Time-Dependent Michaelis–Menten Kinetics for an Enzyme–Substrate–Inhibitor System

S. I. Rubinow and Joel L. Lebowitz

Contribution from the Biomathematics Division, Cornell University Graduate School of Medical Sciences, New York, New York 10021, and the Sloan-Kettering Institute, New York, New York. *Received November 29, 1969*

Abstract: The results of kinetic studies involving an enzyme and two substances competing for the same enzymatic site may be very different when the roles of these substances as substrate and inhibitor are reversed. If the substance used as an inhibitor has a much larger affinity for the enzyme than the substrate, then a plot of inverse reaction velocity of substrate *vs.* inverse initial substrate concentration for different values of initial inhibitor concentration will not have, under regular experimental conditions, the usual Michaelis–Menten form. The results to be expected depend essentially on the time of observation as measured from the start of the reaction. The deviations from the Michaelis–Menten form should be significant in experiments of the type carried out recently by Miller and Balis in their investigation of the enzyme *Escherichia coli* L-asparagine amidohydrolase reacting with the substrates asparagine and glutamine. Other experiments which are expected to require the time-dependent theory for their understanding are indicated.

The results of experiments on simple enzyme activated reactions are usually described and analyzed in terms of the Michaelis–Menten theory.[1] This description predicts a simple linear relationship between the reciprocal reaction velocity and the reciprocal initial substrate concentration s_0^{-1}.[2] The approximate nature of this description is well known; nevertheless it is widely successful in its applications.

The theory was developed initially for a reaction involving an enzyme and a substrate with which it reacts. If there are two substrates present which compete for the same enzymatic site, the reaction is said to be "fully competitive."[3] In such a reaction, the substrate that is singled out for measurement of its reaction velocity is referred to as the "substrate." The second substrate is called the "inhibitor." Obviously, the roles of inhibitor and substrate may be interchanged in a second study.

For such a fully competitive reaction, Michaelis–Menten theory still predicts a linear relation between the reciprocal reaction velocity of the substrate $(v^s)^{-1}$ and s_0^{-1}. As presented by Briggs and Haldane,[4] this relationship takes the form

$$\frac{1}{v^s} = \frac{1}{V_{max}^s}\left[1 + \frac{K_M^s}{s_0}\left(1 + \frac{i_0}{K_M^i}\right)\right] \qquad (1)$$

Here V_{max}^s is the maximum value of the reaction velocity v^s, K_M is the Michaelis constant, i_0 is the initial inhibitor concentration, and the superscripts s and i denote substrate and inhibitor, respectively. The constants K_M and V_{max} depend on various reaction rate constants and the initial enzyme concentration. When a Lineweaver–Burk plot[2] is made of eq 1 for different values of i_0, the result is a family of straight lines with the common intercept $1/V_{max}^s$ on the ordinate axis $s_0^{-1} = 0$.

Recently, Miller and Balis[5] investigated the activities of the enzyme *E. coli* L-asparagine amidohydrolase reacting with the substrates asparagine and glutamine, separately and together. When they utilized asparagine as a substrate in the presence of several concentrations of glutamine, they found that the results of this experiment were in agreement with Michaelis–Menten theory as expressed by eq 1. However, in a second experiment in which glutamine was utilized as a substrate in the presence of several concentrations of asparagine, the results could not be represented by means of eq 1. If asparagine were absent ($i_0 = 0$), the results did agree with eq 1. These authors noted that the maximum reaction velocity for asparagine V_{max} was greater than the maximum reaction velocity for glutamine by a factor of about 15. They therefore suggested that the qualitative basis for the paradoxical difference in the two experiments is that in the second experiment the inhibitor substance asparagine disappears rapidly during the course of the experiment. It was the suggestion of Balis that we investigate theoretically the appropriate modification of eq 1 for such circumstances that motivated the present work.

Time-Dependent Michaelis–Menten Theory

Let S and I denote substrate and inhibitor substances, respectively, which react with an enzyme E at the same enzymatic site. It is assumed that substrate and enzyme react to form an enzyme–substrate complex C_1, which can in turn dissociate to form either the enzyme and substrate, or the enzyme and some products P_1. Similarly, the inhibitor and enzyme react to form an inhibitor–enzyme complex C_2, which dissociates to form either the enzyme and inhibitor or the enzyme and some products P_2. The reverse reactions of products and enzyme to form complexes is assumed to be negligible. These reactions are represented schematically as follows.

$$S + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C_1 \xrightarrow{k_{+3}} E + P_1$$

$$I + E \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} C_2 \xrightarrow{k_{+4}} E + P_2$$

As is well known, the differential equations of the system present a mathematical problem in singular perturba-

