Kinetics of Enzyme Reactions with Competing Alternative Substrates

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SUMMARY

When two alternative substrates are present simultaneously in an enzyme reaction, and one is a better substrate (higher V_{max}) than the other, the combined rate of the two reactions may be greater than, equal to, or less than the rate observed with the better substrate alone. At some concentration of the better substrate, the poorer substrate will appear to have no effect. On a plot of the combined velocity against the concentration of the more effective substrate, the family of curves obtained, each representing a different level of the poorer substrate, will have a common point of intersection. This phenomenon can be exploited effectively to determine whether or not a single enzyme catalyzes two similar reactions, or to determine whether or not two drugs act on the same receptor.

In examining the applicability of these conclusions to more complex enzyme mechanisms, six variations of two major bi-bi (two-substrate, two-product) mechanisms have been studied. It was found that in every case, the formal theory based on the simple Michaelis-Menten mechanism holds, provided that apparent Michaelis constants are used.

INTRODUCTION

The kinetics of a system in which two alternative substrates compete for a single enzyme has been the subject of investigation by many authors since Haldane introduced the case in 1930. The system was originally studied to accommodate the kinetics of a hydrolytic enzyme acting on D and L forms of the substrate (1). Thorn (2) also applied it to determine the ratio of the Michaelis constants of an enzyme for two isotopic forms of the same substrate. Foster and Niemann (3) recognized that the system can be used to determine whether two or more substrates react at the same catalytically active sites of an enzyme molecule. These earlier studies have been reviewed briefly by Segal (4). The term "competitive alternative substrate" implies that two substrates compete for the same catalytic site, and that one can serve as substrate in the absence of the other.

In formulating a molecular basis for the interaction of drugs with a receptor, Ariëns, van Rossum, and Simonis (5, 6) developed an exactly analogous model that explains a certain type of dualism in drug antagonism, and used the term "competitive dualism." They also pointed out that on a plot of combined velocity against concentration of a substrate,[1] the curves share a common intersecting point when the concentration of the substrate with the higher maximal velocity is varied while that of the other substrate is held constant at various levels. This phenomenon is particu-

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[1] In order to maintain uniformity in this paper, enzymological terms are used instead of the corresponding pharmacological ones. Thus, as far as the formal theory goes, the following may be used interchangeably: enzyme and receptor, substrate and drug, alternative substrate and agonist, inhibitor and antagonist, velocity and effect, maximal velocity and efficacy, Michaelis constant and affinity (reciprocal).

larly important as evidence supporting the view that two different reactions can be catalyzed by a single enzyme, since if two different enzymes were involved, the lines would not all intersect at a single point. In fact, the above argument has been successfully employed by several colleagues of the present author: Cohen and Parks (7) showed that 8-aza-GTP and GTP are acted upon by a single enzyme, succinyl adenylate synthetase; Mourad and Parks (8) showed that the purified human erythrocytic nucleoside diphosphokinase is a nonspecific single enzyme rather than a mixture of specific enzymes; Sladek and Mannering[2] investigated the possibility that certain drugs are metabolized by a single enzyme; and recently Henderson *et al.* (9) demonstrated that one enzyme catalyzes the phosphoribosyltransferase reactions with both hypoxanthine and guanine as substrates. Most reactions studied by these investigators were multisubstrate reactions, and therefore the simple equation derived for the single-substrate model was not applicable in the strict sense. However, these authors assumed, without rigorous proof, that the equation is valid if the apparent kinetic parameters are used.

The present paper gives a more explicit analysis of the simplest model, examines the validity of extending the conclusions to two-substrate, two-product (bi-bi) mechanisms, and attempts to identify and differentiate other mechanisms.

THEORY

Single-Enzyme Models

If one measures the rate of disappearance of only one of two alternative substrates, the unmeasured substrate has the effect of a classical competitive inhibitor. In some enzyme reactions one measures the combined rate rather than the individual rates, because the products derived from the alternative substrates are identical or indistinguishable with available assay methods. Likewise, when the formal theory of enzyme kinetics is applied to drug antagonism, the response due to one drug would be indistinguishable from that due to the other drug administered simultaneously. In the present paper, attention is placed entirely on the situation in which the combined rate is measured in the presence of two alternative substrates. The nomenclature of the enzyme mechanisms and the definition of kinetic parameters are adapted from those of Cleland (10).

