Deviations from Michaelis–Menten kinetics

Computation of the probabilities of obtaining complex curves from simple kinetic schemes

Francisco SOLANO-MUÑOZ, Philip B. MCGINLAY, Richard WOOLFSON and William G. BARDSLEY
 Department of Obstetrics and Gynaecology, University of Manchester, St. Mary's Hospital,
 Manchester M13 0JH, U.K.

(Received 7 May 1980/Accepted 15 September 1980)

1. It is possible to calculate the intrinsic probability associated with any curve shape that is allowed for rational functions of given degree when the coefficients are independent or dependent random variables with known probability distributions. 2. Computations of such probabilities are described when the coefficients of the rational function are generated according to several probability distribution functions and in particular when rate constants are varied randomly for several simple model mechanisms. 3. It is concluded that each molecular mechanism is associated with a specific set of curve-shape probabilities, and this could be of value in discriminating between model mechanisms. 4. It is shown how a computer program can be used to estimate the probability of new complexities such as extra inflexions and turning points as the degree of rate equations increases. 5. The probability of 3:3 rate equations giving 2:2 curve shapes is discussed for unrestricted coefficients and also for the substrate-modifier mechanisms. 6. The probability associated with the numerical values of coefficients in rate equations is also calculated for this mechanism, and a possible method for determining the approximate magnitude of product-release steps is given. 7. The computer programs used in the computations have been deposited as Supplement SUP 50113 (21 pages) with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1978) **169**, 5.

We have suggested that Michaelis–Menten kinetics are the exception rather than the rule (Hill *et al.*, 1977), and have supported this by theoretical arguments (Bardsley & Childs, 1975; Bardsley, 1976) and use of experimental results obtained over a wide range of substrate concentrations (Childs & Bardsley, 1975; Waight *et al.*, 1977; Bardsley *et al.*, 1980). It is well known that, where there is no kinetically significant enzyme polymerization, the pseudo-steady-state rate equation in the absence of product is of the form:

$$\frac{v}{[E_0]} = \frac{\sum_{i=1}^m \lambda_i \cdot p_i([S])}{\sum_{i=1}^m p_i([S])} \quad (1)$$

where there are m distinct enzyme intermediates and $[S]$ is the concentration of substrate and $[E_0]$ that of enzyme. In eqn. (1) the $p_i([S])$ values are the enzyme-species distribution polynomials for the i th enzyme species (King & Altman, 1956) and the λ_i

values are zero unless the species in question is product releasing when λ_i is the rate constant for product release from this particular enzyme–product complex. The degree of the polynomials $p_i([S])$ will depend on the number of second-order substrate-addition steps in the directed trees leading to the node in question in the nodal diagram (Volkenstein & Goldstein, 1966; Whitehead, 1979), and by collecting together terms of like degree in these distribution polynomials we may write the initial rate equation as:

$$\frac{v}{[E_0]} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \dots + \alpha_n[S]^n}{\beta_0 + \beta_1[S] + \dots + \beta_n[S]^n + \dots + \beta_{n+r}[S]^{n+r}} \quad (2)$$

When we write eqn. (1) in the form of eqn. (2) we are apt to forget that the numerator coefficients, α_i , are dependent on the denominator coefficients, β_i , and in most theoretical studies it has been tacitly assumed that the α_i and β_i are sufficiently independent to have values giving all curve shapes in v -versus- $[S]$ or

$1/v$ -versus- $1/[S]$ graphs that are possible for the particular values of n and r . However, it is clear that the relationship between α_i and β_i will depend on the particular mechanism, i.e. the sets of directed trees giving rise to the α_i and β_i . An important point is to decide how this restriction might impose itself on the v -versus- $[S]$ profiles possible. This information could be useful in deciding between rival mechanisms. An obvious case where there is a clear relationship between the α_i and β_i is in binding models, where the saturation function is the logarithmic derivative of a binding polynomial (Wyman, 1966, 1972). Because of this the α_i and β_i are related by:

$$\alpha_i = j \cdot \beta_i / n$$

$$\beta_0 = 1$$

where n is the number of binding sites and r is zero.

Now, a number of important questions are suggested by the previous considerations. These questions might be thought of as enquiries about the probable values of α_i and β_i and the probability of obtaining particular curve shapes for selected mechanisms. Although there are the detailed analyses of the random Bi Bi mechanism by King (1956), Ferdinand (1966), Pettersson (1969*a,b*, 1972*a,b*) and others, who began this analysis, there has never been a systematic study of those questions concerning probability. Because of this, we now formulate ten questions that define the problems involved and then proceed to show how these issues may be tackled by a combination of analysis and computation.

Question 1. Analysis shows that rational functions with positive coefficients can give rise to complicated curves in v -versus- $[S]$, $1/v$ -versus- $1/[S]$ or other spaces, and these may be classified by reference to specific geometric features. Is there an intrinsic probability associated with each possible curve shape and can this be calculated?

Question 2. Is it possible to compute approximate values for the intrinsic probabilities?

Question 3. If a computer program is used to work out these probabilities, then how many cycles of randomly generated numbers will be needed before the results obtained converge to give an acceptable estimate of the true probability?

Question 4. In what way does the probability associated with any given curve shape depend on the probability distribution functions from which random values of the coefficients are generated?

Question 5. Is there a substantial difference between intrinsic probabilities computed from random variations of coefficients and the same curve-shape probabilities computed for random variations of rate constants in a mechanism?

Question 6. Does each molecular mechanism have its own specific curve-shape probabilities and, if so,

could this be of use in discriminating between rival mechanisms?

Question 7. A number of special curve-shape features such as sigmoidicity or substrate inhibition have attracted attention as having functional importance in biological systems. What are the intrinsic probabilities of a curve being (a) Michaelis-Menten by decrease in degree, (b) sigmoid in v -versus- $[S]$ plots, (c) of the substrate-inhibition type or (d) concave down in double-reciprocal space?

Question 8. A rational function of degree $n:n$ may give all the shapes possible for degree $n-1:n-1$, $n-2:n-2$ and so on without decreasing in degree, as well as new shapes not possible with the lower-degree functions. What is the probability of an $n:n$ function giving new shapes not possible for lower degrees?

Question 9. In fitting rational functions to experimental enzyme kinetic data, it is important to have estimates of the probable values for α_i and β_i . What are the probable values?

Question 10. It is evident that estimates of coefficients of high-degree rational functions obtained by non-linear regression are not going to be valuable in determining individual rate-constant values. Is there any mechanistic information concerning possible limits on the magnitude of specific rate constants that might be available in the light of a satisfactory answer to question 9?

Systematic analysis of questions (1)–(10)

In the following sections we show how a combination of analysis and computation can be employed to illuminate the subject matter of questions (1)–(10).