(1) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).
(2) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).
(3) M. Dixon and E. C. Webb, "Enzymes," Academic Press, Inc., New York, N. Y., 1964.
(4) G. E. Briggs and J. B. S. Haldane, *Biochem. J.*, **19**, 338 (1925).
(5) H. K. Miller and M. E. Balis, *Biochem. Pharmacol.*, **18**, 2225 (1969).

tion theory.[6] These equations may be solved in a formal way by means of an asymptotic expansion in the small parameter e_0/s_0, where e_0 is the enzyme concentration. (Fortunately, this parameter is always made small in practical investigations of enzyme reactions. For example, in the Miller–Balis experiments previously cited, if we assume that the molecular weight of the enzyme is ~150,000, then $e_0/s_0 \sim 10^{-6}$.) The first term of this expansion, whose derivation is found in the Appendix, is the solution to zero order in e_0/s_0. This solution yields the following expressions for the substrate concentrations $s(t)$ and the inhibitor concentration $i(t)$ as functions of the time t.

$$V_{max}^{s} t = s_0 - s(t) + \frac{V_{max}^{s}}{V_{max}^{i}} i_0 \left\{ 1 - \left[\frac{s(t)}{s_0} \right]^{\delta} \right\} - K_M^{s} \ln \left[\frac{s(t)}{s_0} \right] \quad (2)$$

and

$$i(t) = i_0 \left[\frac{s(t)}{s_0} \right]^{\delta} \quad (3)$$

where

$$\delta = \frac{V_{max}^{i} K_M^{s}}{V_{max}^{s} K_M^{i}} \quad (4)$$

and

$$K_M^{s} = \frac{k_{-1} + k_{+3}}{k_{+1}} \qquad K_M^{i} = \frac{k_{-2} + k_{+4}}{k_{+2}}$$
$$V_{max}^{s} = k_{+3} e_0 \qquad V_{max}^{i} = k_{+4} e_0 \quad (5)$$

Differentiating eq 2 and 3 yields

$$\frac{1}{v^{s}(t)} = \left| \frac{ds(t)}{dt} \right|^{-1} = \frac{1}{V_{max}^{s}} \left[1 + \frac{K_M^{s}}{s(t)} \left(1 + \frac{i(t)}{K_M^{i}} \right) \right] \quad (6)$$

$$\frac{1}{v^{i}(t)} = \left| \frac{di(t)}{dt} \right|^{-1} = \frac{s(t)}{\delta i(t) v^{s}(t)} \quad (7)$$

The assumptions underlying this approximate solution are essentially equivalent to the hypothesis that the system is in a pseudosteady state.[4] The pseudosteady-state hypothesis may be understood physically as follows. Imagine an experiment in which a constant supply of substrate and inhibitor is provided at rates J_s and J_I, respectively, and the reaction products P_1 and P_2 are continuously removed. Then a steady state will be established with the values $\bar{s}$ and $\bar{i}$ for the substrate and inhibitor concentrations, respectively, given by the expressions

$$\bar{s} = \frac{K_M^{s}}{V_{max}^{s}} J_s \left[1 - \frac{J_s}{V_{max}^{s}} - \frac{J_I}{V_{max}^{I}} \right] \quad (8)$$

$$\bar{i} = \frac{\bar{s} J_I}{\delta J_s} \quad (9)$$

These expressions are identical with eq 6 and 7 providing $v^{s}(t)$, $v^{i}(t)$, $s(t)$, and $i(t)$ there are replaced by J_s, J_I, $\bar{s}$, and $\bar{i}$, respectively. Therefore, we may characterize the pseudosteady-state hypothesis as the assumption that, at any instant of time, the relations between the concentrations and the reaction velocities are the same as those that would obtain in the steady-state experimental arrangement envisaged above. It is interesting to note from eq 8 that a steady state is possible only if the quantity

$$\left(\frac{J_s}{V_{max}^{s}} + \frac{J_I}{V_{max}^{I}} \right) < 1$$

It is easy to show that the time interval necessary for the pseudosteady state to be established is of the order of $[k_{+1}(s_0 + K_M^{s})]^{-1}$ and $[k_{+2}(i_0 + K_M^{i})]^{-1}$ for substrate and inhibitor, respectively (see Appendix). For most enzyme–substrate reactions this interval is on the order of a fraction of a second. The times at which v^{s} are usually measured are on the order of minutes, so that the requirements of the theory are readily satisfied in the foregoing respect.

Under usual experimental conditions, the times of measurement are such that $s(t) \approx s_0$. If, in addition, $i(t) \approx i_0$, then eq 6 yields essentially the same result as eq 1. However, as can be seen from (3), $s(t) \approx s_0$ does not assure that $i(t) \approx i_0$ when δ is large. In such a case, the full time dependence of eq 2 and 3 is necessary for interpreting the experiments.

