THE KINETICS OF ENZYME ACTION

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In the endeavours to elucidate the nature of enzyme catalysis a study of reaction kinetics has been of major importance. Most attempts to analyse the kinetics of enzyme action have been based on the postulate of an intermediate complex formed between enzyme and substrate. This theory was formulated in 1913 by MICHAELIS AND MENTEN[1], as the reaction scheme

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + Q \qquad (1)$$

The published investigations of the kinetics of enzyme catalysis have been either studies of the overall reaction or demonstrations of the formation and breakdown of the intermediate complex. The latter experiments, involving the observation of the intermediate complex, necessitate the use of high enzyme concentrations and therefore of special techniques for the measurement of rapid reactions. Under such conditions the overall kinetics show transient phases during the reaction which are not observed at lower enzyme concentrations[2], and these phases have been correlated with changes in the concentration of the enzyme-substrate complex. However, the interpretation of the experimental data is hindered by the absence of an analytical solution of the differential equation obtained on the application of the law of mass action to the postulated reaction scheme. The evaluation of the rate constants of the individual reaction steps requires either the use of an electronic computer[3], or the making of certain assumptions in the formation of soluble but approximate differential equations. The manner in which these approximate equations deviate from the complete equation should be recognised.

The present paper attempts to formulate the solutions of approximate equations in such a manner that further details can be obtained about the individual reaction steps. The validity of the approximate equations is established by comparison with numerical solutions of the complete differential equation. A preliminary communication of this work has already been published[4]. During the development of the present investigation ROUGHTON[5] and GUTFREUND[6] have considered the initial stages of the enzymic reaction when the concentration of the intermediate complex is increasing. The approximate "pre-steady state" equation thus obtained has been experimentally applied to the determination of k_1. Further approximate differential equations have been presented by LAIDLER[7] and MORALES AND GOLDMAN[8].

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THE COMPLETE RATE EQUATION

The reaction scheme of MICHAELIS AND MENTEN[1] is completely described by two independent rate equations. The rate of formation of product is given by

$$\frac{d[Q]}{dt} = k_3 [ES] \tag{2}$$

whilst that of the intermediate complex is

$$\frac{d[ES]}{dt} = k_1 [E][S] - (k_2 + k_3)[ES] \tag{3}$$

The disappearance of substrate is simply the algebraic sum of these two equations.

TABLE I

THE IDENTITIES FOR CONCENTRATION USED IN THE VARIOUS MATHEMATICAL TREATMENTS

Mathematical treatment	*Reactant*			
	E	*S*	*ES*	*Q*
Full equation	$e - p$	$a - x - p$	p	x
"Steady state" approximation of BRIGGS AND HALDANE	$e - p$	$a - x$	p	x
"Pre-steady state" approx. at low enzyme concentration	$e - p$	a	p	x
"Pre-steady state" approx. at high enzyme concentration	$e - p$	$a - p$	p	x

Table I summarises the symbols for concentration that are substituted into equations (2) and (3) in the various mathematical treatments. When the full identities are employed and ES is eliminated between equation (2) and (3), then the overall equation relating product concentration and time is obtained.

$$\frac{d^2x}{dt^2} + k_1(K_m + e + a - x)\frac{dx}{dt} - \frac{k_1}{k_3}\left(\frac{dx}{dt}\right)^2 - k_1 k_3 e(a - x) = 0 \tag{4}$$

This nonlinear differential equation of the second order has not been solved in terms of an explicit relation between x and t. However, it may be simplified by the introduction of new units of time and concentration.

The Michaelis constant $K_m\left(= \frac{k_2 + k_3}{k_1}\right)$ is defined as the unit of concentration that is to be used when considering an enzymic reaction. STRAUSS AND GOLDSTEIN[9] in their generalised treatment of enzyme inhibition term this the "specific concentration". The subscript "s" will be used in the present paper to denote these units. Thus $e_s = e/K_m$, $a_s = a/K_m$, etc.