(1) Analysis of complex kinetic curves

It is presumed that eqns. (1) and (2) are appropriate models in a large number of experimental situations and that therefore complicated v -versus- $[S]$ curves or double-reciprocal plots may result. The classification of 2:2 plots was accomplished by Botts (1958), although most of this analysis is available in text-books (Hardy, 1952; Maxwell, 1966; Archbold, 1970) and was well known to geometers of previous ages. Some preliminary studies of 3:3 functions were reported by Childs & Bardsley (1976) and Katsumata & Goldman (1976), but a comprehensive classification of double-reciprocal plots was first achieved by Bardsley (1977*a*) and Bardsley *et al.* (1980). From this work it follows that classification of curve shape will depend on the sign of polynomials, f_i , in the coefficients of the rational function, and since each coefficient of the rate equation can be expressed as a polynomial in the rate constants for a particular

mechanism we would have a series of functions such as:

$$\begin{aligned} f_i &= f_i(\alpha_i, \alpha_2, \dots, \alpha_n, \beta_0, \beta_1, \dots, \beta_{n+r}) \\ &= g_i(k_1, k_2, \dots, k_i) \end{aligned}$$

to consider. To classify the curves we would need to know the signs of such a set of functions, and it is worthwhile emphasizing that the functions f_i are homogeneous in all the coefficients α_j and β_j and the functions g_i are homogeneous in the rate constants k_j . If we are going to vary the coefficients α_j and β_j or rate constants k_j over an interval in order to see the effect on the sign of f_i or g_i , then the fact that f_i and g_i are homogeneous polynomials means that the absolute value of the range chosen is of no significance. As long as our interest is in curve shapes only and our intention is to vary α_j and β_j or k_j randomly over a range, then the range $0 \leq \alpha_j, \beta_j, k_j < \lambda$, where λ is any positive real number, will suffice. Of course in rate equations for enzyme mechanisms we have $\beta_j > 0$ but $\alpha_j \geq 0$.

To make this clear, let us refer to the graph shapes of Fig. 1, which are the shapes possible for the 2:2 function:

$$\frac{v}{[E_0]} = \frac{\alpha_1[S] + \alpha_2[S]^2}{\beta_0 + \beta_1[S] + \beta_2[S]^2} \quad (3)$$

The homogeneous polynomials to be considered are (following Bardsley, 1976, 1977a,b,c):

$$\begin{aligned} \phi_{21} &= \alpha_2 \beta_1 - \alpha_1 \beta_2 \\ \psi_{11}^{20} &= \alpha_2 \beta_0 - \alpha_1 \beta_1 \\ -R_0^{2:2} &= \alpha_2 \psi_{11}^{20} + \alpha_1^2 \beta_2 \end{aligned}$$

and we have substrate inhibition for $\phi_{21} < 0$, sigmoidicity for $\psi_{11}^{20} > 0$ and a concave-up double-reciprocal plot for $-R_0^{2:2} > 0$. Note that in Fig. 1 and elsewhere in the present paper three signs in brackets, i.e. $[(\pm)(\pm)(\pm)]$ refer to the signs of ϕ_{21} , ψ_{11}^{20} and $-R_0^{2:2}$ in that order. Higher-degree functions require consideration of a greater number of such homogeneous polynomials (Bardsley, 1977a), and there will always be a number of curve-shape determinants that determine, by their sign, necessary and sufficient conditions for specific curve-shape features (Bardsley, 1977b; Bardsley & Waight, 1978).

Second-order rate constants can lie anywhere between zero and $10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}$, whereas first-order ones can theoretically be as high as 10^{13} s^{-1} , although the ones encountered in enzyme-catalysed reactions may tend to lie in more restricted ranges (Gutfreund, 1972) and will be subject to the constraints of detailed balancing (Krupka *et al.*, 1966). We shall examine separately the cases where the coefficients α_i and β_i are varied directly subject to a given probability distribution and also where it

is the rate constants for a selected mechanism that are varied subject to a known probability distribution.

(2) Calculation of curve-shape probabilities

Let us suppose that we have a method for randomly selecting values of α_i and β_i according to some known continuous probability distribution function and we wish to answer questions about possible curve shapes for eqn. (1). As the values of α_i and β_i are selected, then what will the probability of any particular curve-shape feature? Suppose, for instance, we ask what is the probability of a function of degree $n:n+r$ decreasing in degree to $1:1+r$ by cancellation of common factors between numerator and denominator of eqn. (1)?

This will only occur when the appropriate Sylvester resultants of numerator and denominator all vanish (Bardsley, 1977c), i.e. $R_0 = R_1 = \dots = R_{n-2} = 0$.

Now, whatever probability distribution function is chosen for α_i and β_i , there is a corresponding continuous probability distribution function for the resultants R_i , and in a continuous distribution the probability of any discrete value occurring is zero. Hence the probability of choosing α_i and β_i so that decrease in degree occurs is actually zero (i.e. $1/\infty$), and it is not to be expected that a rational function with $n \geq 2$ will give Michaelis–Menten kinetics. If the probability distribution function is discrete, then the probability becomes finite, although very small. Also, if there are molecular constraints operating on k_i values that have the effect of limiting the independence of α_i and β_i , this can increase the probability of decrease in degree but, with genuine enzyme systems, the probability must be vanishingly small.

Suppose we now ask what is the probability that eqn. (1) can give rise to a sigmoid v -versus- $[S]$ curve, i.e. $\psi_{11}^{20} > 0$. Now, a uniformly random choice of α_1 , α_2 , β_0 and β_1 will give the two random numbers $\alpha_2 \beta_0$ and $\alpha_1 \beta_1$ and the probability of $\alpha_2 \beta_0 > \alpha_1 \beta_1$ is $1/2$ (i.e. 50%) and that of $\alpha_2 \beta_0 < \alpha_1 \beta_1$ is also $1/2$. Hence the probability of a sigmoid curve is $1/2$, and, by similar arguments, the probability of obtaining a final maximum when $r = 0$ (i.e. final-partial-substrate inhibition) is the probability that $\phi_{n,n-1} < 0$, which is likewise $1/2$. We shall see below that these probabilities are radically altered when eqn. (2) represents molecular mechanisms and the k_i values are varied. The probability of obtaining other curve-shape features is not so clear, and we now choose to analyse the conditions for a concave-down double-reciprocal plot for eqn. (3) to illustrate this.