In order to compare eq 2 and 3 with experiment in the general case, we note that in practice velocities are often measured by observing $s(t)$ for small times and assuming that a linear expansion of $s(t)$ about the origin is valid. In mathematical terms, a common experimental definition of the reaction velocity is

$$\bar{v}^{s}(t) = \frac{s_0 - s(t)}{t} \quad (10)$$

With this definition and eq 2 and 3 for $s(t)$ and $i(t)$, an expression for $[\bar{v}^{s}(t)]^{-1}$ which generalizes eq 1 is readily found. The result is

$$\frac{1}{\bar{v}^{s}(t)} = \frac{1}{V_{max}^{s}} \left[1 + \frac{K_M^{s}}{s_0} \{ 1 + \epsilon(t) \} \right] \times \left[1 - \frac{i_0 - i(t)}{V_{max}^{i} t} \right]^{-1} \quad (11)$$

where

$$\epsilon(t) = \frac{s_0}{s(t) - s_0} \ln \left[\frac{s(t)}{s_0} \right] - 1 \quad (12)$$

Equation 11 is simplified if we assume, as is usually the case, that the fractional disappearance of $s(t)$ is small during the course of the experiment, $[s_0 - s(t)]/s_0 \ll 1$. If the logarithm term in eq 12 is expanded in powers of $[s_0 - s(t)]/s_0$, then it follows that $\epsilon(t)$ is of the order of the fractional disappearance of $s(t)$, and is small compared to unity. Therefore it may sensibly be neglected in eq 11 for comparison with the results of typical experiments.

Equation 11 together with eq 3 and 12 constitute a convenient time-dependent generalization of Michaelis–Menten theory. Equation 11 reduces to eq 1 in the limit $t \to 0$. If measurements of $\bar{v}^{s}(t)$ are to be made minutes after the start of the reaction, it is necessary to consider whether the time-dependent formalism is needed or not. The decision hinges on the value of δ that appears in eq 3. Thus, if $\delta \lesssim 1$, then during the course of an experiment in which $s(t)$ stays close to s_0, $i(t)$ will not differ very greatly from i_0, so

(6) F. G. Heineken, H. M. Tsuchiya, and R. Aris, *Math. Biosci.*, **1**, 95 (1967).

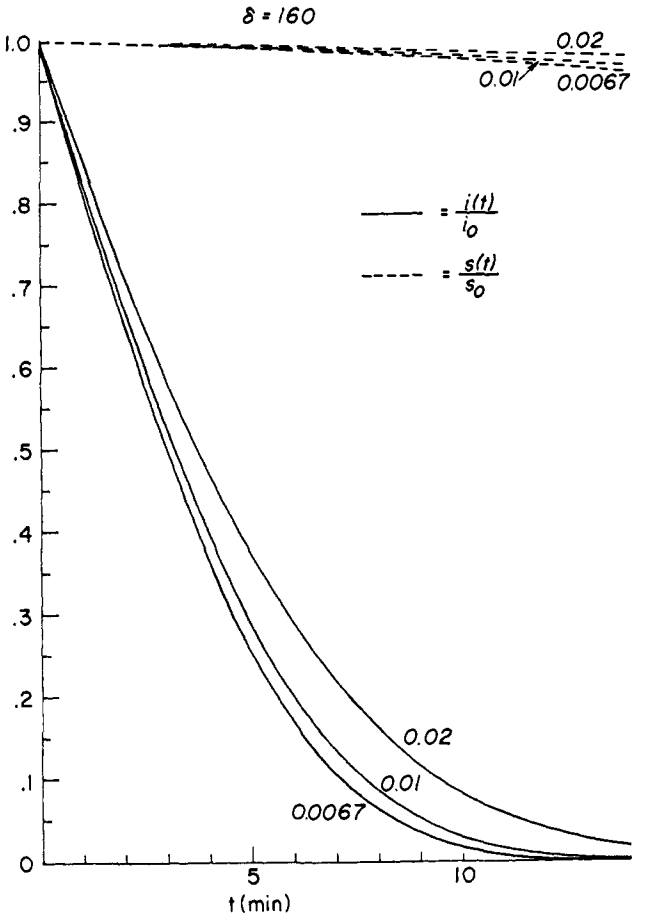

Figure 1. The fractional amounts of substrate $s(t)/s_0$ and inhibitor $i(t)/i_0$ are shown as functions of the time. The curves are based on eq 2 and 3. The parameter values are those of the Miller–Balis experiments: substrate = glutamine, inhibitor = asparagine, $V_{max}^{s} = 0.80 \times 10^{-4}$ mol/(l. min IU), $K_M^{s} = 1.3 \times 10^{-2}$ mol/l., $V_{max}^{i} = 1.3 \times 10^{-3}$ mol/(l. min IU), $K_M^{i} = 1.3 \times 10^{-3}$ mol/l., $\delta = 160$, $i_0 = 5 \times 10^{-3}$ mol/l., $s_0 = 0.02$, 0.01, and 0.00667 mol/l.

