Kinetics of β-Lactamase Inactivation of Penicillins I: Effect of Side-Chain Structure, Ionic Strength, pH, and Temperature

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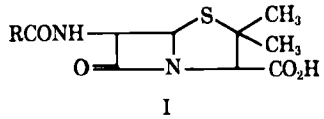
Abstract □ The *Staphylococcus aureus* Tex-2 β-lactamase-catalyzed hydrolysis of several penicillins was shown to follow Michaelis–Menten kinetics. Penicillins with a lipophilic side chain had the highest affinity for the enzyme. Introduction of a polar group (either positively or negatively charged) into the side chain and/or a bulky diester group on the penicillin nucleus drastically lowered the binding. Of the penicillins studied, the α-aminopenicillins were the most susceptible. The effect of ionic strength on the staphylococcal β-lactamase hydrolysis of penicillin G and ampicillin was small, but the effect of pH was conspicuous. At 25°, a V-shaped pH–K_m profile with a pH optimum at 6.8 was obtained for penicillin G, while a sigmoid pH–K_m profile with bell-shaped pH–V_{max} profile at 6.4 was obtained for the amphoteric ampicillin. The heats of activation of penicillin β-lactamase-catalyzed hydrolysis of penicillins was shown to vary with the enzyme species and with the substrate. The amphoteric penicillin had a much lower heat of activation value than that of the monobasic penicillin. For the *S. aureus* Tex-2 catalyzed hydrolysis of penicillin G and ampicillin, the heat of activation values were 7.18 and 5.17 kcal./mole at pH's of 6.8 and 7.4, respectively, while a value of 5.10 kcal./mole was obtained for the *Bacillus cereus* B569 catalyzed hydrolysis of ampicillin at pH 6.2. The temperature optimum for the staphylococcal β-lactamase inactivation of ampicillin at pH 7.4 may be 35–37°.

Keyphrases □ Penicillins—kinetics of β-lactamase hydrolysis, effect of side chain, ionic strength, pH, temperature □ β-Lactamase hydrolysis of penicillins—kinetics, effect of side chain, ionic strength, pH, temperature □ Penicillinase inactivation of penicillins—kinetics, effect of ionic strength, pH, temperature, side chain □ α-Aminopenicillins—kinetics of β-lactamase hydrolysis, effect of side chain, ionic strength, pH, temperature

Considerable research has been conducted on the kinetics of the interactions between the β-lactam antibiotics (the penicillins and cephalosporins) and the β-lactamases produced by various bacteria. Abraham and Newton (1), Banfield (2), Citri and coworkers (3–7), and Hamilton-Miller (8) investigated the kinetics of the bacillary β-lactamases; Knox and Smith (9), Hamilton-Miller (8), Crompton *et al.* (10), Novick (11), Richmond (12), Gourevitch *et al.* (13), Smith *et al.* (14), and Depue *et al.* (15) studied the kinetics of the staphylococcal β-lactamases. The hydrolysis of penicillins by *Klebsiella aerogenes* and *Bacillus licheniformis* enzymes has also been reported (8). Comprehensive reviews on β-lactamases and β-lactamase kinetics were recently published by Citri and Pollock (16), Citri (17), Abraham (18), and Rauenbusch (19).

Of all the penicillins, ampicillin is the most unstable toward the β-lactamases. Penicillin G, phenoxymethyl penicillin (penicillin V), phenethicillin, and propicillin



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are all hydrolyzed at a similar rate and all are more stable than ampicillin (1, 9). Penicillin N, cephalosporin C (1), and 6-aminopenicillanic acid (11) are only slightly inactivated. Methicillin, oxacillin, and cloxacillin (3–7, 11) are resistant.

The ability of a penicillin (I) to resist hydrolysis depends, to a large degree, on the nature of the side chain R (13, 20). Susceptible penicillins have low Michaelis constants (K_m) and very high maximum rates (V_{max}). Moderately resistant penicillins have K_m's 30–80 times larger than those of the susceptible penicillins and have somewhat lower V_{max}'s; the resistant penicillins have very low V_{max}'s but K_m's similar to those of the moderately resistant penicillins (13).

A previous article (21) reported the applicability of a pH-stat alkalimetric titration method in the investigation of β-lactamase activity, substrate specificity, and penicillin stability. The present paper reports the effects of environmental factors, such as ionic strength, temperature, and pH, on the interactions between the β-lactamases and the penicillins. The main interest was the kinetic behavior of the staphylococcal and bacillary β-lactamase (penicillinase) catalyzed hydrolysis of penicillins with different side-chain groups and nucleus structures. It is hoped that such studies will add to the understanding of the mechanism of the penicillinase inactivation of penicillins.