Uni-uni mechanism with an alternative substrate. The simplest case, in which an alternative aubstrate, S', competes with the substrate, S, in a one-substrate, one-product reaction (uni-uni mechanism), may be represented by Model I:

$$E + S \rightleftharpoons ES \rightarrow E + P \qquad \text{(Ia)}$$
$$E + S' \rightleftharpoons ES' \rightarrow E + P' \qquad \text{(Ib)}$$

Let the Michaelis constants for S and S' be K and K', respectively, and the maximal velocities, V and V', respectively. Then the combined rate, v, becomes

$$v = \frac{d(P)}{dt} + \frac{d(P')}{dt} = \frac{\frac{V(S)}{K} + \frac{V'(S')}{K'}}{1 + \frac{(S)}{K} + \frac{(S')}{K'}} \qquad (1)$$

Although Eq. 1 or its equivalent has been derived and analyzed with varying degrees of thoroughness (1, 2, 5–7, 11, 12), it is worthwhile to examine this equation again in view of its potential usefulness. It can be shown that Eq. 1 represents a family of curves that intersect at a common point when the concentration of one substrate is varied while that of the other substrate is held constant, and that the coordinates of the intersecting point on the plot of v against (S) are $KV'/(V - V')$ and V'. Therefore the point of intersection can be seen experimentally only if $V > V'$. On the other hand, if $V < V'$ when $(S') = K'V/(V' - V)$, the velocity becomes independent of (S). If $V' = 0$, or if the measurement of velocity is specific for P or S, the effect of S' is, of course, classical competitive inhibition. A corollary statement is that, if two alternative substrates are present simultaneously, and if one is a better substrate (higher V_{max}) than the other, the

[2] N. E. Sladek and G. J. Mannering, University of Minnesota, personal communications.