that the expression $[i_0 - i(t)]/t$, which appears in the last bracket of eq 11, is approximately equal to $di(t)/dt$ at $t = 0$. In such a case, the use of eq 11 is hardly different from the use of eq 1. However, if δ is sufficiently large, then it is necessary to utilize eq 11 or eq 6, depending on the method of measuring reaction velocity. There are two simplifications in eq 11 that may be made under appropriate conditions. One is the neglect of $\epsilon(t)$ as discussed above. The other results when the time of measurement is sufficiently large so that $i(t)$ is negligible compared with i_0. Then eq 11 assumes the following simple form (assuming $\epsilon(t)$ is also negligible).

$$\frac{1}{\bar{v}^{s}(t)} = \frac{1}{V_{max}^{s}}\left[1 + \frac{K_M^{s}}{s_0}\right]\left[1 - \frac{i_0}{V_{max}^{i} t}\right]^{-1} \qquad (13)$$

Equation 13 predicts that Lineweaver–Burk plots of $[\bar{v}^{s}(t)]^{-1}$ *vs.* s_0^{-1} for different values of i_0, with all measurements made at a fixed time t relative to the onset of the reaction, will yield a family of straight lines with a common intercept at the point $[\bar{v}^{s}(t)]^{-1} = 0$, $s_0^{-1} = -(K_M^{s})^{-1}$. This family includes the straight line of the ordinary Michaelis–Menten theory for the enzyme–substrate system with inhibitor absent. We emphasize that for this equation to be applicable, t must be sufficiently large so that $i(t) \ll i_0$, but not so large as to violate the requirement $[s_0 - s(t)]/s_0 \ll 1$. In other words, it is applicable when most of the inhibitor but very little of the substrate has disappeared.

Discussion

Miller and Balis[5] found that when asparagine was used as a substrate for the enzyme *E.coli* L-asparagine amidohydrolase, the values of the Michaelis–Menten parameters were $K_M^{A} = 1.3 \times 10^{-3}$ mol/l., $V_{max}^{A} = 1.3 \times 10^{-3}$ mol $l.^{-1}$ min^{-1}/IU of enzyme. When glutamine was used as a substrate, the values obtained for these parameters were $K_M^{G} = 1.3 \times 10^{-2}$ mol/l., $V_{max}^{G} = 0.80 \times 10^{-4}$ mol $l.^{-1}$ min^{-1}/IU of enzyme. These values of the Michaelis–Menten parameters determine the value of δ as $^1/_{160}$ if asparagine is used as a substrate and glutamine is used as an inhibitor. In this case, we expect ordinary Michaelis–Menten theory to be applicable, and this is indeed what Miller and Balis found. In the reverse situation in which glutamine is used as a substrate and asparagine is used as an inhibitor, the value of δ is 160. In such a case, we expect ordinary Michaelis–Menten theory not to apply. Rather, it is necessary to apply the time-dependent theory. Figures 1 and 2 are based on eq 2, 3, and 11 for this case, namely, $\delta = 160$.

In Figure 1 we have plotted the functions $s(t)/s_0$ and $i(t)/i_0$ for the parameter values of the Miller–Balis experiments with glutamine as substrate and asparagine as inhibitor. The rapid decrease with time of the inhibitor substance is readily apparent in this figure, even though the fractional decrease of substrate remains small.

In Figure 2 are shown several Lineweaver–Burk plots based on eq 11 for the same experimental values as in Figure 1 and several values of the time of measurement. The value of i_0 was chosen to be 5×10^{-3} mol/l. as in one of the Miller–Balis experiments. The great sensitivity of the curve to the time of measurement is noteworthy. Also shown as dotted lines are two curves based on the simplified eq 13 with $t = 10$ and 20 min. These dotted curves are not very different from the curves that would be obtained using eq 11, except for small values of s_0^{-1}. At these large initial values of substrate concentration, all the curves based on eq 11 converge to a common intercept, not unexpectedly. For a given value of enzyme concentration e_0, the larger the initial value of substrate concentration, the longer it takes for a given fraction of substrate concentration or inhibitor concentration to disappear, and the more eq 11 resembles its zero-time limit, eq 1.

In Figure 3 is shown the fractional amount of inhibitor concentration $i(t)/i_0$ as a function of the fractional disappearance of substrate $(1 - s(t)/s_0)$ at time t, for various values of δ. The curves show clearly how the disappearance of $i(t)/i_0$ increases rapidly with δ for a given amount of substrate disappearance.