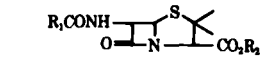
EXPERIMENTAL

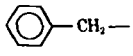
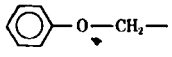
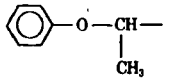
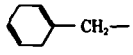
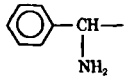
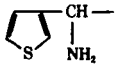
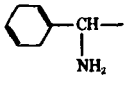
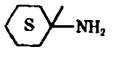
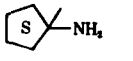
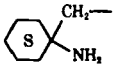
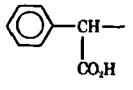
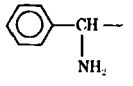
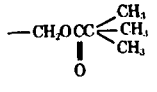
Materials—The following penicillins were used, all of which are either new or established products of this laboratory[1]: the potassium salts of penicillin G, phenoxymethyl penicillin, phenethicillin, ampicillin, and cyclacillin[2]; the sodium salt of 6-[2-(1,4-cyclohexadien-1-yl)acetamido]penicillanic acid[3] (I); and zwitterions of 6-(1-aminocyclopentanecarboxamido)penicillanic acid[4] (II) and 6-[2-(1-aminocyclohexyl)acetamido]penicillanic acid[5] (III). Also used were the disodium salt of carbenicillin[6], zwitterions of epicillin[7] (IV) and 6-[2-amino-(3-thienyl)acetamido]penicillanic acid[8] (V), and the hydrochloride salt of pivampicillin[9].

β-Lactamases—*Staphylococcus aureus* Tex-2 β-lactamase was obtained from the supernatant fluid of a fresh broth culture of this organism[10]. *Bacillus cereus* B569 β-lactamase was obtained commercially[11] in crystalline form and was dissolved in a dilute (0.1%) gelatin A USP solution to the desired strength.

[1] Wyeth Laboratories.
[2] Wy-4508.
[3] Wy-12955.
[4] Wy-7953.
[5] Wy-14903.
[6] Beecham Laboratories.
[7] 6-[2-Amino-(1,4-cyclohexadien-1-yl)acetamido]penicillanic acid, SQ 11,302, Squibb Institute.
[8] BE-P875, Bristol Laboratories.
[9] Leo Laboratories.
[10] Prepared by the Antibiotics Section, Research Division, Wyeth Laboratories.
[11] Riker Co.

Table I—*S. aureus* Tex-2 β-Lactamase-Catalyzed Hydrolysis of Penicillins at 25° and pH 7.0[a]



Number	Penicillin	Side Chain R_1	Side Chain R_2	K_m, μM	V_{max}, μM/min.	Ratio to V_{max} of Penicillin G
1	Penicillin G		H	50	130	1.00
2	Phenoxymethyl penicillin		H	150	165	1.27
3	Phenethicillin		H	100	113	0.87
4	I		H	150	85	0.65
5	Ampicillin		H	550	303	2.33
6	V		H	500	292	2.24
7	IV		H	650	320	2.46
8	Cyclacillin		H	750	78	0.60
9	II		H	350	117	0.90
10	III		H	900	320	2.46
11	Carbenicillin		H	4200	160	1.23
12	Pivampicillin (ampicillin diester)			3000	30–35	0.23–0.27

[a] Substrate concentrations ranging from 2 to 12 × 10^{-3} M and total ionic strength of 0.026 were used for each penicillin except carbenicillin. The K_m and V_{max} were calculated from Lineweaver–Burk plots.

Kinetics—The standardization of the enzyme preparations, the kinetic procedures, and calculations of initial rates were identical to those reported previously (21). The enzyme kinetic parameters were obtained in the following manner.

According to conventional enzyme kinetics (22), the enzyme E and substrate S interact to form an enzyme–substrate complex ES and then decompose to enzyme E and a product P (Scheme I). The

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E$$

Scheme I

rate of formation of product is given by:

$$v = \frac{d(p)}{dt} = \frac{K_3(E)(S)}{(S) + K_m} = \frac{V_{max}(S)}{(S) + K_m} \qquad \text{(Eq. 1)}$$

By taking the reciprocal of Eq. 1 and multiplying through by (S), the following final equation is obtained:

$$\frac{(S)}{v} = \frac{(S)}{V_{max}} + \frac{K_m}{V_{max}} \qquad \text{(Eq. 2)}$$

where (S) is the initial substrate concentration and v is the initial rate. From Lineweaver–Burk plots (23), by plotting the term $(S)/v$ *versus* (S), the V_{max} can be obtained from the slope of the plot and the K_m either by dividing the ordinate intercept by the slope or by extrapolating the slope to the abscissa intercept, which is the same as the negative K_m. A calculator[12] was utilized for the initial rate computations, and the kinetic parameters were obtained graphically.

