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The time course of hydrolysis of a β-lactam antibiotic by intact Gram-negative bacteria possessing a periplasmic β-lactamase

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An equation is derived from first principles for describing the change in concentration with time of a β-lactam antibiotic in the presence of intact Gram-negative bacteria possessing a β-lactamase located in the periplasmic space. The equation predicts a first-order decline in β-lactam concentration of the form $[S] = [S_i]e^{\lambda t}$, where [S] is the exogenous concentration of β-lactam, $[S_i]$ is the value of [S] at time zero, t is the time from mixing of cells and antibiotic and λ (< 0) is the decay constant. The value of λ is exactly described by the theory in terms of experimentally measurable quantities. Quantitative data concerning cephaloridine hydrolysis by intact cells of *Haemophilus influenzae* agreed well with the theory, as did data concerning the uptake of 2-nitrophenyl galactoside by intact cells of *Escherichia coli*. Cephalosporin C hydrolysis by intact cells of *Pseudomonas aeruginosa* did not progress as predicted by the theory. The theory is applicable to any substrate which is acted on by an enzyme that is located solely in the periplasmic space and that obeys the Michaelis–Menten equation of enzyme kinetics.

INTRODUCTION

The movement of hydrophilic compounds across the Gram-negative bacterial outer membrane is one of diffusion through transmembrane pores (Nikaido, 1979; Nikaido & Vaara, 1985). Steady-state kinetic analysis of such transmembrane diffusion (see Nikaido, 1985) has relied on the concept of the outer-membrane 'permeability parameter' (Zimmermann & Rosselet, 1977). In a recent study (Hewinson *et al.*, 1986) anomalous results were obtained with *Pseudomonas aeruginosa* in that this permeability parameter, measured by analysis of steady-state β-lactam hydrolysis according to the method of Zimmermann & Rosselet (1977), varied depending on the concentration of the test compound, cephalosporin C.

R. E. W. Hancock (personal communication) has pointed out that the data of Hewinson *et al.* (1986) could be analysed by plotting $1/v_{intact}$ against $1/[S]$, where v_{intact} is the rate of β-lactam hydrolysis in the presence of intact cells and [S] is the exogenous β-lactam concentration. This plot of the data was similar to what would be expected if a proportion of the β-lactamase were directly accessible to substrate (rather than being located in the periplasmic space and separated from exogenous substrate by the outer membrane). However, the problem with interpretation of plots of this type (e.g. v_{intact} against [S], $1/v_{intact}$ against $1/[S]$ or [S] against time, t) is that there is currently no theory that predicts the shapes of such plots, because they require analyses of integrated rate equations as opposed to analyses of steady states.

Here I derive a theoretical equation, based on the assumptions of Zimmermann & Rosselet (1977), which describes the expected time course of hydrolysis of exogenous β-lactam antibiotic by intact Gram-negative bacteria possessing periplasmic β-lactamase. This equation is used to analyse data from the literature, including the double-reciprocal plot suggested by R. E. W. Hancock (personal communication) of the data of Hewinson *et al.* (1986).

THEORY

Assumptions

The assumptions given below were stated by Zimmermann & Rosselet (1977) and have been analysed extensively by Nikaido (1979, 1985). The assumptions are as follows.

1. That β-lactamase in the suspension of washed intact Gram-negative bacteria is entirely located in the periplasmic space.
2. That the bacteria can be disrupted in order to release all the β-lactamase and expose it to added β-lactam antibiotic.
3. That kinetic parameters measured with released β-lactamase are the same as the kinetic parameters for the enzyme sequestered in the periplasmic space.
4. That the movement of β-lactam compound across the outer membrane is by diffusion only (although it is appreciated that any difference in electric potential across the outer membrane would affect the 'diffusion' of an electrically charged molecule; Nichols *et al.*, 1985).
5. That the rate of disappearance of substrate in the presence of intact cells is equal to the flux of substrate across the outer membrane into the periplasmic space.

Symbols

With the symbols below I have given a consistent set of units so that the quantitative relationships between the different variables and constants can be appreciated. Further symbols are introduced later during the course of the derivation.