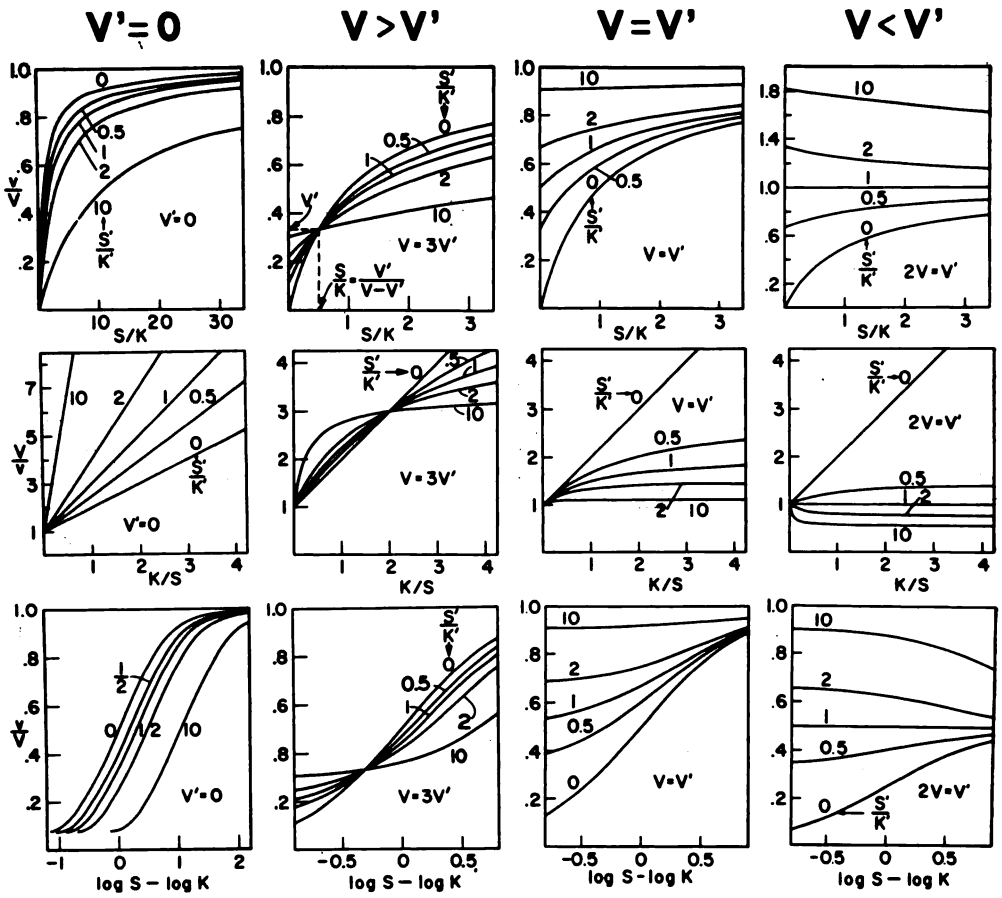


FIG. 1. *Curves represented by*

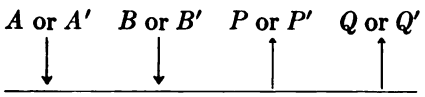
$$v = \frac{\frac{V(S)}{K} + \frac{V'(S')}{K'}}{1 + \frac{(S)}{K} + \frac{(S')}{K'}}$$

The variables are normalized; i.e., v is expressed as a fraction of V, and S as a multiple of K. Thus, the four graphs in the top row correspond to the plot of v against S; in the middle row, to $1/v$ against $1/(S)$; and in the bottom row, to v against log (S). Graphs are drawn faithfully according to the calculations, using the arbitrarily chosen values of V' and $(S')/K'$ indicated.

combined rate of the two reactions may be greater than, equal to, or less than the rate with the better substrate alone. Furthermore, at some concentration of the better substrate, the poorer substrate will appear to have no effect on the rate of reaction. The case of competitive inhibition is extreme. The types of curves on various plots represented by Eq. 1 are shown in Fig. 1. Note that the scales for v and (S) are normalized, i.e., expressed as multiples or fractions of V and K, respectively.

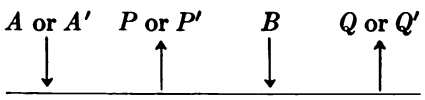
Bi-bi mechanisms. In order to determine whether Eq. 1 is applicable to more complex situations, various bi-bi mechanisms were examined. The steady-state equations were derived by the method of King and Altman (13) for four possible variations of the ordered bi-bi mechanism and two possible variations of the ping-pong bi-bi mechanism caused by the simultaneous presence of an alternative substrate (Model II).

Ordered mechanisms:



(II*a–d*)

Ping-pong mechanisms:



(II*e–f*)

Table 1 lists the schematic representations of each variation and the definitions of the kinetic parameters in the manner suggested by Cleland (10); Table 2 defines the apparent kinetic parameters.

When the rate equations are expressed in terms of apparent kinetic parameters, the equations for all six mechanisms can be written as

$$v = \frac{\frac{V_{app}(S)}{K_{app}} + \frac{V'_{app}(S')}{K'_{app}}}{1 + \frac{(S)}{K_{app}} + \frac{(S')}{K'_{app}}} \qquad (2)$$

Only the definitions of kinetic parameters for each mechanism differ, as listed in Table 1.

Although a separate proof may be required for each reaction mechanism, Eq. 2 appears to be generally applicable to those cases in which a Lineweaver-Burk plot yields a straight line when one substrate is varied in the absence of the alternative substrate. However, it seems appropriate to mention at this point that if the double-reciprocal plots are not straight lines, the predictions based on Eq. 1 or Eq. 2 may