RESULTS AND DISCUSSION

The *S. aureus* Tex-2 β-lactamase-catalyzed hydrolysis was shown to follow Michaelis–Menten kinetics. By using 2.5 units of the enzyme, where the unit of activity of the β-lactamase is defined as the amount of enzyme that will hydrolyze 1.0 μmole of potassium penicillin G in 1 min. at 25° and pH 7.0 (buffer free), and 2.0–12.0 × 10^{-3} M each of penicillin G, phenoxymethyl penicillin, phenethicillin, ampicillin, cyclacillin, Compounds I, II, III, IV, and V, and pivampicillin, essentially pseudo-zero-order kinetics were maintained in each case over most of the reaction, particularly with higher initial substrate concentrations. With carbenicillin, higher (fivefold) substrate concentrations had to be used due to its rather high K_m value. In these studies, except those on carbenicillin and pivampicillin, a constant ionic strength was also maintained for each sample. In almost all cases, rather reproducible initial rates were obtained.

[12] Olivetti Underwood Programma 101.

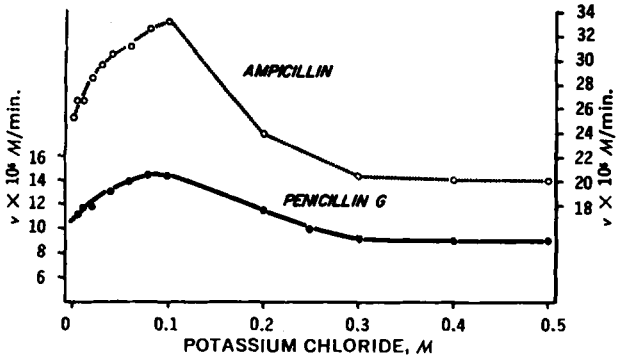


Figure 1—*Effect of ionic strength on the* S. aureus *Tex-2 β-lactamase- (2.5 units) catalyzed hydrolysis of ampicillin and penicillin G at 25°. The pH = 6.8 for penicillin G and 6.4 for ampicillin.* (S) = 2×10^{-3} M *for either penicillin.*

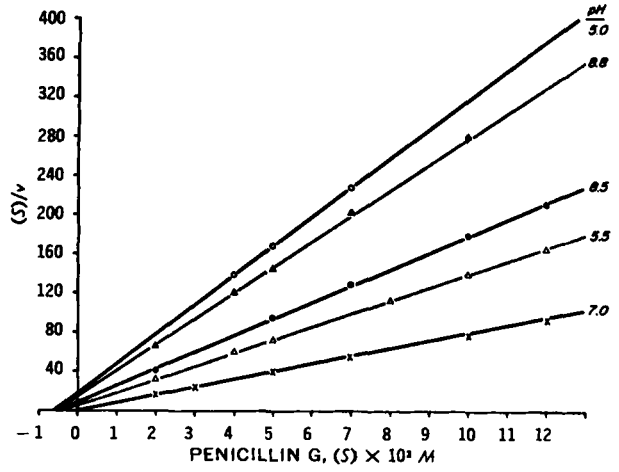


Figure 2—*Effect of substrate concentration* (S) *at various pH's on kinetics of* S. aureus *Tex-2 β-lactamase- (2.5 units) catalyzed hydrolysis of penicillin G at constant temperature (25°).* (S)/v *was plotted* versus (S) *at various indicated pH's to give maximal rates* (V_{max}) *and Michaelis constants* (K_m).

Effect of Penicillin Side Chain on Enzyme Kinetic Parameters—Table I shows the calculated K_m and V_{max} of 12 penicillins studied at pH 7.0 and 25°. Among the penicillins studied, penicillin G, phenoxymethyl penicillin, phenethicillin, and Compound I had the lowest K_m values (50–150 μM); *i.e.*, they have the highest affinity for the active sites on the enzyme. The α-aminopenicillins (ampicillin and Compounds IV and V) and the amino alicyclic penicillins (cyclacillin and Compounds II and III) had a much lower affinity, as reflected in the higher K_m values (350–900 μM). Carbenicillin and pivampicillin had the lowest affinity among the penicillins studied, their K_m values (3000–4000 μM) being about 60–80 times higher than that of penicillin G.

Thus, the penicillins with the highest affinity for β-lactamase are those having aromatic benzyl (penicillin G), phenoxyalkyl, and 1,4-dihydrophenylmethyl (Compound I) side chains. Introduction of a polar group (either positively or negatively charged) into the side-chain group significantly decreases the affinity. A similar finding was previously reported by Depue *et al.* (15), who showed that penicillins with a positively charged nitrogen atom (as in an amino or a nitro group in the side chain) have substantially lower affinity.