A common time scale is obtained by using the reciprocal of the rate constant k_3 as the unit of time for each enzymic reaction. In this way the term $k_3 t$ becomes dimensionless.

The introduction of a new rate parameter "r" as defined by equation (5) allows the other rate constants to be eliminated from mathematical expressions in the above units.

$$k_1 K_m = k_2 + k_3 = r k_3 \tag{5}$$

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The relative magnitudes of k_2 and k_3 are indicated by the value of r. This parameter has a lower limit of unity when $k_2 \ll k_3$, but tends to infinity when $k_2 \gg k_3$.

Equation (4) can now be written in terms of these new units with only the parameter "r" entering into the expression.

$$\frac{d^2x_s}{d(k_3t)^2} + r\left\{(1 + e_s + a_s - x_s)\frac{dx_s}{dk_3t} - \left(\frac{dx_s}{dk_3t}\right)^2 - e_s(a_s - x_s)\right\} = 0 \qquad (6)$$

Equation (6) becomes of the first order when written in terms of the concentration of the intermediate complex.

$$p_s\frac{dp_s}{dx_s} + r\left\{(1 + e_s + a_s - x_s)p_s - p_s^2 - e_s(a_s - x_s)\right\} = 0 \qquad (7)$$

Although equation (7) has no analytic solution relating p_s with x_s, the methods of numerical integration can be employed to obtain a solution for any given set of values of r, e_s and a_s. Alternatively the reversion method of YANG[10] may be used to calculate a particular solution. The variation in the kinetics between different enzymes can then be discussed in terms of the effect of r on the numerical solution.

The procedure that I used for calculating numerical solutions commenced with the fitting of a power series

$$p_s = b_1x_s^{\frac{1}{2}} - b_2x_s + b_3x_s^{\frac{3}{2}} - b_4x_s^2 + \ldots \qquad (8)$$

to equation (7), by which means the coefficients were defined as:

$$\begin{aligned} b_1 &= (2re_sa_s)^{\frac{1}{2}} \qquad (9)\\ 3b_2 &= 2r(1 + e_s + a_s)\\ 2b_1b_3 &= r\left\{b_1^2 + (1 + e_s + a_s)\,b_2 - e_s\right\} - b_2^2\\ 5b_1b_4 &= 2r\left\{2b_1b_2 + (1 + e_s + a_s)\,b_3 - b_1\right\} - 5b_2b_3\\ &\text{etc.} \qquad\qquad\qquad\qquad \text{etc.} \end{aligned}$$

The substitution of a particular set of values of r, e_s and a_s into equation (9) defines the numerical magnitudes of the coefficients b_1, b_2, b_3, etc. Table II gives the values that were thus obtained for three solutions.

TABLE II

DATA OF THE THREE NUMERICAL SOLUTIONS SHOWN IN FIGS. 4 AND 5

a_s	1	1	2
e_s	1	1	1
r	1	2	1
b_1	1.41421	2.00000	2.00000
b_2	2.00000	4.00000	2.66667
b_3	1.06066	3.50000	1.63889
b_4	0.60000	2.80000	0.85926
b_5	0.35650	2.80417	0.58901
b_6	0.24762	2.13810	0.35268
b_7	0.08180	1.22676	0.14924
$p_s^{max.}$	0.310	0.334	0.516
$x_s^{max.}$	0.24	0.16	0.42

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At the beginning of the reaction when x_s is small, values of p_s were calculated to the required degree of accuracy by taking sufficient terms of the power series. Further values of p_s, as x_s was increased by a constant increment, were calculated from the initial values of p_s by a numerical method. MILNE's method of forward and successive integration[11] was used to calculate the results shown in Figs. 4 and 5. An increment in x_s of 0.01 was employed for the calculation of the rapid initial change in p_s, whilst a larger increment of 0.02 was used during the remainder of the reaction. The initial values of p_s were calculated from equation (8) to five significant figures; thus allowing the numerical integration to be carried out to an accuracy in p_s of 0.001.