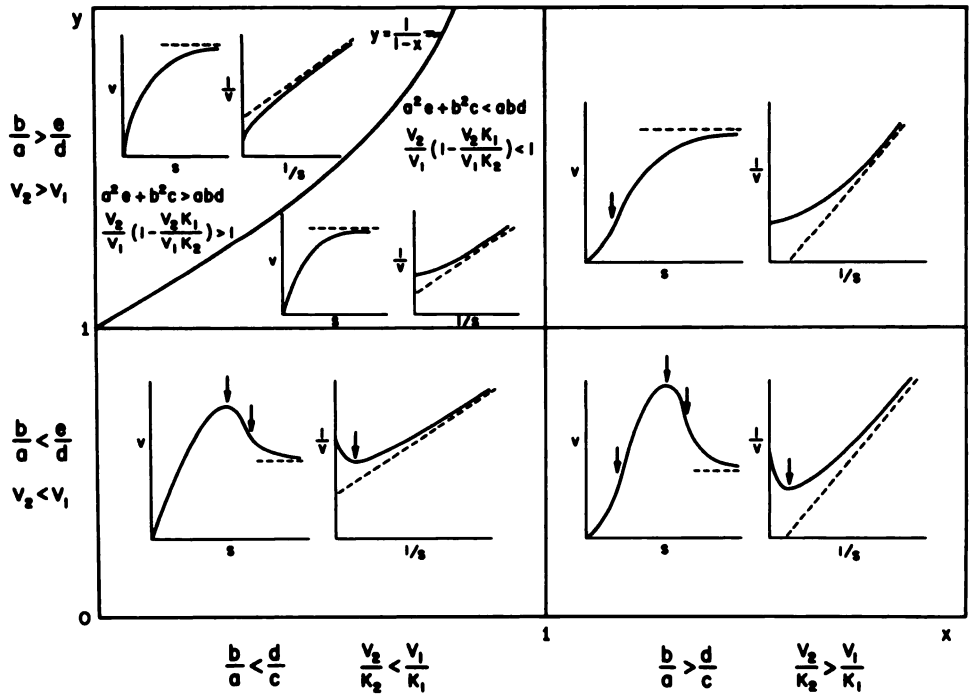


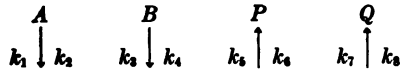
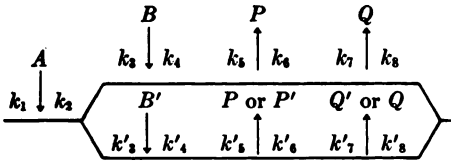
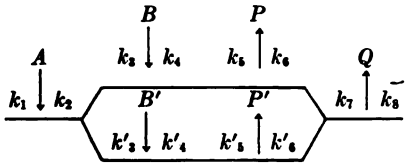
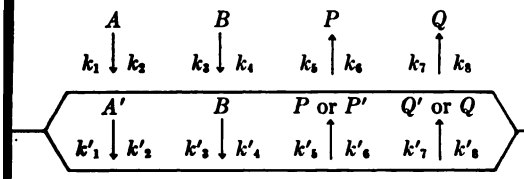
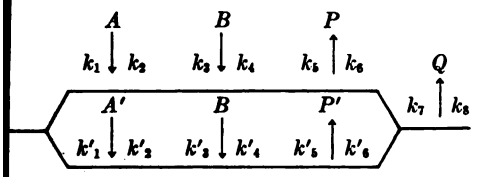
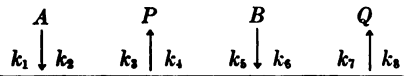
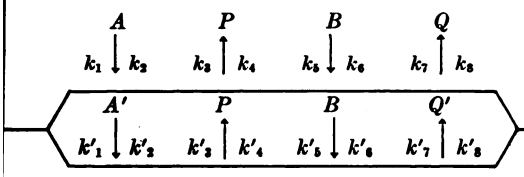
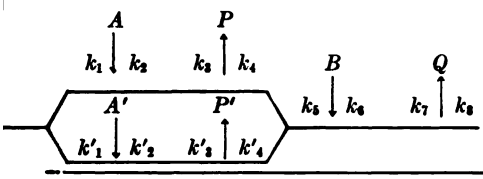
FIG. 2. *Types of curves represented by*

$$v = \frac{a(S) + b(S)^2}{c + d(S) + e(S)^2} \text{ or } v = \frac{\frac{V_1(S)}{K_1} + \frac{V_2(S)^2}{K_1K_2}}{1 + \frac{(S)}{K_1} + \frac{(S)^2}{K_1K_2}}$$

Two different plots, v against (S) and $1/v$ against $1/(S)$, for each type of curve, are drawn in the appropriate areas on the xy-plane, where $x = K_1V_2/K_2V_1$ and $y = V_2/V_1$. Each area represents a set of conditions of the coefficients for each type of curve. Curves are intended to show only the characteristics, such as maximum, minimum, or inflections, as indicated by arrows, or the asymptotes, as indicated by broken lines.