Despite the rather low affinities of the α-aminopenicillins for the enzyme, their maximum rates of hydrolysis were about two times as fast as that of penicillin G or phenoxymethyl penicillin. The kinetic parameters of carbenicillin were even more unusual. Its extremely low affinity seemed to have little effect on its susceptibility toward the enzyme. Cyclacillin, on the other hand, has relatively low affinity for the enzyme and also demonstrates a rather low V_{max} value. However, this property was altered as a result of the addition of a methylene group between the amino alicyclic group and the peptide linkage of cyclacillin as in Compound III. The different kinetic behavior of carbenicillin and Compound III from that of penicillin G and cyclacillin is, no doubt, related to the steric effect of their side chains. In general, the maximum rates of the penicillins obtained in the present studies were in accordance with their relative stability pattern reported previously (21).

The drastically increased stability of pivampicillin could be related to its greatly decreased affinity for the enzyme. Conceivably, the stretched bulky diester group of pivampicillin is playing an important role.

Variation in the penicillin side-chain group (R) could be expected to produce a drastic change in the overall lipophilic and hydrophilic properties of the molecule and in the enzyme kinetic parameters. Variation in the side chain, however, had a much greater effect on the K_m than on the V_{max}. Penicillin G, phenoxymethyl penicillin, and carbenicillin are good examples of this difference; their V_{max} values were essentially identical, but their K_m's differed by a factor of 50–80. Ampicillin and Compounds IV and V, which are all α-aminopenicillins and have similar biological properties[13] (24–26), were equally susceptible toward the β-lactamases. They were inactivated about 2–3 times faster (greater V_{max}) than penicillin G despite their much lower affinity (higher K_m) for the active sites of the enzyme. Thus, hydrolysis may depend on "favorable conformational change" or "favorable orientation" of the active sites in the enzyme which, in turn, may depend on the presence of a charge amino group in the substrate. On the other hand, the significantly increased insusceptibility of cyclacillin and pivampicillin to the β-lactamase is probably due to catalytically "unfavorable orientation" of the active centers in the enzyme as a result of some steric effect of the substrate molecule. The faster and higher serum levels of pivampicillin attained compared to its parent compound (27–29) may be partially due to its higher resistance toward β-lactamase inactivation *in vivo*.

Effect of Ionic Strength—At 25° and the pH optimum (21), *S. aureus* Tex-2 β-lactamase (2.5 units) was allowed to react with penicillin G and ampicillin (2.0×10^{-3} M), the total ionic strength being varied with the range of 0.005–0.5 by adding potassium chloride. The initial rates for both penicillin G and ampicillin were shown to increase directly in proportion to the salt concentration until a maximum rate was reached, then to slow down slightly, and finally to level off with a further increase in salt concentration (Fig. 1). In each case the maximum rate was reached at an ionic strength of 0.1 and began to level off at 0.3. The rate increased about 30% as the apparent potassium chloride concentration progressed from 0 to 0.1 M and decreased about 20% as it continued on to 0.3 M. Accordingly, the neutral salt concentration seemed to have no significant effect on the rate of β-lactamase-catalyzed hydrolysis, at least within the salt range used.

Effect of pH on Kinetic Parameters—As mentioned previously, the hydrophilic and lipophilic groups on the penicillin side chain could effectively influence the kinetic parameters of the β-lactamase-catalyzed hydrolysis of penicillins at a neutral pH. It was of interest to observe the change in kinetic behavior at different pH's, particularly between two distinctly different types of penicillin such as monobasic penicillin G and amphoteric ampicillin. Accordingly, *S. aureus* Tex-2 β-lactamase (2.5 units) was allowed to interact with various initial substrate concentrations of either penicillin G or ampicillin, holding the temperature at 25°, maintaining a constant total ionic strength[14] (0.026 M), and varying the pH from 5 to 9.

Virtually pseudo-zero-order kinetics were obtained for penicillin G at all pH's, *i.e.*, the enzyme apparently was saturated with the substrate. Ampicillin, when applied within the usual range of initial substrate concentrations ($2–12 \times 10^{-3}$ M), appeared quite sufficient to saturate the enzyme at neutral and higher pH's, although at lower pH's (<6.0) a relatively higher initial substrate concentration had to be used. The enzyme kinetic parameters K_m and V_{max} were obtained from Eq. 2 by plotting $(S)/v$ against

[13] All three are acid stable, readily absorbed after oral administration, and have a broad spectrum of antimicrobial activities.