Now eqn. (3) gives a concave-down double-reciprocal plot under the constraint $-R_0^{2:2} < 0$, and we wish to calculate the probability of obtaining this feature when α_1 , α_2 , β_0 , β_1 and β_2 are generated

Fig. 1. 2:2 graph shapes

The convention used in the present paper refers to the shapes illustrated and the signs refer to the sign of the functions ϕ_{21} , ψ_{11}^{20} and $-R_0^{2:2}$. A number of special cases occur when these curve-shape determinants ϕ_{21} , ψ_{11}^{20} and $-R_0^{2:2}$ vanish. When $\phi_{21} = 0$, then $-R_0^{2:2} > 0$, and there is no visible turning point in the v -versus- $[S]$ plot, which may be sigmoid depending on the sign of ψ_{11}^{20} . However, the $1/v$ -versus- $1/[S]$ plot is concave up and has a turning point when $1/[S] = 0$. If $\psi_{11}^{20} = 0$, then $-R_0^{2:2} > 0$, and there is no visible sigmoidicity, since the inflexion is at the origin in v -versus- $[S]$ space. In fact, the v -versus- $[S]$ plot may have a maximum depending on the sign of ϕ_{21} . The graph of $1/v$ versus $1/[S]$ is concave up and the asymptote as $1/[S] \rightarrow \infty$ goes through the origin. Under the constraint $R_0^{2:2} = 0$ a common factor cancels between numerator and denominator of the 2:2 function to give a 1:1 function (i.e. the Michaelis-Menten equation). Note that $\alpha_2 = \beta_2 = 0 \Rightarrow R_0 = 0$, but the special case $\phi_{21} = \psi_{11}^{20} = 0 \Rightarrow -R_0^{2:2} > 0$ and the double-reciprocal plot is convex. When $\alpha_1 = 0 \Rightarrow \psi_{11}^{20} > 0$, $\phi_{21} > 0$, then the v -versus- $[S]$ plot has zero slope at the origin and the $1/v$ -versus- $1/[S]$ plot is asymptotic to a parabola. Also, when $\alpha_2 = 0 \Rightarrow \phi_{21} < 0$, $\psi_{11}^{20} < 0$, then $v(\infty) = 0$ and the $1/v$ -versus- $1/[S]$ plot has a vertical asymptote at $1/[S] = 0$.

randomly according to a uniform continuous rectangular-distribution function.

Let there be random variables X , Y , Z , V and W (i.e. α_1 , α_2 , β_0 , β_1 and β_2) that are independent and uniformly randomly distributed on $[0,1]$. Also let:

$$A = Y^2Z + X^2W - XYV$$

$$B = X$$

$$C = Y$$

$$D = Z$$

$$E = W$$

for then:

$$V = (Y^2Z + X^2W - A)/XY$$

$$= (C^2D + B^2E - A)/BC$$

and the required Jacobian is:

$$\frac{\partial(X,Y,Z,V,W)}{\partial(A,B,C,D,E)} = -\frac{\partial V}{\partial A} = 1/BC$$

Thus the joint probability density function of A, B, C, D, E , say $F(a, b, c, d, e)$, is given by:

$$F(a, b, c, d, e) = (1/BC) \cdot f_1(b) \cdot f_2(c) \cdot f_3(d) \cdot f_4[(c^2d + b^2e - a)/bc] \cdot f_5(e)$$

where f_i are the uniform probability density functions of X, Y, Z, V, W . Thus the probability of a concave-down double-reciprocal plot is given by:

$$P\{A \leq 0\} = \int_{-\infty}^0 \int_0^1 \int_0^1 \int_0^1 \int_0^1 F(a, b, c, d, e) \cdot de \cdot dd \cdot dc \cdot db \cdot da$$

$$= \int_{-\infty}^0 \int_0^1 \int_0^1 \int_0^1 \int_0^1 \frac{1}{bc} \cdot f_4\left(\frac{c^2d + b^2e - a}{bc}\right) \cdot de \cdots da$$

After some lengthy algebra, this multiple integral may be evaluated and is found to be:

$$P\{A \leq 0\} = 1/8$$

a result that is surprising at first sight since it might be felt intuitively that a 2:2 double-reciprocal plot would be equally likely to be concave up or concave down from a random choice of α_i and β_i .

In principle, it is possible to calculate probabilities for all curve shapes subject to evaluating such multiple integrals, but, in practice, the labour involved and possibilities of error are such that an alternative approach, based on computation, seems more attractive. So let us turn to discuss such a method.

(3) Computation of curve-shape probabilities

It is not possible to have a continuous distribution for purposes of computation, since all real numbers are generated by computers in decimal representation up to a certain number of places. We have used a variety of computer programs to generate random numbers with six-figure precision, since this should give a good approximation to a continuous distribution. All of these used the RANF(A) procedure on the Manchester CYBER 72 system to generate numbers that were rectangularly distributed in the range 0.000000 to 0.999999. However, calling these numbers $Z(I)$, $I = 1, 2, \dots, N$, then a variety of FORTRAN programs were used to calculate curve-shape probabilities for independent α_i and β_i and also for k_i of mechanisms. For this purpose it was convenient to transform the numbers $Z(I)$, as is now discussed.

Type I distribution. The numbers $Z(I)$ were

randomly generated and new distributions were defined according to:

$$Y(I) = \lambda Z(I)$$

where λ was a scalar typically 1, 10, 1000 etc., and so $0 \leq Y(I) < \lambda$. As discussed above, this scalar factor λ is quite irrelevant in calculating curve-shape probabilities for α_i and β_i varied or k_i varied, but was required for other purposes, as becomes clear below.

Type II distribution. For some purposes it is useful to increase the proportion of smaller numbers in a given range, and one unsymmetrical distribution that was used employed random numbers $W(I)$ calculated according to:

$$W(I) = 10^{Y(I)}$$

so that $1 \leq W(I) < 10^\lambda$.

Type III distribution. When the values of k_i are chosen for a mechanism it is sometimes useful to restrict the choice of individual rate constants to ranges that are likely on chemical grounds. Thus, in studying model mechanisms, we have sometimes used the integer part of $Y(I)$ with $\lambda = 10$, but with the series truncated to give a range of integers between 0 and 6 according to:

$$V(I) = [10Z(I)] \text{ (but integer part } \leq 6)$$

so that $V(I) = 0, 1, 2, 3, 4, 5$ or 6 .

Then rate constants were generated by the routine:

$$k_i = 10^{\mu + V(I)}$$

the scalar μ being chosen so that k_i was in the range required for the particular model mechanism under consideration.

In model mechanisms one constant is calculated for each cycle according to detailed balancing (Krupka *et al.*, 1966). The computer programs written for mechanisms and using this particular distribution rejected those samples where calculated rate constants fell outside a mechanistically realistic range as specified by us.

The mechanisms studied were as shown in Fig. 2, and rate equations were calculated by a program supplied by Dr. Keith Indge (see the Appendix).

Fig. 3 shows the results for a uniform random choice of α_i and β_i and the 2:2 function of eqn. (3). For each random choice of α_i and β_i values the computer determined the sign of ϕ_{21} , ψ_{11}^{20} and $R_0^{2:2}$. The cumulative occurrence of the different curve-shape types is plotted as a function of the number of choices of sets of α_i and β_i for both type I and type II distributions over 200 cycles in Fig. 3.