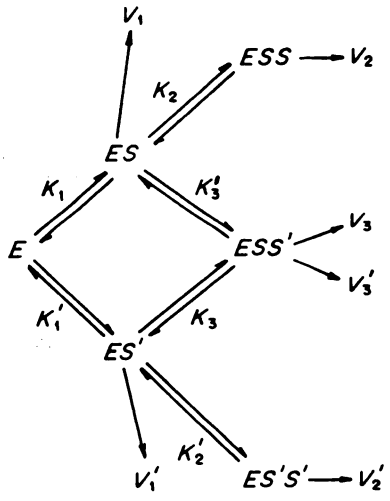
TABLE 1

Definition of kinetic parameters in bi-bi mechanisms with alternative substrates

Mechanisms	V or V'	K_{ia} or K'_{ia}	K_a or K'_a	K_b or K'_b
Ordered bi-bi mechanisms				
	$\frac{k_5k_7}{k_5+k_7}$	$\frac{k_2}{k_1}$	$\frac{k_5k_7}{k_1(k_5+k_7)}$	$\frac{(k_4+k_5)k_7}{k_3(k_5+k_7)}$
	$\frac{k'_5k'_7}{k'_5+k'_7}$	$\frac{k_2}{k_1}$	$\frac{k'_5k'_7}{k_1(k'_5+k'_7)}$	$\frac{(k'_4+k'_5)k'_7}{k'_3(k'_5+k'_7)}$
	$\frac{k'_5k_7}{k'_5+k_7}$	$\frac{k_2}{k_1}$	$\frac{k'_5k_7}{k_1(k'_5+k_7)}$	$\frac{(k'_4+k'_5)k_7}{k'_3(k'_5+k_7)}$
	$\frac{k'_5k'_7}{k'_5+k'_7}$	$\frac{k'_2}{k'_1}$	$\frac{k'_5k'_7}{k'_1(k'_5+k'_7)}$	$\frac{(k'_4+k'_5)k'_7}{k'_3(k'_5+k_7)}$
	$\frac{k'_5k_7}{k'_5+k_7}$	$\frac{k'_2}{k'_1}$	$\frac{k'_5k_7}{k'_1(k'_5+k_7)}$	$\frac{(k'_4+k'_5)k_7}{k'_3(k'_5+k_7)}$
Ping-pong bi-bi mechanisms				
	$\frac{k_3k_7}{k_3+k_7}$		$\frac{(k_2+k_3)k_7}{k_1(k_3+k_7)}$	$\frac{k_3(k_5+k_7)}{k_5(k_3+k_7)}$
	$\frac{k'_3k'_7}{k'_3+k'_7}$		$\frac{(k'_2+k'_3)k'_7}{k'_1(k'_3+k'_7)}$	$\frac{k'_3(k'_5+k'_7)}{k'_5(k'_3+k'_7)}$
	$\frac{k'_3k_7}{k'_3+k_7}$		$\frac{(k'_2+k'_3)k_7}{k'_1(k'_3+k_7)}$	$\frac{k'_3(k_5+k_7)}{k_5(k'_3+k_7)}$

not be applicable, even if two substrates, S and S', react at the same catalytic sites. A model that might present such complications is discussed below.

Two interacting catalytic centers. In this model, there are two catalytically active centers on a single molecule of enzyme. Each active center is capable of catalyzing a one-substrate, one-product reaction of the type $E + S \rightleftharpoons ES \rightarrow E + P$, but either the maximal velocity or the Michaelis constant or both may differ, depending on whether or not the other site is occupied, and an alternative substrate, S', may be acted upon. Under the usual equilibrium assumptions, similar to those of the Michaelis-Menten theory, the reaction pathway may be represented by Model (III).