J: $\mu mol \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; flux of substrate across the outer membrane, positive flux is from the exogenous compartment into the periplasmic compartment.

v_{intact}: $\mu mol \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; rate of disappearance of substrate in a suspension of intact cells. Assumption 5 above states that $v_{intact} = J$.

[S]: μM, i.e. $\mu mol \cdot dm^{-3}$; substrate concentration in the exogenous compartment.

$[S_p]$: μM; substrate concentration in the periplasmic compartment.

$[S_i]$: μM; initial substrate concentration in the exogenous compartment at zero time.

C: $dm^3 \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; outer-membrane permeability parameter for the substrate, first defined by Zimmermann & Rosselet (1977). C is equivalent to PA in Nikaido's (1979, 1985) analyses. The units of C arise because of the units chosen for J and [S]. The cubic length dimension, dm^3, occurs because C derives from the product of diffusivity (L^2T^{-1}), area per unit mass of cells (L^2M^{-1}) and reciprocal length (L^{-1}) (West & Page, 1984; Nichols *et al.*, 1985).

ρ: $(g\ dry\ wt.) \cdot dm^{-3}$, i.e. $(g\ dry\ wt.) \cdot l^{-1}$; density of cells in the reaction mixture.

ω: $dm^3 \cdot (g\ dry\ wt.)^{-1}$, i.e. $l \cdot (g\ dry\ wt.)^{-1}$; aqueous volume of the periplasmic compartment.

K_m: μM; and V: $\mu mol \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; Michaelian enzyme kinetic parameters.

U: $dm^3 \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; physiological efficiency of the enzyme, V/K_m.

t: min; time from the start of an experiment, i.e. when the substrate is mixed with a suspension of intact cells.

Derivation

Three equations are necessary. The first one arises from the basic equation of Zimmermann & Rosselet (1977):

$$J = C([S]-[S_p])$$

This equation is derived from Fick's first law, which states that the rate of transfer of diffusing substance through unit cross-sectional area is proportional to the concentration gradient of that substance measured at right-angles to the section (see Crank, 1975). The form of the Zimmermann & Rosselet (1977) equation assumes that the concentration gradient across the bacterial outer membrane is linear, and equal to $\Delta[S]/\Delta x$, where $\Delta[S]$ is the difference in concentration of substrate between the exogenous and periplasmic compartments ($[S_p]-[S]$) and Δx is the thickness of the outer membrane. In using the above equation, I also assume that a linear transmembrane concentration gradient of diffusing substance exists at any instant during the progress of the reaction. A second expression is also required for the first equation: the rate of decrease of [S] is equal to the flux across the outer membrane expressed as a function of cell mass multiplied by the density of cells in suspension, i.e. $d[S]/dt = -J\rho$. Combining the above two expressions yields, with rearrangement, the first equation:

$$[S_p] = [S] + \frac{1}{C\rho} \cdot \frac{d[S]}{dt} \quad (1)$$

The second one is obtained by differentiating eqn. (1) with respect to time:

$$\frac{d[S_p]}{dt} = \frac{d[S]}{dt} + \frac{1}{C\rho} \cdot \frac{d^2[S]}{dt^2} \quad (2)$$

Eqn. (3) was previously explained as follows: 'The rate of change of concentration of the β-lactam substrate in the periplasmic space is . . . the sum of two terms: the influx due to diffusion and the loss due to enzyme-catalysed hydrolysis' (Hewinson *et al.*, 1986):

$$\frac{d[S_p]}{dt} = \frac{1}{\omega} \cdot \left(C([S]-[S_p]) - \frac{V[S_p]}{K_m+[S_p]} \right) \quad (3)$$

This equation assumes that, at any instant, the periplasmic enzyme-catalysed reaction is in a steady state so that the Michaelis–Menten equation is valid (Cornish-Bowden, 1979), and that the achievement of the initial steady state is instantaneous. One further criterion is necessary to obtain the simplified differential equation in [S]: that is, that $[S_p] \ll K_m$. It will be seen from the subsequent analyses of work already published that indeed it is common that $[S_p] \ll K_m$ (Lindberg & Normark, 1986). If, for a particular bacterium/β-lactam combination, a measurement of $[S_p]$ by the method of Zimmermann & Rosselet (1977) shows $[S_p] \geqslant K_m$, then the final integrated eqn. (11) below is not applicable. When $[S_p] \ll K_m$, the enzyme kinetic term of eqn. (3) may be rewritten as:

$$V[S_p]/(K_m+[S_p]) = U[S_p]/(1+([S_p]/K_m) \simeq U[S_p]$$

Substitution in eqn. (3) yields:

$$\frac{d[S_p]}{dt} = \frac{1}{\omega} \cdot \left(C([S]-[S_p]) - U[S_p] \right) \quad (4)$$