The mathematical explanation of the Miller–Balis phenomenon indicates that a similar breakdown in the applicability of ordinary Michaelis–Menten theory will occur in the usual investigation of the kinetics of enzyme–substrate–inhibitor systems of a fully competitive type whenever the parameter δ is large compared to unity. Thus, the enzyme xanthine oxidase oxidizes the antimetabolito-6-mercaptopurine to produce 6-thiouric acid. It was found[7] that the parametric values of

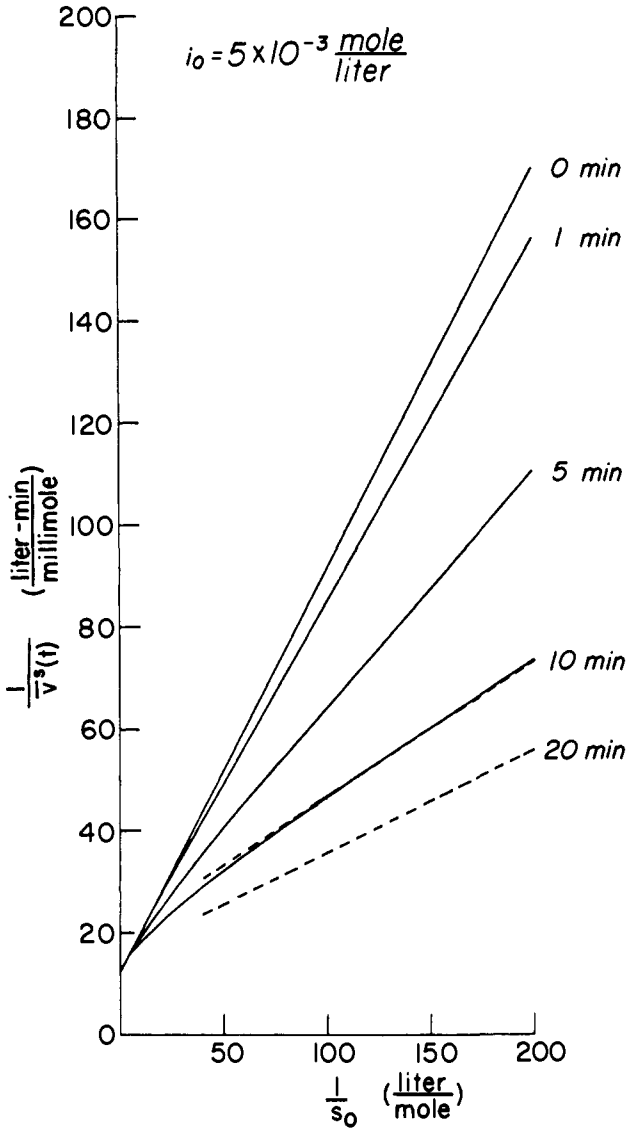

Figure 2. The reciprocal mean reaction velocity $[\bar{v}^s(t)]^{-1}$ is shown as a function of the reciprocal initial substrate concentration s_0^{-1} for different values of the time. The solid curves are based on eq 11 for the same experimental values of the parameters as those quoted for Figure 1. The dashed curves are based on the approximate form of eq 11 (eq 13), for t = 10, 20 min. This form is valid for t sufficiently large so that $i(t) \ll i_0$, while $[s_0 - s(t)]/s_0 \ll 1$.

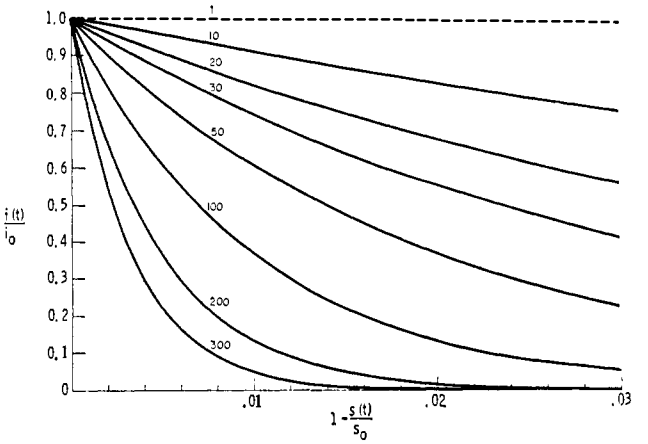

Figure 3. The fractional amount of inhibitor concentration $i(t)/i_0$ is shown as a function of the fractional disappearance of substrate concentration $(1 - s(t)/s_0)$ at a given time, for different values of δ. The figures are based on eq 3.

this reaction are $K_M = 17.5 \times 10^{-6}$ M and $V_{max} = 0.083$ μmol hr^{-1}/0.4 unit enzyme. For the catalytic oxidation of xanthine to uric acid by xanthine oxidase, the parametric values of the reaction are $K_M = 5.4 \times 10^{-6}$ M, and $V_{max} = 9.40$ μmol hr^{-1}/0.4 unit of enzyme. The competitive inhibition of xanthine oxidation by 6-mercaptopurine in the presence of xanthine oxidase was observed to obey ordinary Michaelis–Menten theory for reactions of a fully competitive type.[7] We readily calculate $\delta = 0.0027$ for this reaction, so that the agreement with ordinary theory is expected. At the same time, we note that in the reverse situation in which xanthine inhibits the oxidation of 6-mercaptopurine, $\delta = 370$. Such an experiment has not been performed to the best of our knowledge. When it is, we predict that ordinary Michaelis–Menten theory will not be applicable and that the time-dependent formalism will be applicable. With regard to the observations, it is recommended that the time at which observations are made be carefully recorded. Other substrates of xanthine oxidase, such as 2,6-diaminopurine, are "slow" when compared with xanthine,[8] so that utilizing xanthine as an inhibitor with them would also result in a large value for δ.