The numerical method of obtaining a solution of equation (7) is not suited to the direct evaluation of the individual rate constants from experimental data. For this purpose solutions of approximate equations are more useful. The numerical solutions however, are required when considering the range of validity of the approximate equations.

APPROXIMATE EQUATIONS AT LOW ENZYME CONCENTRATION

At low concentrations of enzyme certain assumptions may be made to simplify equation (4). In their classical treatment BRIGGS AND HALDANE[12] suppose that the total concentration of enzyme, and hence of ES, is negligibly small when compared with Q and S. Furthermore since ES is small compared with Q and S its rate of change must, except during the first instant of the reaction, be negligible when compared with that of Q and S.

Using the appropriate identities from Table I to substitute into equations (2) and (3), a simpler overall equation is obtained.

$$\frac{d^2x}{dt^2} + k_1(K_m + a - x)\frac{dx}{dt} - k_1k_3e(a - x) = 0 \qquad (10)$$

The second restriction that dp/dx and therefore d^2x/dt^2 may be neglected in equation (10)[13], reduces it to the familiar expression for the velocity of an enzymic reaction.

$$\frac{dx}{dt} = k_3p = \frac{k_3e(a - x)}{K_m + (a - x)} \qquad (11)$$

This second assumption is valid over the whole of the reaction except for the initial rapid formation of the intermediate complex. During the remainder of the reaction the concentration of substrate falls but before it becomes comparable with ES the reaction is virtually complete. The "steady state" approximation is often formally written as $dp/dt = 0$. This implies that the concentration of the intermediate complex is constant throughout the reaction. In fact equation (11) describes its variation with substrate concentration and hence its decrease during the course of the reaction!

The "steady state" treatment evaluates only the two reaction parameters contained in equation (11); the maximal velocity k_3e, and the Michaelis constant K_m. The reaction must be studied in other kinetic regions to obtain values for the remaining parameters.

The kinetics of the initial rapid formation of the enzyme-substrate complex may be used to evaluate k_1. The assumption is made in deriving the following equations that little of the substrate has been consumed during this "pre-steady state"

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of the reaction (*i.e.* S $\gg$ ES + Q). The identities for concentration that are substituted into equations (2) and (3) under this assumption are given in Table I. The overall rate equation so obtained is a linear differential equation with constant coefficients.

$$\frac{d^2x}{dt^2} + k_1(K_m + a)\frac{dx}{dt} - k_1k_3ea = 0 \tag{12}$$

The solution of equation (12) is

$$x = \frac{k_3ea}{K_m + a}\left\{t + \frac{\exp[-k_1(K_m + a)t] - 1}{k_1(K_m + a)}\right\}, \tag{13}$$

whilst the concentration of ES is given by

$$p = \frac{ea}{K_m + a}\left\{1 - \exp[-k_1(K_m + a)t]\right\} \tag{14}$$

The exponential increase of ES to the initial value given by the "steady state" treatment is reflected as a lag in the rate of the formation of product. The rate of disappearance of substrate however, being the sum of ES and Q, shows a lead phase. Fig. 1 illustrates these initial changes in the concentrations of substrate and product. The curves were calculated from equations (13) and (14) under the arbitarily chosen condition that

$$\frac{k_1(K_m + a)}{k_3} = r(1 + a_s) = 3$$

The units along the time and concentration axes of Fig. 1 are proportional to k_3 and e respectively.

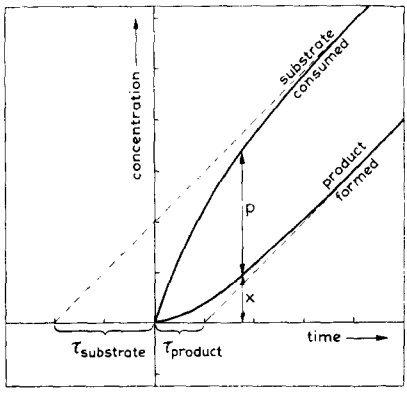

Fig. 1. Pre-steady state kinetics.