[14] The ionic strengths contributed by the substrates were calculated using pK 2.64 as the dissociation constant for the carboxyl group of penicillins and 7.25 for the amino group of ampicillin (30).

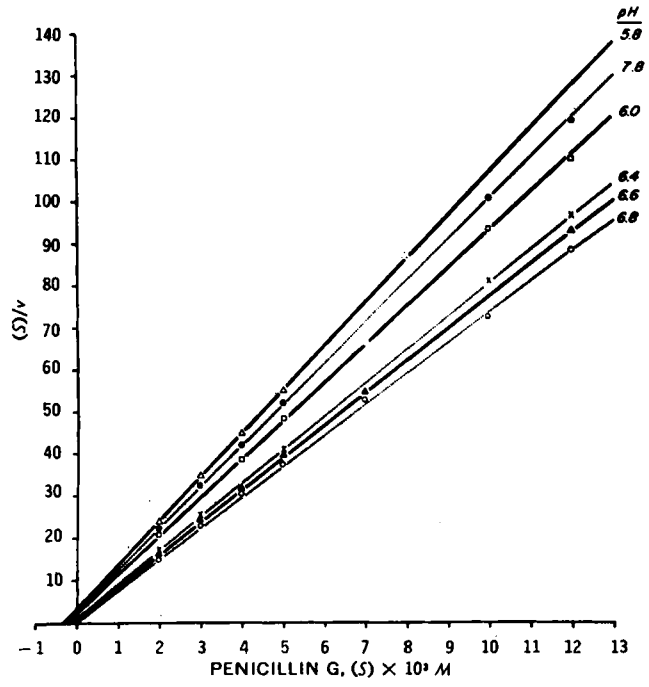


Figure 3—*See Fig. 2 for explanation.*

(*S*). Figures 2 and 3 are the resulting plots for penicillin G; similar plots were obtained for ampicillin but are not shown.

Penicillin G had the highest affinity for the active sites of the *S. aureus* Tex-2 β-lactamase or a lowest K_m value at pH 6.8 than at other pH's. The K_m values were increased about 15-fold at pH's of 5 and 8.5 over that at pH 6.8. By plotting the K_m against the pH (Fig. 4), a V-shaped pH–K_m profile was obtained. This type of curve may be typical for the monobasic penicillin G type of drugs. By plotting the V_{max} *versus* pH (right-hand side of Fig. 4), a nearly bell-shaped pH–V_{max} profile was obtained which is also in accordance with the pH optimum value of *S. aureus* Tex-2 reported previously (21). The greatest affinity occurred at the point of highest rate. At pH's other than optimum, the hydrolysis rate decreases significantly and simultaneously diminishes its affinity for the enzyme.

The effect of pH on the affinity characteristics of ampicillin were quite different from those of penicillin G. As shown in Fig. 5, a sigmoid pH–K_m profile was obtained for this amphoteric penicillin, although its pH–V_{max} curve was bell-shaped like those of all the other penicillins studied (21). Since it depends on the state of ionization of the substrate (and possibly on the free enzyme and enzyme–substrate complex), the pH has a significant effect on the K_m. Thus, at a pH of about 5, ampicillin exists virtually in zwitterionic form and has the highest K_m (>2000 μ*M*) or the lowest affinity for the

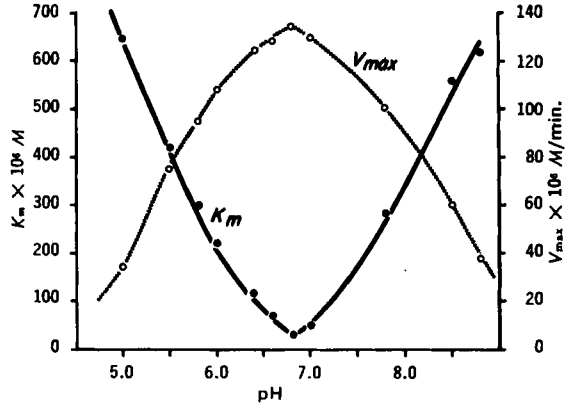


Figure 4—*Effect of pH on maximal rate* (V_{max}) *and Michaelis constant* (K_m) *for the* S. aureus *Tex-2 β-lactamase- (2.5 units) catalyzed hydrolysis of penicillin G at 25°.*