It is now possible to replace $[S_p]$ and $d[S_p]/dt$ in eqn. (4) with expressions in [S], $d[S]/dt$ and $d^2[S]/dt^2$ by using eqns. (1) and (2). With rearrangement, the following expression is obtained:

$$\frac{d^2[S]}{dt^2} + \frac{d[S]}{dt} \cdot \left(\frac{U}{\omega} + \frac{C}{\omega} + C\rho \right) + [S] \cdot \left(\frac{C\rho U}{\omega} \right) = 0 \quad (5)$$

Eqn. (5) is a linear homogeneous second-order differential equation with constant coefficients of the form:

$$d^2y/dx^2 + a(dy/dx) + by = 0$$

The general solution of this equation when there are two real roots of the 'characteristic equation', $\lambda^2 + a\lambda + b = 0$, is given by Kreysig (1983); and that solution, with [S] in place of y, is:

$$[S] = Ae^{\lambda_1 t} + Be^{\lambda_2 t} \quad (6)$$

where:

$$\lambda_1 = \tfrac{1}{2}(-a + \sqrt{a^2-4b})\ min^{-1} \quad (7)$$

$$\lambda_2 = \tfrac{1}{2}(-a - \sqrt{a^2-4b})\ min^{-1} \quad (8)$$

and

$$a = \frac{U}{\omega} + \frac{C}{\omega} + C\rho\ min^{-1} \quad (9)$$

$$b = \frac{C\rho U}{\omega}\ min^{-2} \quad (10)$$

The constants A and B (μM) were evaluated by reference to initial conditions when $[S] = [S_i]$ and, from eqn. (1) setting $[S_p]$ equal to zero, $d[S]/dt$ (zero time) $= -C\rho[S_i]$. At zero time then: from eqn. (6), $[S_i] = A + B$; and, by differentiating eqn. (6), $-C\rho[S_i] = \lambda_1 A + \lambda_2 B$. These two equations are solved for A and B, whence the following integrated form of eqn. (5):

$$[S] = [S_i] \cdot \left[\left(\frac{\lambda_2 + C\rho}{\lambda_2 - \lambda_1} \right) \cdot e^{\lambda_1 t} + \left(\frac{\lambda_1 + C\rho}{\lambda_1 - \lambda_2} \right) \cdot e^{\lambda_2 t} \right] \quad (11)$$

Eqn. (11) describes the time course of hydrolysis of a β-lactam compound which is at concentration $[S_i]$ when initially mixed with the suspension of intact Gram-negative bacteria. λ_1 and λ_2 can be evaluated by reference to eqns. (7)–(10). In fact eqn. (11) is more complex than the equation which is experimentally

applicable because, as is seen in the analyses below, the second term in the equation is negligible and $(\lambda_2+C\rho)/(\lambda_2-\lambda_1) \simeq 1$. Thus eqn. (11) simplifies to a first-order decline in [S] governed by the kinetic constant λ_1.

TESTS OF THE THEORY

This section consists of the application of eqn. (11) to three published experiments.

β-Lactam hydrolysis by *Haemophilus influenzae*

The first experiment to be studied is that of the hydrolysis of cephaloridine by *Haemophilus influenzae* (Coulton *et al.*, 1983). In place of g dry wt. as their unit of cell mass, these authors used 10^9 cells; in the work below I shall refer to 10^9 cells as 1 'cell unit'.

It is possible to make a quantitative comparison between what is predicted by eqn. (11) and what is observed in Fig. 2(*b*) of the paper of Coulton *et al.* (1983). Fig. 2(*b*) of that paper plotted v_{intact} against [S] for cephaloridine hydrolysis at cephaloridine concentrations between 100 and 500 μM inclusive. The graph was a straight line through the origin of slope that I calculate to be 1.371×10^{-5} dm^3·min^{-1}·(cell unit)$^{-1}$.