Equation (13) is not directly amenable to the evaluation of k_1 from experimental data. ROUGHTON[5] therefore further simplified the expression to

$$x = k_1k_3eat^2/2, \tag{15}$$

for the initial part of the lag phase when $t \ll 1/k_1(K_m + a)$. Now on differentiation, equation (15) gives

$$\frac{dx}{dk_3t} = k_1eat = \left(\frac{2k_1ea}{k_3}\right)^{\frac{1}{2}}x^{\frac{1}{2}} \tag{16}$$

which on conversion to specific units becomes recognisable as the first term of the power series given as equations (8) and (9).

$$\frac{dx_s}{dk_3t} = p_s = (2re_sa_s)^{\frac{1}{2}}x_s^{\frac{1}{2}} \tag{17}$$

Thus the use of equation (15) to determine k_1 is limited, by the numerical values of the coefficients b_1, b_2, b_3, etc., to experimental observations at such small product concentrations that the terms in higher powers of x_s may be neglected. However, measurements at the initial part of the lag phase are unlikely to be accurate because of the limitations of chemical purity and experimental technique.

A more useful graphical method for evaluating k_1 utilises experimental data

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obtained at $t \gg 1/k_1 (K_m + a)$. This is the reverse of the condition used in deriving equation (15). The exponential term of equation (13) vanishes to leave an expression for a straight line, which on extrapolation makes an intercept (τ_{product}) with the time axis,

where
$$\tau_{\text{product}} = \frac{1}{k_1(K_m + a)} \tag{18}$$

This method of calculating k_1 from an experimentally determined intercept has been successfully employed by GUTFREUND[6].

If the experimental technique used determines the change in substrate concentration instead of the formation of the product, then, as illustrated in Fig. 1, a negative intercept is obtained. This is given by

$$k_1 \tau_{\text{substrate}} = \frac{1}{K_m + a} - \frac{k_1}{k_3} \tag{19}$$

The experimental determination of this intercept at various substrate concentrations allows both k_1 and k_3 to be independently obtained using equation (19).

Although the concentration of enzyme does not enter directly into equations (18) and (19), it is evident from Fig. 1 that it must be sufficiently great for ES and Q to be of comparable magnitude. For greater enzyme concentrations the assumption that $S \gg ES + Q$, made in deriving equation (12), may no longer be valid for the initial stages of the reaction even when $S \gg Q$. In this case the "pre-steady state" equations derived in the following section must be employed.

APPROXIMATE EQUATIONS AT HIGH ENZYME CONCENTRATION

When the concentrations of enzyme, substrate and product are comparable, the assumption of the previous section that the concentration of intermediate complex is small is no longer valid. Nevertheless modified "steady state" and "pre-steady state" approximations can be used to obtain soluble differential equations in which no restriction is placed on the magnitude of the intermediate complex.

For the "pre-steady state", the assumption is made that only a few % of the substrate has been converted to product (*i.e.* $S \gg Q$). This gives the differential equation.

$$\frac{d^2x}{dt^2} + k_1(K_m + e + a)\frac{dx}{dt} - \frac{k_1}{k_3}\left(\frac{dx}{dt}\right)^2 - k_1 k_3 e a = 0 \tag{20}$$

The "steady state" approximation is defined by the condition that the first term of equation (4) be neglected in comparison with the magnitude of the other terms. The following quadratic in the reaction velocity is then obtained.

$$\left(\frac{dx}{dt}\right)^2 - k_3(K_m + e + a - x)\frac{dx}{dt} + k_3^2 e(a - x) = 0 \tag{21}$$

The validity of equations (20) and (21) will be considered in the discussion. At present only the solutions are given. These may be simplified by the introduction of two further symbols.

$$\theta = [(K_m + e + a - x)^2 - 4e(a - x)]^{\frac{1}{2}} \tag{22}$$

$$\Phi = \frac{K_m + e + a - x + \theta}{2} \tag{23}$$

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Both these quantities have the units of concentration and may be converted to specific units through division by K_m. Since these functions depend on the concentration of the product, they change during the reaction from their initial values of θ° and Φ° when $x = 0$.