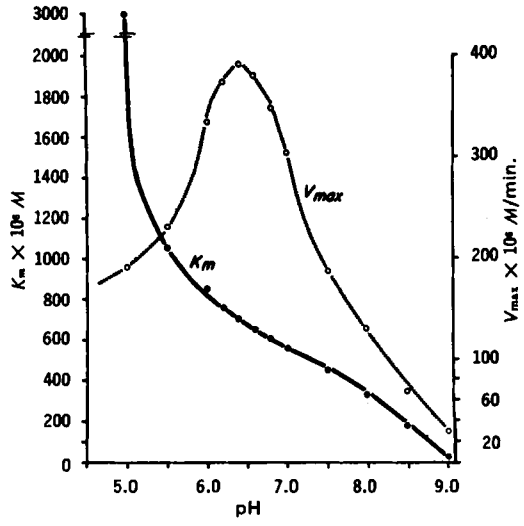


Figure 5—*Effect of pH on maximal rate* (V_{max}) *and Michaelis constant* (K_m) *for the* S. aureus *Tex-2 β-lactamase- (2.5 units) catalyzed hydrolysis of ampicillin at 25°.*

active sites of the enzyme, but at pH 6–7 it exists in both zwitterionic and anionic species and its affinity is increased about fivefold; at a pH above 8.5, essentially all of the ampicillin molecules are in anionic form and the K_m (<200 μ*M*) drops about 10–20-fold compared with that at a pH of 5. Accordingly, further evidence is provided that only the positively charged amino group on the side chain of ampicillin is needed to account for the decreased affinity for the enzyme.

The maximum *S. aureus* Tex-2 β-lactamase activity occurred at a slightly lower pH with ampicillin than with penicillin G (6.4 instead of 6.8). The difference probably involves the state of ionization of the enzyme–substrate complex. The maximum rate (V_{max}) was shown to be nearly halved at pH 5 and decreased about 10-fold at pH 9 compared with the value at the pH optimum (6.4). The decreased V_{max} at pH's other than optimum observed in the present studies may have been due to a conformational change or to the state of ionization of the enzyme–substrate complex rather than to an acid–base inactivation of the enzyme protein.

Effect of Temperature on Rate—Experiments were conducted on the *S. aureus* Tex-2 β-lactamase-catalyzed hydrolysis of penicillin

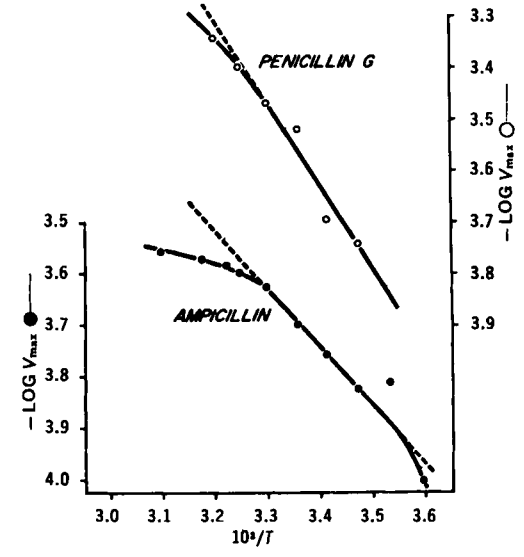


Figure 6—*Effect of temperature on the maximal rate* (V_{max}) *of the* S. aureus *Tex-2 β-lactamase-catalyzed hydrolysis of two penicillins. The logarithm of the maximal rate (log* V_{max}*) is plotted against the reciprocal of the absolute temperature (10³/T°). Ampicillin was hydrolyzed at pH 7.4 with 2.5 units of the enzyme; penicillin G was hydrolyzed at 6.8 with 5.0 units of the enzyme.*

Table II—Effect of Temperature on *S. aureus* Tex-2 Kinetic Parameters[a]

Temperature	Penicillin G		Ampicillin	
	$K_m \times 10^6$ *M*	$V_{max} \times 10^6$ *M*/min.	$K_m \times 10^6$ *M*	$V_{max} \times 10^6$ *M*/min.
5°	—	—	800	100
10°	—	—	750	155
15°	200	180	650	160
20°	80	202	600	175
25°	35	268	460	200
30°	25	340	350	235
35°	30	398	320	245
37°	—	—	300	258
40°	50	455	—	—
42°	—	—	340	267
50°	—	—	500	278

[a] Substrate concentration ranging from 40 to 240 μmoles ($2 \times 12 \times 10^{-3}$ *M*) and a total ionic strength of 0.026 were maintained closely for each penicillin. The *S. aureus* Tex-2 catalyzed hydrolysis of penicillin G and ampicillin was conducted at pH 6.8 and 7.4, respectively. The strength of the enzyme was 5.0 units for penicillin G and 2.5 units for ampicillin.

