prior to absorption, reports of relative absorption of the two compounds where the nonspecific assay was used are probably accurate. This does not necessarily apply to other ampicillin precursors. A separation method of analysis, along with employment of the pharmacokinetic approach used in the present study, is necessary for elucidation of the actual chemotherapeutic value of antibiotic precursors.

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ACKNOWLEDGMENTS AND ADDRESSES