A number of conclusions can be drawn from this Figure as follows. (i) After some 50 cycles the cumulative probabilities settle down to values close to the asymptotic ones. In other words, the program is giving results that converge to the true limits fairly

Fig. 2. Mechanisms discussed in the text

Mechanism 1. This is a one-site/two-state mechanism with no provision for enzyme-substrate/enzyme-product interconversion rate constants. It is essentially an implicit version (Waight & Bardsley, 1977) of the Monod-Wyman-Changeux (Monod *et al.*, 1965) mechanism. Mechanism 2. This allows for first-order steps interconverting substrate and product on the enzyme, and is this an explicit Monod-Wyman-Changeux model monomer mechanism. Mechanism 3. This is the random Bi Bi mechanism. Mechanism 4. This is an implicit version of the substrate-modifier mechanism (Bardsley *et al.*, 1980).

quickly. (ii) In Table 1 are the results for a larger number of cycles, and this suggests that, in general, there is no need to proceed beyond 200 cycles to approach acceptable estimates of probabilities. (iii) The main difference between type I and type II distributions is that a curve that is non-sigmoid, has no substrate inhibition and is concave up in double-reciprocal space (+ - +) is much less probable for type II distribution than for type I, whereas the curve (+ - -) behaves in the opposite manner.

(4) Studies of curve-shape probabilities for the one-site/two-state mechanism

Having defined, in Fig. 3 and Table 1, the intrinsic probabilities with α_i and β_i varied uniformly

randomly, we now turn to mechanisms 1 and 2 of Fig. 2. These involve only one site for binding of substrate, but the enzyme can exist in two conformational forms. It is therefore the simplest example of Monod-Wyman-Changeux model (Monod *et al.*, 1965), and, as discussed elsewhere (Bardsley *et al.*, 1980), this particular mechanism exists in two formulations. The implicit formulation (mechanism 1) has no provision for substrate-product interconversion, and is therefore a compression of the explicit formulation (mechanism 2). Both give a 2:2 rate equation, but Figs. 4 and 5 show the results obtained when the k_i values were varied in the range $0 \leq k_i < 1000$ (Figs. 4a and 5a) according to a type I distribution, or $1 \leq k_i < 10^{10}$

Fig. 3. Probability of curve shapes for the general 2:2 function

$v/[E_0] = (\alpha_1[S] + \alpha_2[S]^2)/(\beta_0 + \beta_1[S] + \beta_2[S]^2)$. (a) Type I distribution with $0 \leq \alpha_i, \beta_i < 1$. (b) Type II distribution with $1 \leq \alpha_i, \beta_i < 10^{10}$. The symbols are for the following scheme:

	$\phi^{2:1}$	ψ_{11}^{20}	$-R_0^{2:2}$
○	+	—	+
△	+	—	—
□	—	—	+
●	+	+	+
▲	—	+	+

and the corresponding curve shapes are those in Fig. 1. Here and in all following Figures N refers to the number of cycles of the computer program.

Fig. 4. Probability of curve shapes for mechanism 1 of Fig. 2 (Monod–Wyman–Changeux model implicit) (a) Type I distribution with $0 \leq k_i < 1000$. (b) Type II distribution with $1 \leq k_i < 10^{10}$. Symbols are as for Fig. 3.

to each other, but quite distinct from the pair Figs. 3(a) and 3(b).

If we now compare Fig. 4 with Fig. 5, we discover, surprisingly, that the curve-shape behaviour of mechanisms 1 and 2 are very similar. In this instance, it would appear that replacing an explicit scheme by an implicit one results in no distinct differences, despite the greater number of degrees of freedom in the explicit formulation. However, it is evident in a most striking manner that the expectation of complex curves from a mechanism is totally different from that expected by randomly varying α_i and β_i . We showed above that a concave-down double-reciprocal plot had an intrinsic probability of 1/8 with α_i and β_i varied randomly, but, from Figs. 4 and 5, it is clear that this particular curve shape is the most probable for the Monod–Wyman–Changeux-model monomer (mechanisms 1 and 2) when the k_i values are varied directly and hence the α_i and β_i indirectly.

From Table 1 we can make further conclusions.

(Figs. 4b and 5b) for a type II distribution. A number of distinctive points are noted when we compare Figs. 3 and 4. (i) In going from Fig. 4(a) to Fig. 4(b) we notice that the most probable curves in either set do not change their orders of occurrence very much, and this compares with Figs. 3(a) and 3(b), which show similar behaviour. (ii) Two of the least probable curves in Fig. 3 become the most probable in Fig. 4. (iii) Three shapes have very low probability in Fig. 4(a) and one in Fig. 4(b). (iv) Clearly the probability patterns of Figs. 4(a) and 4(b) are related

Table 1. *Intrinsic curve-shape probabilities*

The first line refers to the mechanism, which was either an arbitrary 2:2 function with α_i and β_i varied, or else it was mechanism 1 [Monod–Wyman–Changeux (MWC) model implicit], mechanism 2 (Monod–Wyman–Changeux model explicit) or mechanism 3 (random Bi Bi) when k_i values were varied. The second line refers to the types of probability distribution function as described in the text, and the third line gives the total numbers of cases generated by the computer. The signs $(+)$ $(+)$ $(+)$ refer to the signs of ϕ_{21} , ψ_{11}^{20} and $-R_0^{2,2}$, and values give the percentage of cases with each curve shape.

Mechanism	...	2:2 function		1 (MWC model implicit)			2 (MWC model explicit)			3 (random Bi Bi)	
Distribution	...	I	II	I	II	III	I	II	III	I	II ($0 < [B] < 0.1$)
Total cases	...	10000	10000	10000	9990	10219	10000	9982	8372	10000	9987
+ - +		7.99	0.12	39.44	25.89	39.61	40.94	31.12	34.28	64.37	41.86
- - +		29.34	29.21	1.02	7.03	1.84	0.72	7.81	6.42	20.55	29.99
+ - -		12.81	20.93	59.01	57.25	52.3	58.21	54.57	53.58	15.08	26.49
+ + +		28.9	28.81	0.53	9.59	6.23	0.13	6.19	5.62	0	1.32
- + +		20.96	20.93	0	0.14	0.010	0	0.13	0.09	0	0.21
+ (+) (+)		49.70	49.86	98.98	92.73	98.14	99.28	92.06	93.49	79.45	69.8
(+) (+) (+)		49.86	49.74	0.53	9.73	6.24	0.13	6.32	5.71	0	1.53
(+) (+) +		87.19	79.07	40.99	42.75	47.7	41.79	45.43	46.42	84.92	73.51

Fig. 5. Probability of curve shapes for mechanism 2 of Fig. 2 (Monod–Wyman–Changeux model explicit) (a) Type I distribution with $0 \leq k_i < 1000$. (b) Type II distribution with $1 \leq k_i < 10^{10}$. Symbols are as for Fig. 3.