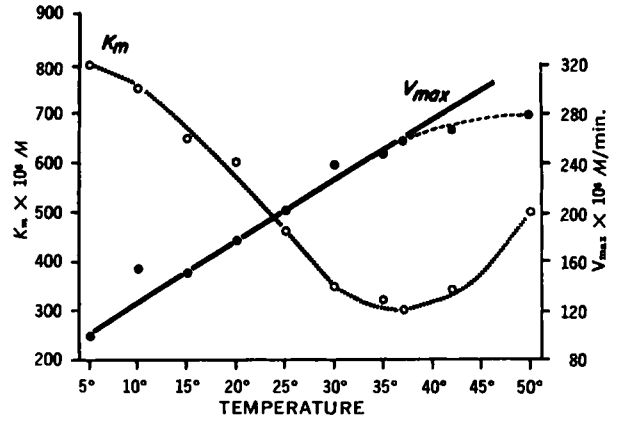


Figure 7—*Effect of temperature on the* S. aureus *Tex-2 β-lactamase-(2.5 units) catalyzed hydrolysis of ampicillin at pH 7.4. The Michaelis constant* (K_m) *is shown on the left-hand scale; the maximum rate* (V_{max}) *is shown on the right-hand scale.*

G at 15–40° and pH 6.8 and of ampicillin at 5–50° and pH 7.4. A constant enzyme concentration was maintained (5.0 units for penicillin G and 2.5 units for ampicillin) as well as a nearly constant ionic strength (0.026 *M*), but the substrate concentration was varied ($2\text{–}12 \times 10^{-3}$ *M*). The V_{max} values for different temperatures were obtained from Lineweaver–Burk plots (Eq. 2). Figure 6 was constructed by plotting log V_{max} *versus* $1/T$ for both penicillin G and ampicillin. The heats of activation for the staphylococcal β-lactamase hydrolysis of these two antibiotics, as calculated from the Arrhenius equation, were 7.18 and 5.17 kcal./mole, respectively.

The *B. cereus* B569 β-lactamase- (5.0 units) catalyzed hydrolysis of ampicillin was also investigated at 4–45° and pH 6.2. From the plot of the logarithms of the initial rates *versus* the reciprocals of the absolute temperatures (not shown), the heat of activation for this hydrolysis was 5.10 kcal./mole.

The curves plotted in Fig. 6 show a slight downward concavity, which indicates that either the breakdown of the enzyme–substrate complex or the affinity of the substrate for the enzyme, or both, may depend on the heat of activation over the indicated range of temperatures. The room temperature range of 15–30° was chosen for calculating the heat of activation, simply because within this range the rates of hydrolysis fall essentially on a straight line. As the temperature was increased toward the so-called "optimum" for the enzyme, the heat of activation dropped to values somewhat lower than those in the room temperature region. For penicillin G at 30–40°, it dropped from 7.18 to about 5.0–6.0 kcal./mole. At 15° and below, on the other hand, it increased from 7.18 to about 9.5 kcal./mole. Similar variations were noted for the interactions of ampicillin with *S. aureus* Tex-2 and *B. cereus* B569 β-lactamases.

Prior to the present study, Smith and Hamilton-Miller (31) had already presented data showing that the heat of activation of the penicillin hydrolysis is higher when catalyzed by β-lactamases from Gram-positive than from Gram-negative bacteria (7–9 *versus* about 3–5 kcal./mole). No one had previously demonstrated, however, that the heat of activation also varies from enzyme to enzyme (species) in accordance with the temperature and the type of substrate used. The variance of heat of activation with temperature will be discussed further in the following section.

Effect of Temperature on Kinetic Parameters—With the pH for the *S. aureus* Tex-2 β-lactamase-catalyzed hydrolysis of ampicillin held at 7.4 and of penicillin G held at 6.8, the temperature was varied and the resulting kinetic parameter values, were obtained (Table II). The affinity of penicillin G and ampicillin for the enzyme (as shown by the decrease in K_m) increased with the increase in temperature, the highest affinity falling in the region of 30–35° for penicillin G and 35–37° for ampicillin and thereafter decreasing with any further increase in temperature. Similarly, the V_{max} increased proportionately with an increase in temperature up to the body temperature region for both penicillins, and then the rate increased only slightly with a further increase in temperature. The effect of temperature, like pH and the penicillin side chain, was shown to be definitely greater on the K_m than on the V_{max}. Figure 7 clearly depicts the effect of temperature on the kinetic parameters of the *S. aureus* Tex-2 β-lactamase-catalyzed hydrolysis of ampicillin at pH 7.4. In this system, both the rate and affinity are at a maximum at an apparent temperature optimum of the body temperature (35–37°).