The "steady state" reaction velocity derived from equation (21) can now be written as

$$\frac{dx}{dt} = \frac{k_3 e(a - x)}{\Phi} \tag{24}$$

By measuring the rate of product formation (dx/dt) in the "steady state" region of the reaction, Φ can be experimentally determined from equation (24). For this calculation $k_3 e$ is obtained from the value of the maximal velocity ($k_3 e$) at low enzyme concentration by multiplying by the ratio of the enzyme concentration at which Φ is being determined to that at which the maximal velocity was originally measured. The magnitude of Φ varies between $(K_m + e + a - x)$ and one half of this value. It approaches the former value both at high and at low enzyme concentrations. When either $K_m \gg e$ or $(a - x) \gg e$, then $\Phi = (K_m + a - x)$ and the reaction velocity reduces to equation (11).

By rearranging equation (23), an expression is obtained which may be used to calculate the molar concentration of enzyme from an experimentally determined value of Φ. Thus

$$e = \frac{\Phi(\Phi - K_m - a + x)}{\Phi - a + x} \tag{25}$$

The percentage error in this calculated value of the enzyme concentration is given in equation (26) as a function of the experimental error in Φ.

$$\frac{\delta e}{e}\,\% = \frac{\Phi(\Phi - a + x) - (a - x)(\Phi - K_m - a + x)}{(\Phi - K_m - a + x)(\Phi - a + x)} \cdot \frac{\delta\Phi}{\Phi}\,\% \tag{26}$$

The variation of this error function with enzyme concentration is plotted in Fig. 2 for several values of $(a_s - x_s)$. Reasonable accuracy in the calculation of the molar concentration of enzyme is obtained only above a certain range of enzyme concentration.

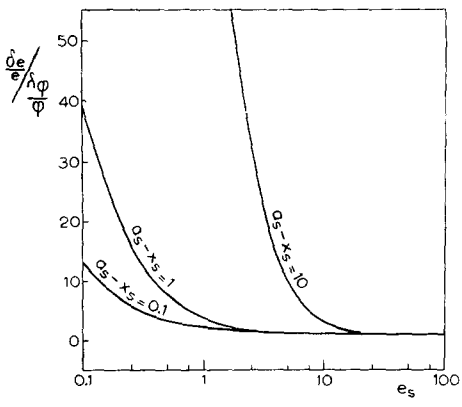

Fig. 2. Error function in the calculation of the molar enzyme concentration.

The preceding equations have been based on the measurement of the product concentration x. If the analytical method determines instead the change in substrate concentration, expressions in terms of $(x + p)$ must be used. The "steady state" assumption that d^2x/dt^2 and therefore dp/dt is negligibly small, reduces the complete equation to

$$\frac{d(x + p)}{dt} = \frac{k_3 e(a - x - p)}{K_m + a - x - p} \tag{27}$$

This can only be used to derive values of $k_3 e$ and K_m by the traditional methods.