Another example of a fully competitive reaction in which δ is rather small is the catalysis by the enzyme adenosine deaminase of the dechlorination of 6-chloropurine ribonucleoside to yield inosine and chloride ions, in the presence of adenosine as inhibitor. The reaction parameters for adenosine diaminase with adenosine as substrate are $V_{max} = 400$ μmol min^{-1}/mg of enzyme, $K_M = 8.3 \times 10^{-5}$ M; with 6-chloropurine ribonucleoside as substrate, $V_{max} = 100$ μmol min^{-1}/mg of enzyme, $K_M = 6.4 \times 10^{-4}$ M. With adenosine as a substrate and 6-chloropurine ribonucleoside as inhibitor, $\delta = 0.032$, so that the time-independent theory should apply as is observed.[9] The reverse situation in which 6-chloropurine ribonucleoside is utilized in the presence of adenosine diaminase with adenosine as an inhibitor has a value of $\delta = 31$ associated with it. This value is not as large as in the previously cited example, although we would still expect some deviations from the classical theory if the experiment is performed.

Conclusions

When two substances react with an enzyme in a fully competitive manner, the theoretical expressions for the temporal disappearance of each of them assume a symmetric form. In kinetic studies of such a system, the substance whose reaction velocity is measured is called the substrate and the other substance is called the inhibitor. Thus, there is a dual choice as to which substance plays the role of substrate and which substance plays the role of inhibitor. The parameter δ is a measure of the relative "fastness" or "slowness" of the two substances. When δ is less than unity, the "slow" substance is being utilized as inhibitor. When δ is greater than unity, the "fast" substance is being utilized as inhibitor. If δ is less than or comparable with unity, then ordinary Michaelis–Menten theory may be

(7) H. R. Silberman and J. B. Wyngaarden, *Biochim. Biophys. Acta*, 47, 178 (1961).

(8) J. B. Wyngaarden, *J. Biol. Chem.*, 224, 453 (1957).

(9) J. G. Cory and R. J. Suhadolnik, *Biochemistry* 4, 1733 (1965).

expected to be sufficient to understand the kinetic aspects of the study. If δ is large compared to unity, then the time-dependent theory presented herein is needed for a proper interpretation of the results.

Acknowledgment. We are grateful to M. Earl Balis for suggesting this problem to us and for many helpful discussions of the work while it was in progress. This work was supported in part by NCI Grant No. CA-08748 at Sloan-Kettering Institute and by USAFOSR Grant No. 68-1416 at Belfer Graduate School of Science, Yeshiva University.

Appendix. Zero-Order Solution of the Kinetic Equations

Let e, s, i, c_1, and c_2 represent the concentrations of the quantities enzyme, substrate, inhibitor, substrate–enzyme complex, and inhibitor–enzyme complex, respectively, at any time. Let the subscript zero attached to a symbol denote its value at the initial time $t = 0$. Introduce the following dimensionless variables and parameters.

$$\begin{gathered} t' = k_1 e_0 t \qquad s' = s/s_0 \qquad i' = i/i_0 \\ c_1' = c_1/e_0 \qquad c_2' = c_2/e_0 \qquad \alpha = e_0/s_0 \\ \beta = i_0/s_0 \qquad \gamma = k_{+2}/k_{+1} \\ K_1 = (k_{-1} + k_{+3})/k_{+1}s_0 \qquad K_2 = (k_{-2} + k_{+4})/k_{+2}i_0 \\ U_1 = k_{+3}/k_{+1}s_0 \qquad U_2 = k_{+4}/k_{+2}i_0 \end{gathered} \tag{1}$$

If we now drop the primes, the kinetic equations assume the following form.

$$\frac{ds(t)}{dt} = -s + (s + K_1 - U_1)c_1 + sc_2 \tag{2}$$

$$\alpha\frac{dc_1(t)}{dt} = s - (s + K_1)c_1 - sc_2 \tag{3}$$

$$\frac{di(t)}{dt} = \gamma[-i + (i + K_2 - U_2)c_2 + ic_1] \tag{4}$$

$$\alpha\frac{dc_2(t)}{dt} = \beta\gamma[i - (i + K_2)c_2 - ic_1] \tag{5}$$

These equations are to be solved subject to the initial conditions

$$\begin{gathered} s(0) = i(0) = 1 \\ c_1(0) = c_2(0) = 0 \end{gathered} \tag{6}$$