The slope predicted by eqn. (11) is also calculable from data in the same paper. Values for K_m (500 μM), V [0.020 μmol·min^{-1}·(cell unit)$^{-1}$] and C [2.12×10^{-5} dm^3·min^{-1}·(cell unit)$^{-1}$] are given in Table 1 of Coulton *et al.* (1983). Measurements of v_{intact} were made at a cell density of 10^3 (cell unit)·dm^{-3}. The periplasmic volume of *H. influenzae* is uncertain, so here I use a value that has been obtained for the periplasmic volume of *Salmonella typhimurium* (Stock *et al.*, 1977) of 0.001 dm^3·(g dry wt.)$^{-1}$, i.e. 1×10^{-7} dm^3·(cell unit)$^{-1}$. Eqn. (11) then becomes:

$$[S] = [S_i](0.999988e^{-0.01386t} + 0.000012e^{-612.0t}) \quad (12)$$

The second term is negligible, so this simplifies to:

$$[S] = [S_i]e^{-0.01386t} \quad (13)$$

Differentiating eqn. (13) gives $d[S]/dt = -0.01386[S]$, and because, as pointed out above, $d[S]/dt = -J\rho$ and $v_{intact} = J$, $v_{intact} = 0.01386[S]/10^3$. The slope of the graph of v_{intact} against [S] is thus predicted by the theory to be 1.386×10^{-5} dm^3·min^{-1}·(cell unit)$^{-1}$. This differs from the measured slope by 1%.

2-Nitrophenyl galactoside uptake by *Escherichia coli*

Page & West (1981) have shown that the lactose-transport system of *Escherichia coli* behaves in such a way that Michaelian kinetic parameters of K_m (app.) and V (app.) can be assigned to it. This means that the uptake of β-galactosides by whole cells should follow the theory developed here. This proposal can be tested by analysing data on the kinetics of uptake of 2-nitrophenyl galactoside by whole cells of *E. coli* (West & Page, 1984). These authors determined experimentally the shape of the v_{intact}-versus-v_{intact}/[S] graph for this uptake process (Fig. 1*b* of West & Page, 1984). When the exponential term governed by λ_2 is neglected, the theory presented here states that, as [S] is lowered, the ratio v_{intact}/[S] should become constant with a value of $-\lambda_1/\rho$. A constant ratio of v_{intact}/[S] at low [S] was indeed observed by West & Page (1984), and the value was 4.3×10^{-3} dm^3·s^{-1}·(g dry wt.)$^{-1}$. A predicted value of $-\lambda_1/\rho$ obtained by using eqns. (7)–(10) can be calculated from the following experimental data. V and K_m were taken from West & Page (1984), and were 6.9 μmol·s^{-1}·(g dry wt.)$^{-1}$ and 933 μM respectively; therefore U was 7.58×10^{-3} dm^3·s^{-1}·(g dry wt.)$^{-1}$. ρ was taken to be the midpoint of the range quoted by Page & West (1982), i.e. 0.75 (g dry wt.)·dm^{-3}. The assumed value of ω was again 10^{-3} dm^3·(g dry wt.)$^{-1}$, and a value of C of 1.20×10^{-2} dm^3·s^{-1}·(g dry wt.)$^{-1}$ was obtained from experimental results reported by West & Page (1984; see Table 1) at a 2-nitrophenyl galactoside uptake rate of 1.00 μmol·s^{-1}·(g dry wt.)$^{-1}$. The theoretical value of $-\lambda_1/\rho$ calculated by using these data was 4.57×10^{-3} dm^3·s^{-1}·(g dry wt.)$^{-1}$, which agrees well with the published experimental value (a difference of about 6%).