For instance, either substrate inhibition or sigmoidicity have a 50% possibility for eqn. (3) with α_i and β_i varied directly and randomly. However, the Monod–Wyman–Changeux-model the monomer

has a probability of substrate inhibition of less than 5% and a probability of sigmoidicity of less than 10%. The probability of a concave-down curve is about 50%.

In our previous discussion of this mechanism (Bardsley *et al.*, 1980) we pointed out that it was extremely difficult to obtain a v -versus- $[S]$ curve with both sigmoidicity and a maximum (i.e. $- + +$). Although this curve has a 20% chance of occurring if α_i and β_i vary uniformly randomly, the chance of getting this curve from a Monod–Wyman–Changeux-model monomer is less than 1% when the k_i values are varied.

It might be argued that the findings would be different if the k_i values were chosen by some procedure that kept the values close to those expected from chemical arguments. Table 1 also lists the results obtained according to these constraints:

$$10^2 < k_{+1}, k_{+3} < 10^8$$

$$10^{-1} < k_{-1}, k_{-3}, k_p, k_q \text{ (mechanism 2)} < 10^5$$

$$10^{-3} < k_{+2}, k_{-2}, k_{-4}, k_{+7} < 10^3$$

$$10^{-4} < k_p, k_q \text{ (mechanism 1)}, k_{+5}, k_{-5}, k_{+6}, k_{-6} < 10^2$$

$$10^{-6} < k_{-4}, k_{-7} < 10^6 \text{ (otherwise rejected)}$$

k_{+1} and k_{+3} units of $M^{-1} \cdot s^{-1}$, but all other rate constants have units of s^{-1} . A type III distribution was used in this program.

Values of k_{-4} or k_{-7} were calculated by using:

$$k_{+1} k_{-2} k_{-3} k_{+4} = k_{-1} k_{+2} k_{+3} k_{-4}$$

and

$$k_{+4} k_{-5} k_{+6} k_{-7} = k_{-4} k_{+5} k_{-6} k_{+7}$$

and when values occurred outside the range $10^{-6} \leq k_i \leq 10^6$ then the set was rejected. From Table 1 we see that this procedure does not lead to any substantial differences from the previous ones. It

seems as if there are ways in which the topological features of the mechanism impress themselves on the potential for leading to complex curves irrespective of the probability distribution functions used to generate the random values for k_i . There are two effects operating to bring this about. First, varying the k_i values in a prescribed way for a given mechanism defines a probability distribution for α_i and β_i . However, restricting the ranges of k_i values imposes a further constraint on the distribution of α_i and β_i and hence on the probability of realizing possible curve shapes.

The Monod–Wyman–Changeux binding scheme is often supposed to be associated with sigmoid saturation curves and concave-up double-reciprocal plots, although other features are possible (Bardsley & Waight, 1978). Here we have analysed the behaviour of a steady-state mechanism which is one-site/two-state and is thus a Monod–Wyman–Changeux-model monomer. This mechanism has a 50% chance of giving concave-down reciprocal plots, it is unlikely to be sigmoid or show a maximum in v -versus- $[S]$ plots, and there is a vanishingly small probability of obtaining a curve that is both sigmoid and of the substrate-inhibition type. This suggests the intuitive feeling that highly complex curves have low intrinsic probabilities and suggests that where this mechanism is suspected we should expect to see featureless v -versus- $[S]$ profiles.

(5) Studies of curve-shape probabilities for the random Bi Bi mechanism

Here a slight complication is that, in calculating curve-shape probabilities for mechanism 3 of Fig. 2, we also have to regard the second substrate as varied along with the second-order rate constant associated with it. The results of the computation are shown in Table 1.

Evidently the most probable curve is of the hyperbolic type in v -versus- $[S]$ space, although substrate inhibition is possible (20–30%). The double-reciprocal plot will tend to be concave up (75–85%), and sigmoidicity is not to be expected (<2%). Again, the highly complex curves with sigmoidicity and substrate inhibition that had a probability of 21% when α_i and β_i were varied uniformly now have a negligible chance of occurring.

(6) Curve-shape probabilities for the 3:3 function

It has been shown that the 3:3 function:

$$\frac{v}{[E_0]} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \alpha_3[S]^3}{\beta_0 + \beta_1[S] + \beta_2[S]^2 + \beta_3[S]^3} \quad (4)$$

can have 27 double-reciprocal plots (Bardsley, 1977a; Bardsley *et al.*, 1980), but five of these shapes are actually those that are possible for eqn. (3). In other words, even without decreasing in degree, we may have $R_0^{3:3} \neq 0$, $R_1^{3:3} \neq 0$ and still

have five shapes that are indistinguishable from the five possible 2:2 shapes as regards turning points and inflexions. Of course, these 3:3 curves would not be conic sections, and the best-fit 2:2 curves obtained by non-linear regression might not be very good fits either by eye or by inspecting the sum of weighted residuals squared.

There is, however, a new geometric feature possible with degree 3:3 that is not allowed for degree 2:2; $d^2(1/v)/d(1/[S])^2$ may have up to two positive zeros. So, rather than computing the probabilities for all 3:3 curve shapes, we shall content ourselves with working out the separate probabilities of a 3:3 function having zero, one or two inflexions in the double-reciprocal plot. These are also the same as the probabilities for the same number of inflexions in Scatchard or Hanes plots.

A computer program was written to vary the α_i and β_i of eqn. (4), calculate the coefficients of the numerator of $d^2(1/v)/d(1/[S])^2$ and then find the positive roots of $d^2(1/v)/d(1/[S])^2 = 0$. The results are shown in Fig. 6 for a type I distribution and for a type II distribution. Clearly the two types of distribution do not make much difference, and we conclude that the probability of one inflexion is of the order of 60%, whereas that of two is about 10%. Hence the probability of a 3:3 function of the type of eqn. (4) giving a curve that cannot be given by a 2:2 function like eqn. (3) is about 70%.

(7) Studies of curve-shape probabilities for the substrate-modifier mechanism

The mechanism under consideration is mechanism 4 of Fig. 2, and the computation of probabilities

Fig. 6. Probability of inflexions in double-reciprocal plots for the general 3:3 function $v/[E_0] = (\alpha_1[S] + \alpha_2[S]^2 + \alpha_3[S]^3)/(\beta_0 + \beta_1[S] + \beta_2[S]^2 + \beta_3[S]^3)$.

Type I distribution with $0 \leq \alpha_i, \beta_i < 1$ (○, △ and □).

Type II distribution with $1 \leq \alpha_i, \beta_i < 10^{10}$ (●, ▲ and ■).

○ and ●, No inflexions in the double-reciprocal plot; △ and ▲, one inflexion in the double-reciprocal plot; □ and ■, two inflexions in the double-reciprocal plot.

is displayed in Fig. 7 for a type I distribution and for a type II distribution.