The equations of the "pre-steady state", being based on the assumption that

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$a \gg x$ contain only the initial values θ° and Φ°. Thus on integrating equation (20) the following expression is obtained for the reaction velocity:

$$\frac{dx}{dt} = k_3 p = \frac{k_3 ea\, \Phi^\circ [1 - \exp(-k_1 \theta^\circ t)]}{(\Phi^\circ)^2 - ae \exp(-k_1 \theta^\circ t)} \tag{28}$$

On integrating equation (28), the relation between product concentration and time is obtained.

$$x = \frac{k_3 ea}{\Phi^\circ} \left\{ t - \frac{\Phi^\circ}{k_1 ea} \ln \frac{(\Phi^\circ)^2 - ea \exp(-k_1 \theta^\circ t)}{\theta^\circ \Phi^\circ} \right\} \tag{29}$$

This equation is analogous to that derived for low enzyme concentrations. An intercept on the time axis of

$$\tau_{\text{product}} = \frac{\Phi^\circ}{k_1 ea} \ln \frac{(\Phi^\circ)^2 - ea}{\theta^\circ \Phi^\circ} \tag{30}$$

is obtained on extrapolation of the experimental data subsequent to the lag phase (*i.e.* for which $t \gg 1/k_1\theta^\circ$). Equation (30) may be simplified to

$$\tau_{\text{product}} = \frac{1}{k_1 \Phi^\circ}$$

when the function $ae/(\Phi^\circ)^2$, shown in Fig. 3, is less than 0.1. Using equation (30), k_1 may be calculated if e as well as K_m and a are known and τ_{product} has been experimentally determined. However the extrapolation of the experimental data is unlikely to be linear at these high enzyme concentrations. Hence the best procedure is to fit a quadratic, $t = A + Bx + Cx^2$, to the experimental data subsequent to the lag phase. The coefficient of x is known and is given by the expression $B = \Phi^\circ/k_3 ea$. The required intercept, τ_{product}, is the constant term A.

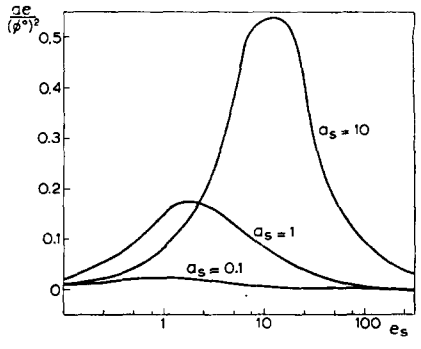

Fig. 3. Variation of the function $\frac{ae}{(\Phi^\circ)^2}$.

The experimental measurement of the concentration of substrate rather than that of the product, requires the above equations to be reformulated in terms of $(x + p)$. This leads to an intercept obtained by extrapolation of the data on the change in substrate concentration, which is given by

$$\tau_{\text{substrate}} = \frac{\Phi^\circ}{k_1 ea} \ln \frac{(\Phi^\circ)^2 - ea}{\theta^\circ \Phi^\circ} - \frac{1}{k_3} \tag{31}$$

This again is an analogous expression to that derived for low enzyme concentrations.

DISCUSSION

In order to ascertain the extent to which the equations given in the previous section deviate from the complete equation, several numerical solutions of equation (7) have been calculated. These are shown in Figs. 4 and 5 together with the solutions of the "pre-steady state" and "steady state" equations at high enzyme concentration (equations (20) and (21) respectively). The progress of the enzymic reaction is recorded as the change in the specific concentration of the intermediate complex p_s accom-

panying the formation of product x_s. Expressed in this manner, the kinetic form of the complete solution depends only on the rate parameter "r" for given initial concentrations of enzyme and substrate. The "steady state" solution, however, is independent of r, being given by

$$p_s = \frac{dx_s}{dk_3 t} = \frac{e_s(a_s - x_s)}{\Phi_s} \qquad (32)$$

Fig. 4 shows the extent of the deviation between the "steady state" approximation and the numerical solution for the limiting condition that $r = 1$ (*i.e.* $k_2 \ll k_3$). When r is very large (*i.e.* $k_2 \gg k_3$), the initial rise of p_s occurs before an appreciable concentration of product is formed. Thereafter the kinetics follow closely the "steady state" approximation, which itself becomes identical with the equilibrium treatment[14]. An intermediate solution, where $r = 2$ (*i.e.* $k_2 = k_3$), is also illustrated in Fig. 4.