The enzyme concentration e satisfies the relation

$$c_1 + c_2 + e = 1 \tag{7}$$

Equations 2–5 are nonlinear and not susceptible to solution in closed form. We shall assume that α is very small compared to unity. This condition is satisfied in the usual experimental situation. In addition we assume that the parameters β and γ are 0(1). This suggests that a solution to eq 2–5 be sought by means of an expansion in α. However, the small parameter α multiplies the highest derivative term in two of the equations and therefore the problem presented is classified in the theory of singular perturbations. According to this theory, the solution to eq 2–5 in terms of an expansion in α is asymptotic; *i.e.*, it tends to the true solution as $\alpha \to 0$, although the series solution probably diverges. The solution we shall present parallels the asymptotic solution presented in ref 6 for an enzyme–substrate system. The reader is referred there for a fuller discussion of the solution and the method for obtaining it.

We proceed formally by seeking a solution to eq 2–5 as a power series in α, *e.g.*

$$(s,i,c_1,c_2) = \sum_{n=0}^{\infty} (s^{(n)},i^{(n)},c_1^{(n)},c_2^{(n)})\alpha^n \tag{8}$$

If we substitute (8) into (2)–(5) and equate to zero the coefficients of like powers in α, we find that the terms which are zero order in α satisfy the equations

$$\frac{ds^{(0)}}{dt} = -s^{(0)} + (s^{(0)} + K_1 - U_1)c_1^{(0)} + s^{(0)}c_2^{(0)} \tag{9}$$

$$\frac{di^{(0)}}{dt} = \gamma[-i^{(0)} + (i^{(0)} + K_2 - U_2)c_2^{(0)} + i^{(0)}c_1^{(0)}] \tag{10}$$

$$0 = s^{(0)} - (s^{(0)} + K_1)c_1^{(0)} - s^{(0)}c_2^{(0)} \tag{11}$$

$$0 = i^{(0)} - (i^{(0)} + K_2)c_2^{(0)} - i^{(0)}c_1^{(0)} \tag{12}$$

Equations 11 and 12 are algebraic equations which are readily solved for $c_1^{(0)}$ and $c_2^{(0)}$. The resulting expressions may be substituted into eq 8 and 9. The latter are then also readily solvable. The result is

$$c_1^{(0)}(t) = \frac{K_2 s^{(0)}(t)}{K_1 i^{(0)}(t) + K_2 s^{(0)}(t) + K_1 K_2} \tag{13}$$

$$c_2^{(0)}(t) = \frac{K_1 i^{(0)}(t)}{K_1 i^{(0)}(t) + K_2 s^{(0)}(t) + K_1 K_2} \tag{14}$$

$$s^{(0)}(t) + \frac{U_1}{U_2}[s^{(0)}(t)]^{\gamma U_2 K_1/U_1 K_2} + K_1 \ln s^{(0)}(t) + C = -U_1 t \tag{15}$$

$$i^{(0)}(t) = [s^{(0)}(t)]^{\gamma U_2 K_1/U_1 K_2} \tag{16}$$

where C is a constant to be determined. We do not impose on the solution the requirement that it satisfy the initial conditions because the assumed expansion is not valid in the neighborhood of $t = 0$. In fact, it can be seen that $c_1^{(0)}(t)$ and $c_2^{(0)}(t)$ cannot satisfy the initial conditions. Rather, we recognize that the expansion (8) constitutes an "outer" expansion, valid only for t "sufficiently large." We must find another expansion, the "inner" expansion, which is valid for t small.

To this end we introduce the new time scale τ and new variables defined by

$$\begin{gathered} \tau = t/\alpha \qquad S(\tau) = s(\alpha\tau,\alpha) \qquad I(\tau) = i(\alpha\tau,\alpha) \\ C_1(\tau) = c_1(\alpha\tau,\alpha) \qquad C_2(\tau) = c_2(\alpha\tau,\alpha) \end{gathered} \tag{17}$$

In terms of these variables, eq 2–5 become

$$\frac{dS}{d\tau} = \alpha[-S + (S + K_1 - U_1)C_1 + SC_2] \tag{18}$$

$$\frac{dI}{d\tau} = \alpha\gamma[-I + (I + K_2 - U_2)C_2 + IC_1] \tag{19}$$

$$\frac{dC_1}{d\tau} = S - (S + K_1)C_1 - SC_2 \tag{20}$$

$$\frac{dC_2}{d\tau} = \beta\gamma[I - (I + K_2)C_2 - IC_1] \tag{21}$$

As before, we assume a solution to these equations of the form

$$(S,I,C_1,C_2) = \sum_{n=0}^{\infty}(S^{(n)},I^{(n)},C_1^{(n)},C_2^{(n)})\alpha^n \quad (22)$$