Immediately we can see a dramatic difference between Figs. 6 and 7. In Fig. 6 one inflexion was the most probable, whereas in Fig. 7 it is the curve with no inflexions that has become the most probable. Once again the mechanism has stamped an indelible imprint on the curve-shape probabilities, as judged by the comparison between varying α_i and β_i or k_i . This has occurred to such an extent that no examples giving two inflexions was

Fig. 7. Probability of inflexions in double-reciprocal plots for mechanism 4 of Fig. 2 (implicit substrate-modifier mechanism).

Type I distribution with $0 \leq k_i < 1000$ (○ and △). Type II distribution with $1 \leq k_i < 10^{10}$ (● and ▲). Symbols are as for Fig. 6. Note that the probability of two inflexions with this mechanism, though very small, is finite, and has been checked by numerical examples.

uncovered during the generation of random k_i values, and yet this was a likely outcome of varying α_i and β_i in eqn. (4). We showed previously (Bardsley *et al.*, 1980) that the substrate-modifier mechanism can give two inflexions in $1/v$ -versus- $1/[S]$ plots, and, suspecting that this could have been mistaken due to rounding-off error, we have now recalculated the zeros of $d^2(1/v)/d(1/[S])^2$ for the k_i values reported. There were two positive zeros, and we conclude that the intrinsic probability of two inflexions is extremely low. Here again the mechanism results in a lower probability for highly complex curves than would be possible if the α_i and β_i were varied independently.

(8) Numerical values of coefficients of rational functions encountered in enzyme kinetics

The motivation for this investigation is the fact that the finite λ_i values of eqn. (1) are likely to be fewer in number than the total number of enzyme species involved in the catalysis. This is because some species are involved in substrate addition, some are concerned with first-order reactions converting substrate into product, and some involve conformational changes and therefore only a few will be product-releasing leading to numerator terms. In general, we would expect the numerator coefficients to be larger than those in the denominator or smaller, depending on whether the rate constants representing product-release steps are greater than or less than unity. Also, since the numerator coefficients are simpler expressions than those in the denominator, we might expect them to

Table 2. Numerical values of coefficients in the rate equation for the substrate-modifier mechanism
Mechanism 4 of Fig. 2 is an implicit version of the substrate-modifier mechanism that gives a 3:3 rate equation. Values of the coefficients α_i and β_i are given for type I and type II probability distribution functions together with other relevant statistical data. k_{+1} , k_{+2} , k_{+3} and k_{+4} have units of $M^{-1} \cdot s^{-1}$, but all other rate constants have units of s^{-1} .

Type I distribution with $0 \leq k_i < 1000$ ($i \neq -4$)				
	No. of cases	Mean	Standard deviation	Range of values
α_1	200	8.1×10^{12}	6.0×10^{13}	$3.9 \times 10^7 - 7.3 \times 10^{14}$
α_2	200	6.7×10^{12}	5.0×10^{13}	$7.1 \times 10^9 - 4.5 \times 10^{14}$
α_3	200	1.4×10^{11}	1.8×10^{11}	$9.5 \times 10^7 - 1.1 \times 10^{12}$
β_0	200	1.8×10^{10}	1.2×10^{11}	$3.8 \times 10^6 - 1.3 \times 10^{12}$
β_1	200	3.3×10^{10}	2.2×10^{11}	$6.6 \times 10^7 - 2.2 \times 10^{12}$
β_2	200	1.4×10^{10}	8.9×10^{10}	$6.2 \times 10^7 - 7.9 \times 10^{11}$
β_3	200	2.7×10^8	2.9×10^8	$1.6 \times 10^6 - 1.6 \times 10^9$
Type II distribution with $1 \leq k_i < 10^{10}$ ($i \neq -4$)				
α_1	200	7.7×10^{46}	1.1×10^{48}	$6.6 \times 10^5 - 1.5 \times 10^{49}$
α_2	200	1.2×10^{41}	1.1×10^{42}	$1.1 \times 10^{15} - 1.2 \times 10^{43}$
α_3	200	1.4×10^{33}	1.3×10^{34}	$1.8 \times 10^9 - 1.6 \times 10^{35}$
β_0	200	1.2×10^{38}	1.6×10^{39}	$1.1 \times 10^7 - 2.3 \times 10^{40}$
β_1	200	1.8×10^{40}	2.6×10^{41}	$2.4 \times 10^{10} - 3.7 \times 10^{42}$
β_2	200	8.4×10^{36}	5.3×10^{38}	$3.0 \times 10^{10} - 7.3 \times 10^{39}$
β_3	200	3.4×10^{26}	4.1×10^{27}	$1.2 \times 10^6 - 5.7 \times 10^{28}$

fluctuate rather more widely. The denominator coefficients, especially those of lower degree, are buffered, so to speak, because of their complexity. Table 2 lists the means and standard deviations of the coefficients obtained for the substrate-modifier mechanism when k_i values are varied uniformly. The standard deviations are large and do not seem to fit in too well with the intuitive feeling outlined above. However, the means of the numerator coefficients are, without exception, greater than the means of the denominator coefficients, and this suggests a possible way of obtaining information about the magnitude of product-releasing steps.

In any mechanism with m species, there will be directed trees, $T_{ij}S^j$, of degree j in substrate where T_{ij} represents the homogeneous polynomial in k_i values representing steps leading to species i . If now there are a number of product-releasing steps, k_{pi} , from node i , then the constant term β_0 will be the sum of all directed trees of degree zero in S , i.e.

$$\beta_0 = \sum_{i=1}^m T_{i0}$$

Here T_{i0} is the sum of all directed trees of zero degree in S that terminate on species i .

Similarly β_1 will be given by:

$$\beta_1 = \sum_{i=1}^m T_{i1}$$

but the expression for α_1 will only have a limited number of these trees, say involving nodes a , b and c . Thus the expression for α_1 will be:

$$\alpha_1 = k_{pa}T_{a1} + k_{pb}T_{b1} + k_{pc}T_{c1}$$

with similar expressions for the other α_i and β_i . If the coefficients β_i are homogeneous of order q in the constants k_i , then the coefficients α_i will be homogeneous of order $q+1$ in the k_i and, very approximately, it is to be expected that the value of α_i/β_i will be of an order comparable with that of the largest product-release constant. This suggests a possible use for the values of the coefficients α_i and β_i calculated by non-linear regression. If a non-linear regression program is used to calculate best-fit coefficients α_i and β_i , then there is little hope of using these to calculate k_i values. Indeed, if a mechanism is assumed, it is far better anyway to regress to the values of k_i rather than α_i and β_i . Our approach previously has been to regard non-linear regression as an aid to establishing minimum degree of rate equations (Bardsley *et al.*, 1980), but it is clear that, if a mean was taken of the estimates of α_i/β_i , then this would, in many circumstances, give an estimate of the order of magnitude of product-releasing steps. In this context it should be emphasized that the product-release steps estimated would be genuine product-release steps from enzyme–product com-

plexes. Typically they should be of the order of magnitude of k_{-1} , and hence the interpretation would involve explicit schemes only as in mechanism 2.