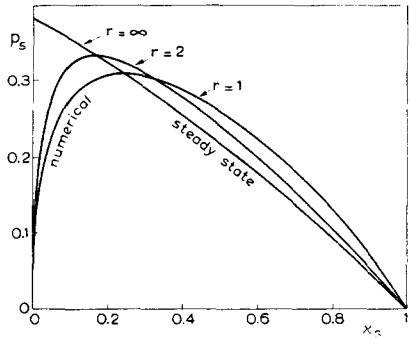

Fig. 4. Solutions of the rate equations for $a_s = 1$, $e_s = 1$.

Each numerical solution has a maximum in the concentration of the intermediate complex p_s^{max}, which occurs at a particular product concentration x_s^{max}. The values of p_s^{max} and x_s^{max} are given in Table II together with other data for the numerical calculations. The magnitude of x_s^{max} is dependent on r, becoming smaller as r increases. The experimental observations of x_s^{max} could therefore be used as an indication of the relative magnitudes of k_2 and k_3.

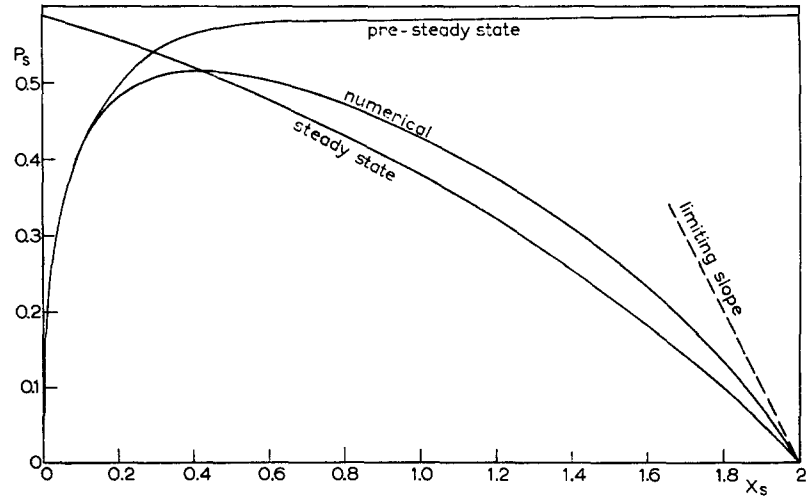

Fig. 5. Solutions of the rate equations for $a_s = 2$, $e_s = 1$ and $r = 1$.

Fig. 5 illustrates solutions obtained at twice the substrate concentration used in Fig. 4. The numerical solution calculated for $r = 1$ at $a_s = 2$ is independent of that derived for $a_s = 1$. However, the "steady state" approximation is simply a backward extrapolation of the curve given in the previous figure. The "pre-steady

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state" solution which is also indicated in Fig. 5, is calculated from equation (33) relating x_s and p_s.

$$2rx_s = \ln \left\{ \frac{p_s^2 - (1 + e_s + a_s)\, p_s + e_s a_s}{e_s a_s} \right\} - \frac{1 + e_s a_s}{\theta^\circ_s} \ln \frac{\Phi^\circ_s\, (a_s e_s - \Phi^\circ_s p_s)}{a_s \sigma_s\, (\Phi^\circ_s - p_s)} \qquad (33)$$

This equation is obtained on integrating equation (7) after applying the "pre-steady state" approximation that $a_s \gg x_s$.

The figures under discussion illustrate that the "steady state" approximation is the locus of p_s^{max} as r is varied. At the beginning of an enzymic reaction the values of p_s are well below those predicted by the "steady state" approximation but agree with the "pre-steady state" equation. At values of $x_s > x_s^{max}$, the magnitude of p_s exceeds that of the "steady state" treatment but does not deviate greatly from it.