(III)

Here K_1, K_2, etc., represent the dissociation constants of each step indicated by double arrows, and V_1, V_2, etc., represent the maximal velocities hypothetically obtainable when the concentration of the corresponding product-releasing enzyme species reaches that of the total enzyme. The combined rates of formation of the products from S and S' may be approximated by the following rate equation (14, 15):

$$v = \frac{\frac{V_1(S)}{K_1} + \frac{V_2(S)^2}{K_1K_2} + \frac{(V_3 + V'_3)(S)(S')}{K_1K'_3} + \frac{V_1(S')}{K'_1} + \frac{V'_2(S')^2}{K'_1K'_2}}{1 + \frac{(S)}{K_1}\left(1 + \frac{(S)}{K_2}\right) + \frac{(S)(S')}{K_1K'_3} + \frac{(S')}{K'_1}\left(1 + \frac{(S')}{K'_2}\right)} \quad (3)$$

In the absence of the competing alternative substrate, i.e., when $(S') = 0$, Eq. 3 reduces to

$$v = \frac{\frac{V_1(S)}{K_1} + \frac{V_2(S)_2}{K_1K_2}}{1 + \frac{(S)}{K_1} + \frac{(S)^2}{K_1K_2}} \quad (3a)$$

The types of curves represented by this equation on both the v against (S) plot and the $1/v$ against $1/(S)$ plot are illustrated in Fig. 2. Note that on the Lineweaver-Burk plot Eq. 3*a* may yield a concave upward curve with or without a minimum, a straight line, or a concave downward curve. The plot of Eq. 3 with (S) varied and (S') held constant yields curves of a similar nature.

When such curves are observed experimentally, one might be tempted to ignore the points at high concentrations of S or S', to extrapolate the straight portion of each curve, and to make predictions based on Eq. 1. However, the possibility that the curves might intersect nowhere near the predicted coordinates could lead to the false conclusion that the two substrates are acted upon by different enzymes. One such case has been thoroughly explored in this laboratory for the human erythrocytic purine nucleoside phosphorylase, with inosine and deoxyinosine as the competing substrates (14).

Multienzyme Models

When two different enzymes act on two different substrates, but the assay method does not distinguish one reaction from the other, the kinetics may resemble that of alternative-substrate cases. However, careful examination can differentiate one mechanism from another. As shown below, in no multienzyme systems do all the curves on a plot of combined velocity against the concentration of a substrate intersect at one point.

Two different enzymes, a simple case.

TABLE 2

Definition of apparent kinetic parameters in bi-bi mechanisms with alternative substrates

For all ping-pong mechanisms, set $K_{ia} = 0$ and $K'_{ia} = 0$.

$V_{app} = \frac{V}{1 + \frac{K_a}{(A)}}$ or $\frac{V}{1 + \frac{K_b}{(B)}}$	$V'_{app} = \frac{V'}{1 + \frac{K'_a}{(A)}}$ or $\frac{V'}{1 + \frac{K'_b}{(B)}}$
$K_{a(app)} = \frac{K_a\left(1 + \frac{K_{ia}K_b}{K_a(B)}\right)}{1 + \frac{K_b}{(B)}}$	$K'_{a(app)} = \frac{K'_a\left(1 + \frac{K'_{ia}K'_b}{K'_a(B)}\right)}{1 + \frac{K'_b}{(B)}}$
$K_{b(app)} = \frac{K_b\left(1 + \frac{K_{ia}}{(A)}\right)}{1 + \frac{K_a}{(A)}}$	$K'_{b(app)} = \frac{K'_b\left(1 + \frac{K'_{ia}}{(A)}\right)}{1 + \frac{K'_a}{(A)}}$

The simplest model for a mixture of two enzymes, each acting on a different substrate, S or S', may be represented by Model IV:

$$E_1 + S \rightleftharpoons E_1S \rightarrow E_1 + P \quad \text{(IV}a\text{)}$$
$$E_2 + S' \rightleftharpoons E_2S' \rightarrow E_2 + P' \quad \text{(IV}b\text{)}$$

The combined rate becomes

$$v = v_1 + v_2 = \frac{\frac{V_1(S)}{K}}{1 + \frac{(S)}{K}} + \frac{\frac{V_2(S')}{K'}}{1 + \frac{(S')}{K'}} \quad (4)$$

In this model, the plot of $1/v$ against $1/(S)$, as well as that of $1/v$ against $1/(S')$, gives a straight line, but the plot in the presence of a constant amount of the alternative substrate always yields a concave downward curve. This model is easily distinguishable from others, because the rates are strictly additive, according to Eq. 4, and the curves representing various concentrations of S' will not intersect at a single point.