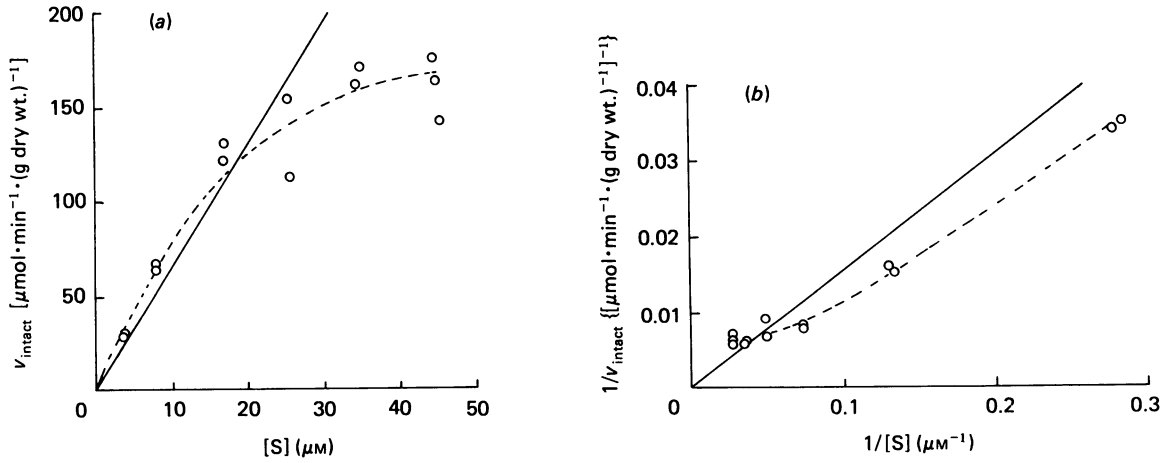

Fig. 1. Plots of (*a*) v_{intact} against [S] and (*b*) $1/v_{intact}$ against 1/[S] for cephalosporin C hydrolysis by intact cells of *Pseudomonas aeruginosa* 3-Pre

The data are from Table 1 of Hewinson *et al.* (1986). The straight lines predicted by the theory (eqn. 14) for the two graphs are drawn as continuous lines. The best-fit curves as judged by eye are drawn as broken lines.

β-Lactam hydrolysis by *Pseudomonas aeruginosa*

Fluxes of β-lactam antibiotics across the outer membrane of *Pseudomonas aeruginosa* have been analysed successfully by using the Zimmermann & Rosselet (1977) approach (Angus *et al.*, 1982; Yoshimura & Nikaido, 1982; Hancock & Wong, 1984). However, in one study anomalous results were obtained in that the value of C was unexpectedly high, and the value varied smoothly with exogenous β-lactam concentration (Hewinson *et al.*, 1986). It is the anomalous study that I analyse here in terms of the new kinetic theory.

A plot of v_{intact} against [S] for *Ps. aeruginosa* 3-Pre is given in Fig. 1(*a*) and plot of $1/v_{intact}$ against 1/[S] in Fig. 1(*b*). In this experiment $V = 1010\ \mu mol \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$, $K_m = 30.1\ \mu M$, ρ was 0.035 (g dry wt.)·dm^{-3} and the measured value of C varied between 3 and 12 $dm^3 \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$; here I use a value of 8 $dm^3 \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$ in the calculations of what is predicted by eqn. (11). Again, in the absence of a measured value for the volume of the periplasmic space of *Ps. aeruginosa*, I use the value of $10^{-3}\ cm^3 \cdot (g\ dry\ wt.)^{-1}$. As was the case for *H. influenzae*, the second exponential term is negligible, so that for *Ps. aeruginosa* 3-Pre the above data predict:

$$[S] = [S_i]e^{-0.226t} \quad (14)$$

Thus the predicted slope of the v_{intact}-versus-[S] plot ($-\lambda_1/\rho$) is 6.46 $dm^3 \cdot min^{-1} \cdot (g\ dry\ wt.)^{-1}$ and the predicted slope of the $1/v_{intact}$-versus-1/[S] plot ($-\rho/\lambda_1$) is 0.155 $dm^{-3} \cdot min \cdot (g\ dry\ wt.)$. These two predicted lines are drawn in Figs. 1(*a*) and 1(*b*), as are the best-fit curves drawn by eye (broken lines).