Table 3 shows that, as far as the explicit form of the one-site/two-state model is concerned, the mean of α_1/β_1 and α_2/β_2 gives a good estimate of k_p and k_q if these are of comparable value and less than 10^2 . If one of k_p or k_q is larger than the other, then this procedure will still give a tolerable estimate of the larger constant as long as this is less than 10^2 . This technique should be used cautiously, since the standard deviations were large.

Discussion

We now discuss possible answers to questions 1–10 formulated in the introduction.

Answer to question 1. Once a classification of complex curves has been achieved, there will be an intrinsic probability associated with each possible shape for any given distribution of the coefficients α_i and β_i . These probabilities can be expressed as multiple integrals if the probability distribution functions generating the α_i and β_i are known and if the necessary and sufficient conditions for curve-shape features can be shown to depend on the sign of functions of α_i and β_i . This has been done for the 2:2 function in the present paper.

Answer to question 2. In practice, the evaluation of such multiple integrals may not be possible and, at best, is likely to be tedious. We have shown how computation may be used to give approximate values for these intrinsic probabilities.

Answer to question 3. The computer programs used for the purpose of approximating to intrinsic probabilities were shown to converge rapidly. In most cases, a good estimate was obtained by 50 cycles.

Answer to question 4. It was shown that the probabilities for alternative curve shapes were not strongly influenced by the choice of probability distribution functions for α_i and β_i or k_i .

Answer to question 5. In all cases the curve-shape probabilities obtained by varying α_i and β_i were considerably different from those obtained by varying k_i .

Answer to question 6. It seems that each molecular mechanism is associated with a characteristic set of curve-shape probabilities. This could be of value in discriminating between alternative mechanisms. For instance, suppose one suspected mechanism 1 of Fig. 1 and the experimental data were of the form of a v -versus- $[S]$ curve that was sigmoid and showed substrate inhibition over a wide variety of conditions of temperature, ionic strength, pH or modifier concentration. In this circumstance, the choice of mechanism would be open to doubt.

Although mechanism 1 can indeed give such a curve shape, it is highly improbable, and it is difficult to find appropriate sets of k_i values. It would be expected that varying the k_i by altering experimental conditions would lead to a change of curve shapes.

Answer to question 7. The probability of a complex mechanism decreasing in degree to give a Michaelis–Menten curve is very small. Actual experimental data may be difficult to distinguish from Michaelis–Menten kinetics under one particular set of experimental conditions, but, by varying experimental conditions, the k_i values should change sufficiently to betray high-degree terms in the rate equation. With α_i and β_i varied, there is a probability of 1/2 for obtaining a sigmoid or substrate-inhibition type of curve. However, the mechanisms we have studied have a much lower probability than this. Also, mechanisms 1 and 2 seem to have probabilities of concave-down double-reciprocal plots near to 1/2 when the k_i values are varied.

Answer to question 8. In the case of the 3:3 rate equation for the substrate-modifier mechanism, the shapes with inflexions in double-reciprocal space are not very probable when the k_i are varied. Hence it would seem that this mechanism is very likely to give curves similar to those possible for 2:2 functions. It would be difficult on curve shape alone to detect cubic terms in this particular rate equation, and, in general, we suspect that, although highly complex curves are possible with high-degree rational functions, the intrinsic probabilities associated with such curves will be low.

Answer to question 9. In rate equations, one coefficient is always arbitrary, and so the important question is not really the absolute magnitude of the α_i and β_i but the scale separating the lowest and highest values. This is important in non-linear regression, where it is required to set limits within which the parameters may be allowed to vary. Some relevant information is in Tables 2 and 3.

Answer to question 10. Taking the mean of α_i/β_i fitted by non-linear regression to experimental data may give an approximate measure of the largest product-release constant in a mechanism. The reliability of this procedure decreases markedly for product-release constants greater than approx. 10^2 , and interpretation must always be in terms of explicit schemes.

Table 3. Estimates of the mean of α_i/β_i for the explicit version of the one-site/two-state mechanism

In the Monod–Wyman–Changeux explicit monomer mechanism (mechanism 2 of Fig. 2) k_p and k_q are true product-release constants, and this Table represents the statistical data supporting the contention that the value α_i/β_i is of the order of the larger of k_p or k_q . In each case 50 cycles of a type III distribution were used to generate random values of k_i (k_{-4} and k_{-7} being calculated), and the coefficients of the rate equation were computed. The constants k_p and k_q were fixed for each set of 50 cycles, as indicated in the left-hand column, and all other rate constants were in the numerical ranges: $10^2 \leq k_{+1}$, $k_{+3} \leq 10^8$, $10^{-3} \leq k_{+2}$, k_{-2} , k_{+4} , k_{-4} , k_{+7} , $k_{-7} \leq 10^3$, $10^{-1} \leq k_{-1}$, $k_{-3} \leq 10^5$, $10^{-4} \leq k_{+5}$, k_{-5} , k_{+6} , $k_{-6} \leq 10^2$. k_{+1} and k_{+3} have units of $\text{M}^{-1} \cdot \text{s}^{-1}$, but all other rate constants have units of s^{-1} .

k_q	k_p	$\left[\left(\frac{\alpha_1}{\beta_1} + \frac{\alpha_2}{\beta_2} \right) / 2 \right] (\text{mean} \pm \text{s.d.})$
1	10^{-6}	0.1 ± 0.2
1	10^{-4}	0.1 ± 0.2
1	10^{-2}	0.1 ± 0.2
1	1	0.3 ± 0.3
1	10^2	2.6 ± 6.0
1	10^4	4.8 ± 11.2
1	10^6	5.1 ± 11.6
10^{-6}	10^{-6}	$4.4 \times 10^{-7} \pm 4.1 \times 10^{-7}$
10^{-4}	10^{-4}	$4.4 \times 10^{-5} \pm 4.1 \times 10^{-5}$
10^{-2}	10^{-2}	$4.0 \times 10^{-3} \pm 3.9 \times 10^{-3}$
1	1	0.3 ± 0.3
10^2	10^2	5.3 ± 9.0
10^4	10^4	9.3 ± 16.6
10^6	10^6	9.5 ± 16.8

Conclusion

If the aim of an enzyme-kinetic study is merely to predict the approximate fluxes of metabolites in a cellular system, then two-parameter equations such as those of Michaelis & Menten, Hill and Monod, Wyman & Changeux are probably sufficient. On the other hand, if detailed kinetic studies are undertaken with a view to unravelling details of molecular mechanisms, then it is likely that information will also be available from physical and chemical studies. Increasingly such physical studies of enzymes reveal modifier or allosteric binding sites, identify conformational effects or other sources of co-operativity and sometimes even suggest mnemonic or hysteretic features. Pseudo-steady-state rate equations calculated for such mechanisms that do not involve kinetically significant enzyme polymerization steps will be rational functions of degree $\geq 2:2$ and complex curves are to be expected. The present paper is the first to deal specifically with questions of the probability of obtaining such complex curves. It is offered as a contribution to developing a deeper understanding of deviations from Michaelis–Menten kinetics, so that curved double-reciprocal plots should not be regarded as a nuisance, but should be seen as normal behaviour and hence analysed more systematically as an aid to understanding the physical chemistry of enzyme-catalysed reactions.