The heat of activation varying with temperature in β-lactamase kinetics is not unexpected, since β-lactamases, like other biologically active enzymes, are proteinaceous macromolecules. The active sites on the enzyme and their three-dimensional structures are extremely sensitive to environmental factors, particularly pH and temperature. The observed high and low heat of activation values at relatively lower and higher temperatures in comparison with that at room temperature in the present staphylococcal β-lactamase studies was believed due to the physical structure changes of the enzyme. It is conceivable that at the apparent optimal conditions (pH optimum and temperature optimum) of an enzyme, its active form molecules are overwhelming; accordingly, a faster reaction rate, higher affinity for a substrate, and lower heat of activation for the reaction are characteristic.

SUMMARY

The *S. aureus* Tex-2 β-lactamase-catalyzed hydrolysis of several penicillins was shown to follow Michaelis–Menten kinetics. Variations in side-chain structure as well as pH and temperature had a much greater effect on the K_m than on the V_{max}. Either a positively or negatively charged group on the penicillin side chain caused a drastic decrease in binding to the active sites of the enzyme. The α-aminopenicillins were the most readily hydrolyzed of the penicillins studied. Some side-chain groups may introduce a steric effect which renders the penicillin molecule less accessible to the enzyme, or the same reduced accessibility may result from esterification of the penicillin nucleus carboxyl group. The effect of a neutral salt on the enzyme kinetics was insignificant, but the effect of pH was conspicuous. At 25°, a V-shaped pH–K_m profile with a pH optimum at 6.8 was obtained for penicillin G, while a sigmoid-shaped pH–K_m profile with a maximum rate at pH 6.4 was obtained from ampicillin. In the temperature range of 15–30°, the heat of activation values for the *S. aureus* Tex-2 β-lactamase-catalyzed inactivation of penicillin G and ampicillin were 7.18 and 5.17 kcal./mole at pH's of 6.8 and 7.4, respectively, while that for the *B. cereus* B569 β-lactamase-catalyzed hydrolysis of ampicillin was 5.10 kcal./mole at pH 6.2. The heat of activation varies from enzyme to enzyme species in accordance with temperature and the type of substrate used. The temperature optimum for the *S. aureus* Tex-2 β-lactamase may be 35–37°.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 6, 1972, from the *Pharmacy Research and Development Division, Wyeth Laboratories, Inc., Radnor, PA 19087*

Accepted for publication December 5, 1972.

Presented to the Division of Basic Pharmaceutics, 31st International Congress of Pharmaceutical Sciences, Washington, D. C., September 1971.

The authors express their appreciation to Dr. S. Rosenman for supplying the *S. aureus* Tex-2 β-lactamase preparations and to Miss G. Moffitt and Mr. H. Garber for their excellent technical assistance.

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Fenfluramine Blockade of CNS Stimulant Effects of Amphetamines

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Abstract □ Fenfluramine, 15 mg./kg. s.c., produced a slight but significant decrease in spontaneous motor activity in male Swiss mice as measured in a circular photocell activity cage. Treatment with fenfluramine 15 min. prior to either dextroamphetamine or methamphetamine, 2.5 mg./kg. i.p., significantly diminished the hyperactivity caused by these compounds. In a second experiment in mice, fenfluramine prolonged pentobarbital sleeping time, whereas dextroamphetamine and methamphetamine shortened barbiturate narcosis. Pretreatment with fenfluramine reversed the reduction of pentobarbital sleeping time induced by amphetamine. In a final experiment, it was found that fenfluramine did not produce amphetamine stereotypy in male Wistar rats. Fenfluramine administered prior to dextroamphetamine or methamphetamine, however, prevented the appearance of typical compulsive gnawing behavior in 80% of the animals. These results, using three different indexes of CNS excitation, demonstrate that fenfluramine reliably antagonizes amphetamine-induced stimulation in mice and rats. The observed reduction of the CNS activity of amphetamines by fenfluramine points to a possible clinical application of this agent in the prophylactic management of amphetamine abuse. Preliminary clinical trials using various psychological tests tend to confirm that fenfluramine decreases the effects of dextroamphetamine in man.

Keyphrases □ Amphetamine-induced CNS stimulation—blockade effect of fenfluramine, rats, mice □ Fenfluramine blockade—amphetamine-induced CNS stimulation, rats, mice □ CNS stimulation, amphetamine induced—blockade effect of fenfluramine, rats, mice

The anorexigenic agent fenfluramine, *N*-ethyl-α-methyl-*m*-(trifluoromethyl)phenethylamine hydrochloride, differs from its structural analog amphetamine (1–3) in that it does not produce CNS stimulation yet may produce a sedative action (4). Furthermore, fenfluramine reduction of amphetamine toxicity in grouped mice has been demonstrated (5).

Recently, Jonsson *et al.* (6) showed the blockade of intravenous amphetamine euphoria in man by oral pretreatment with α-methyl-*p*-tyrosine, an inhibitor of