DISCUSSION

The theory described here in the form of eqn. (11) was derived mathematically from first principles based on the physical model proposed by Zimmermann & Rosselet (1977). The physical meaning of the two exponential declines, one of them extremely rapid and for a small proportion of the added β-lactam compound and the other one a slower decline of virtually all of the added antibiotic, is unclear. It is tempting to speculate that the more rapid exponential fall in antibiotic concentration, i.e. that governed by λ_2, represents the fall in exogenous β-lactam concentration required to bring the periplasmic concentration to its pseudo-steady-state value.

Regardless of the physical meaning of the exponential term governed by λ_2, the examples of work from the published literature show that this term can be neglected (e.g. eqn. 12). Thus in the presence of intact Gram-negative bacteria possessing a periplasmic β-lactamase active against a particular β-lactam antibiotic the fall in concentration on mixing the cells and antibiotic is predicted to be first-order. Moreover, if the constants C, ρ, U and ω are measured, the first-order decay constant, λ_1, is quantitatively described (eqn. 7) and may be compared with the value obtained from the slope of the plot of ln[S] against t. It is also worth pointing out that the theory has a more general applicability. It should describe the loss from the medium of any substrate acted on by a periplasmic enzyme obeying Michaelis–Menten kinetics (as long as $[S_p] \ll K_m$). This statement thus includes transport systems as well as other periplasmic hydrolytic enzymes.

Eqn. (11) describes well the data concerning the hydrolysis of cephaloridine catalysed by β-lactamase in intact cells of *H. influenzae* (Coulton *et al.*, 1983). It also agrees well with the kinetics of 2-nitrophenyl galactoside uptake by *E. coli* (West & Page, 1984). Eqn. (11) does not describe very well the data concerning the hydrolysis of cephalosporin C catalysed by β-lactamase in intact cells of *Ps. aeruginosa* strain 3-Pre (Figs. 1*a* and 1*b*; Hewinson *et al.*, 1986). However, it was already known that these data did not fit the Zimmerman & Rosselet (1977) model because the measured permeability parameter, which is expected to be constant, varied as a function of the concentration of the substrate, cephalosporin C (Hewinson *et al.*, 1986). On the basis of these three applications to published data, the equations presented here distinguish between data that fit the Zimmermann & Rosselet (1977) model and those that do not.

Although eqn. (11) fitted the data of Coulton *et al.* (1983) and West & Page (1984), it must be emphasized that its validity depends on the steady state of the enzyme-catalysed reaction being achieved instantaneously at the start of an experiment (eqn. 3). This is probably incorrect for the exponential term governed by λ_2, but this term was negligible in the above three examples.

It may be thought that the values of ω (the periplasmic volume) used in the calculations were not very near the real values. Although Stock *et al.* (1977) gave values of around $10^{-3}\ dm^3 \cdot (g\ dry\ wt.)^{-1}$ for *S. typhimurium*, this might not be applicable to other bacteria. In fact the value of ω is not critical because λ_1 is not very sensitive to ω in the realistic range. For example, the data of Coulton *et al.* (1983) gave λ_1 (to five significant figures) $= -0.13856\ min^{-1}$ when ω was taken to be $10^{-3}\ dm^3 \cdot (g\ dry\ wt.)^{-1}$. The same data gave $\lambda_1 = -0.013855\ min^{-1}$ and $-0.013856\ min^{-1}$ when ω was taken to be 10^{-2} and $10^{-4}\ dm^3 \cdot (g\ dry\ wt.)^{-1}$ respectively.

Eqn. (11) in the form of the equation that arises from neglecting the second exponential, i.e. $[S] = [S_i]e^{\lambda_1 t}$, predicts the shapes of three simple plots of progress-curve or initial-rate data obtained by mixing intact cells and a solution of β-lactam compound. First, a plot of ln[S] against t should yield a straight line of slope, λ_1. Furthermore, the two plots analysed in the Tests of the theory section (see Fig. 1) of v_{intact} against [S] and of $1/v_{intact}$ against 1/[S] should yield straight lines through the origin with slopes of $-\lambda_1/\rho$ and $-\rho/\lambda_1$ respectively. This was so for the cephaloridine hydrolysis data of Coulton *et al.* (1983). If the first-order equation is correct, rates of β-lactam hydrolysis at various values of [S] on a progress curve can be computed by the chord-drawing method of Waley (1981). The slope of the chord intersecting the progress curve at $[S_1]$ and $[S_2]$ is parallel to the tangent, and hence reaction rate, at $[S_3] = ([S_2] - [S_1])/\ln([S_2]/[S_1])$.