F. S.-M. thanks the Fundacion Cultural 'Esteban Romero', Murcia, Spain, for a maintenance grant. We thank Dr. Keith J. Indge (University of Manchester Institute of Science and Technology) for advice on computation.

References

- Archbold, J. W. (1970) *Algebra*, 4th edn., p. 134, Pitman Publishing, London
- Bardsley, W. G. (1976) *Biochem. J.* **153**, 101–117
- Bardsley, W. G. (1977a) *J. Theor. Biol.* **65**, 281–316
- Bardsley, W. G. (1977b) *J. Mol. Biol.* **113**, 573–578
- Bardsley, W. G. (1977c) *J. Theor. Biol.* **67**, 121–139
- Bardsley, W. G. & Childs, R. E. (1975) *Biochem. J.* **149**, 313–328
- Bardsley, W. G. & Waight, R. D. (1978) *J. Theor. Biol.* **70**, 135–156
- Bardsley, W. G., Leff, P., Kavanagh, J. P. & Waight, R. D. (1980) *Biochem. J.* **187**, 739–765
- Botts, J. (1958) *Trans. Faraday Soc.* **54**, 593–604
- Childs, R. E. & Bardsley, W. G. (1975) *Biochem. J.* **145**, 93–103
- Childs, R. E. & Bardsley, W. G. (1976) *J. Theor. Biol.* **63**, 1–18
- Ferdinand, W. (1966) *Biochem. J.* **98**, 278–283
- Gutfreund, H. (1972) *Enzymes: Physical Principles*, pp. 157–161, Wiley–Interscience, New York
- Hardy, G. H. (1952) *Course of Pure Mathematics*, 10th edn., p. 238, Cambridge University Press, Cambridge
- Hill, C. M., Waight, R. D. & Bardsley, W. G. (1977) *Mol. Cell. Biochem.* **15**, 173–178
- Katsumata, M. & Goldman, A. W. (1976) *J. Biochem. (Tokyo)* **79**, 605–611
- King, E. L. (1956) *J. Phys. Chem.* **60**, 1378–1381
- King, E. L. & Altman, C. (1956) *J. Phys. Chem.* **60**, 1375–1378
- Krupka, R. M., Kaplan, H. & Laidler, K. J. (1966) *Trans. Faraday Soc.* **62**, 2754–2759
- Maxwell, E. A. (1966) *Advanced Algebra*, part 1, p. 137, Cambridge University Press, Cambridge
- Monod, J., Wyman, J. & Changeux, J.-P. (1965) *J. Mol. Biol.* **12**, 88–118
- Pettersson, G. (1969a) *Acta Chem. Scand.* **23**, 2717–2726
- Pettersson, G. (1969b) *Acta Chem. Scand.* **23**, 3243–3247
- Pettersson, G. (1972a) *Acta Chem. Scand.* **26**, 3935–3942
- Pettersson, G. (1972b) *Biochim. Biophys. Acta* **276**, 1–11
- Volkenstein, M. V. & Goldstein, B. N. (1966) *Biochim. Biophys. Acta* **115**, 471–477
- Waight, R. D. & Bardsley (1977) *Biochem. Soc. Trans.* **5**, 758–761
- Waight, R. D., Leff, P. & Bardsley, W. G. (1977) *Biochem. J.* **167**, 787–798
- Whitehead, E. P. (1979) *J. Theor. Biol.* **80**, 355–382
- Wyman, J. (1966) *J. Am. Chem. Soc.* **89**, 2202–2218
- Wyman, J. (1972) *Curr. Top. Cell. Regul.* **6**, 209–226

APPENDIX

The computer programs used in the present paper have been deposited as Supplement SUP 50113 with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K. We are grateful to Dr. K. J. Indge for transferring to our

files a number of unpublished programs that he has developed for calculating rate equations for complex mechanisms and printing out coefficients after sorting in order to facilitate checking procedures. Using these programs we have noted one or two misprints in earlier papers, as now detailed.

Corrections to *Biochem. J.* (1980) **187**, 739–765:

p. 763, l. 16 up	$k_{-2} k_{-3} k_{-4} k_{+7} k_q$	should be	$k_{-2} k_{-3} k_{+4} k_{+7} k_q$
p. 764, l. 4 down	$k_{+1} k_{+4} k_{+5} k_{+6} k_q$	should be	$k_{+1} k_{-2} k_{+5} k_{+6} k_{+7}$
p. 764, l. 8 down	$k_{+1} k_{-2} k_{+4} k_{-5} k_{-6}$	should be	$k_{+1} k_{-2} k_{+4} k_{-5} k_{+6}$
p. 764, l. 8 down	$k_{+1} k_{-2} k_{-3} k_{+5} k_{+7}$	should be	$k_{+1} k_{-2} k_{+4} k_{+6} k_p$
p. 764, l. 9 down	$k_{+1} k_{-2} k_{+5} k_{+7}$	should be	$k_{+1} k_{-2} k_{-3} k_{+5} k_{+7}$
p. 764, l. 10 down	$k_{+2} k_{-3} k_{+4} k_{-6} k_{+7}$	should be	$k_{+2} k_{+3} k_{+4} k_{-6} k_{+7}$
p. 765, l. 34 down	$k_{+2} k_{+3} k_{-4} k_{+5} k_q$	should be	$k_{+1} k_{+3} k_{-4} k_{+5} k_q$

Corrections to *Biochem. Soc. Trans.* (1977) **5**, 785–789

p. 786, l. 30 down	$k_{+3} k_{+6} (k_{+5} + \dots)$	should be	$k_{+3} k_{+6} (k_{-7} + \dots)$
p. 786, l. 31 down	add to the expression for b' the term $k_{-3} k_{+7} (k_{-6} + k_{+9})$		

Corrections to *Biochem J.* (1975) **145**, 93–103:

The corrections to the rate equation are discussed by Indge & Childs (1976) *Biochem. J.* **155**, 567–570

Corrections to *Biochem. J.* (1977) **167**, 787–798:

p. 789, column 2, l. 23 down α_3 should be as follows:

$$\alpha_3 = 2k_{+1} k_{+2} k_{+4} k_{+6} k_{+8} (k_{+5} + k_{+7})$$

p. 789, column 2, l. 27 down change k_{-6} to k_{+6}