CHANCE[2] in considering the kinetics of the enzyme-substrate complex, makes a sharp distinction between the "steady state" and a "post-steady state" region of the reaction. Figs. 4 and 5 show that the deviation between the "steady state" approximation and the numerical solutions is not confined to any final region of the reaction. The above data also renders invalid LAIDLER's suggestion[7] that the general solution deviates considerably from the "steady state" curve in the region of p^{max}, but corresponds at the end of the reaction.

Some measure of the observed deviation at the end of the reaction may be obtained on comparing the limiting slope

$$-\left(\frac{dp_s}{dx_s}\right)_{x_s = a_s}$$

of the complete equation with that of the "steady state" approximation. By substitution of

$$-\left(\frac{dp_s}{dx_s}\right)_{x_s = a_s} = \underset{x_s \to a_s}{\text{Limit}} \left(\frac{p_s}{a_s - x_s}\right)$$

into equation (7), the limiting slope of the complete equation is derived as

$$-\left(\frac{dp_s}{dx_s}\right)_{x_s = a_s} = \frac{r(1 + e_s) - [r^2(1 + e_s)^2 - 4re_s]^{\frac{1}{2}}}{2} \qquad (34)$$

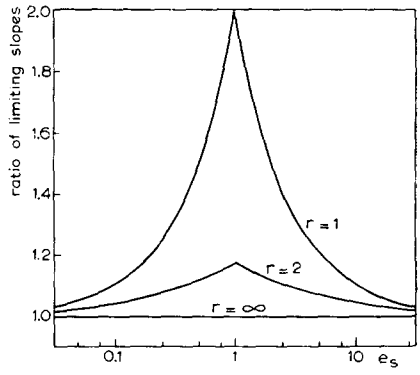

Fig. 6. Dependence of the ratio of limiting slopes on e_s.

The limiting slope of the "steady state" approximation however, is given by

$$-\left(\frac{dp_s}{dx_s}\right)_{x_s = a_s} = \frac{e_s}{1 + e_s} \qquad (35)$$

The ratio of these limiting slopes, obtained by division of equation (34) by equation (35), is plotted against the enzyme concentration in Fig. 6.

As already suggested the maximum deviation is observed when $r = 1$. The ratio of limiting slopes also shows a marked dependence on the enzyme concentration, being greatest when $e_s = 1$. This concentration was therefore chosen for the calculation of the numerical solutions in order that the maximum extent of the deviation might be indicated. When $e_s \gg 1$ or $e_s \ll 1$, the "steady state" approximation holds true at the end of the reaction. The extent of the deviation during the course of the

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reaction is not given by the limiting slopes but could be obtained by the use of an electronic computer.

ACKNOWLEDGEMENTS

I much appreciate the advice and encouragement which I received from Drs. E. M. CROOK, H. KESTELMAN AND R. A. BUCKINGHAM of University College, London (England). I also gratefully acknowledge a grant from the Medical Research Council.

SUMMARY

The differential equation describing the Michaelis-Menten scheme is modified by a change of time and concentration units so as to contain only one rate parameter. In this form it is solved numerically. Solutions are obtained by the "steady state" and "pre-steady state" approximations and are compared with the numerical solutions. Methods are suggested for the derivation of individual rate constants and the molar concentration of enzyme from kinetic experiments on the overall reaction.

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Received April 6th, 1956

THE OUTPUT OF ^{45}Ca FROM FROG MUSCLE

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It is well known that calcium exists in biological materials in both ionic and bound states. It was thought that the application of the calcium isotope ^{45}Ca might reveal to what extent the element is bound in frog muscle and what factors affect its subsequent liberation. Earlier work (TAUBMANN[1]; BERWICK[2]) has shown that little muscle Ca is removed by perfusion with Ca-free solution, or even by grinding in KCl solution. It therefore seemed likely that only a small proportion of the total Ca would be exchangeable, as proves to be the case. Recently GILBERT AND FENN[3] using ^{45}Ca have reached similar conclusions.

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