The limitation of the theory described by eqn. (11) is that it only holds when $1 + ([S_p]/K_m)$ can be approximated to unity. However, for β-lactam compounds, Lindberg & Normark (1986) state that indeed $[S_p] \ll K_m$ in many cases of interest. Moreover, Nikaido (1985) has pointed out that measurements of permeability parameters are most precise when $[S_p]$ is low, so it is likely that the above condition will also hold in most experiments. I have not attempted to analyse mathemati-

cally how high $[S_p]$ can be relative to K_m without any significant effect on the accuracy of eqn. (11), but the experiments described by West & Page (1984; Fig. 1*b*) show that the ratio $v_{intact}/[S]$ for 2-nitrophenyl galactoside uptake by *E. coli* deviated from its constant value at $v_{intact} \simeq 0.17V$, i.e. at $[S_p] \simeq 0.20K_m$. It appears then that the theory presented in this paper holds when $[S_p] \leqslant 0.2K_m$.

The treatment described here for the hydrolysis of β-lactam antibiotics by intact Gram-negative bacteria makes no assumptions or predictions about any inhibition of bacterial growth. A kinetic theory has been developed (Waley, 1987*a*,*b*) which relates the minimum inhibitory concentration of a β-lactam antibiotic to outer-membrane permeability and periplasmic β-lactamase activity, but the theory also requires analysis of rates of inactivation of the β-lactam-sensitive penicillin-binding proteins. Readers interested in the quantitative basis of growth inhibition by β-lactam compounds are referred to those papers.

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REFERENCES

Angus, B. L., Carey, A. M., Caron, D. A., Kropinski, A. M. B. & Hancock, R. E. W. (1982) Antimicrob. Agents Chemother. **21**, 299–309

Cornish-Bowden, A. (1979) Fundamentals of Enzyme Kinetics, p. 20, Butterworths, London

Coulton, J. W., Mason, P. & Dorrance, D. (1983) J. Antimicrob. Chemother. **12**, 435–449

Crank, J. (1975) The Mathematics of Diffusion, 2nd edn., pp. 1–10, Oxford University Press, Oxford

Hancock, R. E. W. & Wong, P. G. W. (1984) Antimicrob. Agents Chemother. **26**, 48–52

Hewinson, R. G., Lane, D. C., Slack, M. P. E. & Nichols, W. W. (1986) J. Gen. Microbiol. **132**, 27–33

Kreysig, E. (1983) Advanced Engineering Mathematics, pp. 67–68, John Wiley & Sons, New York

Lindberg, F. & Normark, S. (1986) Rev. Infect. Dis. **8**, suppl. 3, S292–S304

Nichols, W. W., Hewinson, R. G., Slack, M. P. E. & Walmsley, H. L. (1985) Biochem. Soc. Trans. **13**, 697–698

Nikaido, H. (1979) in Bacterial Outer Membranes: Biogenesis and Functions (Inouye, M., ed.), pp. 361–407, John Wiley and Sons, New York

Nikaido, H. (1985) Pharmacol. Ther. **27**, 197–231

Nikaido, H. & Vaara, M. (1985) Microbiol. Rev. **49**, 1–32

Page, M. G. P. & West, I. C. (1981) Biochem. J. **196**, 721–731

Page, M. G. P. & West, I. C. (1982) Biochem. J. **204**, 681–688

Stock, J. B., Rauch, B. & Roseman, S. (1977) J. Biol. Chem. **252**, 7850–7861

Waley, S. G. (1981) Biochem. J. **193**, 1009–1012

Waley, S. G. (1987*a*) Microbiol. Sci. **4**, 143–146

Waley, S. G. (1987*b*) Biochem. Soc. Trans. **15**, 235–236

West, I. C. & Page, M. G. P. (1984) J. Theor. Biol. **110**, 11–19

Yoshimura, F. & Nikaido, H. (1982) J. Bacteriol. **152**, 636–642

Zimmermann, W. & Rosselet, A. (1977) Antimicrob. Agents Chemother. **12**, 368–372

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