Substituting eq 22 into (18)–(21) and equating coefficients of α^0, we obtain

$$\frac{dS^{(0)}}{d\tau} = 0 \quad (23)$$

$$\frac{dI^{(0)}}{d\tau} = 0 \quad (24)$$

$$\frac{dC_1^{(0)}}{d\tau} = S^{(0)} - (S^{(0)} + K_1)C_1^{(0)} - S^{(0)}C_2^{(0)} \quad (25)$$

$$\frac{dC_2^{(0)}}{d\tau} = \beta\gamma[I^{(0)} - (I^{(0)} + K_2)C_2^{(0)} - I^{(0)}C_1^{(0)}] \quad (26)$$

The solution to these equations which does satisfy the initial conditions is

$$S^{(0)} = 1 \quad (27)$$

$$I^{(0)} = 1 \quad (28)$$

$$C_1^{(0)} = \frac{K_2}{K_1 + K_2 + K_1K_2} - Ae^{\lambda_+\tau} - Be^{\lambda_-\tau} \quad (29)$$

$$C_2^{(0)} = \frac{K_1}{K_1 + K_2 + K_1K_2} + (1 + K_1 + \lambda_+)Ae^{\lambda_+\tau} + (1 + K_1 + \lambda_-)Be^{\lambda_-\tau} \quad (30)$$

where

$$A = -[\{1 + K_1 - \beta\gamma(1 + K_2)\}^2 + 4\beta\gamma]^{-1/2}\left[1 + \frac{K_2\lambda_-}{K_1 + K_2 + K_1K_2}\right] \quad (31)$$

$$B = [\{1 + K_1 - \beta\gamma(1 + K_2)\}^2 + 4\beta\gamma]^{-1/2}\left[1 + \frac{K_2\lambda_+}{K_1 + K_2 + K_1K_2}\right] \quad (32)$$

$$\lambda_\pm = {}^1/_2\{1 + K_1 + \beta\gamma(1 + K_2) \pm [\{1 + K_1 - \beta\gamma(1 + K_2)\}^2 + 4\beta\gamma]^{1/2}\} \quad (33)$$

It may be observed parenthetically that if $\beta\gamma \ll 1$, $\lambda_+ \approx -(1 + K_1)$ and $\lambda_- \approx -\beta\gamma(1 + K_2)$. The inner expansion and the outer expansion must be asymptotically equal in their common domain of validity. We assume that there is such a common domain for some intermediate values of t. Therefore, if the inner expansion is expanded for large values of τ and the outer expansion is expanded for small values of t, the resulting expansions must be asymptotically equal. Upon doing this, we find that the constant C in eq 15 is determined as

$$C = -1 - U_1/\gamma U_2 \quad (34)$$

The complete asymptotic solution to a given order in α may be constructed from the inner and outer solutions (see ref 6 for details). We denote this solution to zero order in α by $\bar{s}^{(0)}$, $\bar{\imath}^{(0)}$, $\bar{c}_1^{(0)}$, $\bar{c}_2^{(0)}$. The result is

$$\bar{s}^{(0)}(t) = s^{(0)}(t) = -U_1t + 1 + U_1/\gamma U_2 - (U_1/\gamma U_2)[s^{(0)}(t)]^{\gamma U_2K_1/U_1K_2} - K_1 \ln s^{(0)}(t) \quad (35)$$

$$\bar{\imath}^{(0)}(t) = i^{(0)}(t) = [s^{(0)}(t)]^{\gamma U_2K_1/U_1K_2} \quad (36)$$

$$\bar{c}_1^{(0)}(t) = \frac{K_2s^{(0)}(t)}{K_1i^{(0)}(t) + K_2s^{(0)}(t) + K_1K_2} - Ae^{\lambda_+t/\alpha} - Be^{\lambda_-t/\alpha} \quad (37)$$

$$\bar{c}_2^{(0)}(t) = \frac{K_1i^{(0)}(t)}{K_1i^{(0)}(t) + K_2s^{(0)}(t) + K_1K_2} + (1 + K_1 + \lambda_+)Ae^{\lambda_+t/\alpha} + (1 + K_1 + \lambda_-)Be^{\lambda_-t/\alpha} \quad (38)$$

where A, B, and $\lambda_\pm$ are given by eq 31–33. If the expressions for $\bar{s}^{(0)}(t)$ and $\bar{\imath}^{(0)}(t)$ above are expressed in terms of dimensional variables by the use of (1), they become eq 2 and 3, respectively, of the text.

By comparing (37) and (38) with (13) and (14), respectively, we see that the time needed for the pseudo-steady state to be established is just the time necessary for the exponential terms in (37) and (38) to be negligible compared with the nonexponential terms.