Two different enzymes, each specific for one substrate, but inhibited by the other substrate. Two enzymes, one (E_1) specific for the substrate S but competitively inhibited by the second substrate S', and the other (E_2) specific for S' and competitively inhibited by S, are represented by Model V:

$$E_1 + S \rightleftharpoons E_1S \rightarrow E_1 + P \quad \text{(V}a\text{)}$$
$$E_1 + S' \rightleftharpoons E_1S' \quad \text{(V}b\text{)}$$
$$E_2 + S' \rightleftharpoons E_2S' \rightarrow E_2 + P' \quad \text{(V}c\text{)}$$
$$E_2 + S \rightleftharpoons E_2S \quad \text{(V}d\text{)}$$

$$v = \frac{\frac{V_1(S)}{K_1}}{1 + \frac{(S)}{K_1} + \frac{(S')}{K'_1}} + \frac{\frac{V_2(S')}{K'_2}}{1 + \frac{(S')}{K'_2} + \frac{(S)}{K_2}} \quad (5)$$

The plots of v against $1/(S)$ or $1/(S')$ will be a straight line in the absence of the alternative substrate, and will be a concave downward curve in its presence. The curves will not cross at a single point, however, and the combined rates will be less than the sum of the individual rates with either substrate alone.

Two nonspecific enzymes. Another conceivable model is one in which two nonspecific enzymes act on S as well as S', but with different degrees of effectiveness; i.e., S is a better substrate than S' for E_1, but a poorer substrate for E_2 (Model VI).

$$E_1 + S \rightleftharpoons E_1S \rightarrow E_1 + P \quad \text{(VI}a\text{)}$$
$$E_1 + S' \rightleftharpoons E_1S' \rightarrow E_1 + P' \quad \text{(VI}b\text{)}$$
$$E_2 + S \rightleftharpoons E_2S \rightarrow E_2 + P \quad \text{(VI}c\text{)}$$
$$E_2 + S' \rightleftharpoons E_2S' \rightarrow E_2 + P' \quad \text{(VI}d\text{)}$$

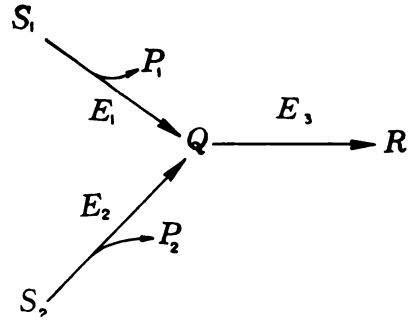
The combined rate equation is

$$v = \frac{\frac{V_1(S)}{K_1} + \frac{V'_1(S')}{K'_1}}{1 + \frac{(S)}{K_1} + \frac{(S')}{K'_1}} + \frac{\frac{V_2(S)}{K_2} + \frac{V'_2(S')}{K'_2}}{1 + \frac{(S)}{K_2} + \frac{(S')}{K'_2}} \quad (6)$$

In this case, even in the absence of the alternative substrate, the Lineweaver-Burk plot does not give a straight line, but a concave downward curve. The shapes of the curves in the presence of the alternative substrate are similar to those for Eq.

4, and they do not intersect at a common point.

Different enzyme systems linked to a common chain. Another complicated case is one in which two or more substrate systems feed into a common chain, such as an electron transport system. The simplest case of this kind may be represented by the following diagram (Model VII):



(VII)

If the common step is not rate-limiting, the steady-state concentration of Q will be very low compared to the Michaelis constant of Q for E_3, the rates of disappearance of S_1 and S_2 will be independent of each other, and the rate of formation of R will be simply the sum of the individual reaction rates. If, on the other hand, the common step is rate-limiting, the simultaneous presence of two substrates will result in mutual inhibition, even though the two reactions are catalyzed by different enzymes, E_1 and E_2. In this case, the steady-state concentration of Q in the presence of both substrates will be always higher than that in the presence of either substrate alone (in the same concentration as in the mixture). The combined rate (v_3 in the presence of both S_1 and S_2) is therefore always higher than the greater of the two individual rates (either v_1 with S_1 alone or v_2 with S_2 alone), and less than the sum of the two rates (v_1 with S_1 alone plus v_2 with S_2 alone). Thus one substrate behaves as a competitive inhibitor as far as the rate of disappearance of the other is concerned, but the type of dualism seen in Model I does not hold.

DISCUSSION

As shown above, the use of a competing alternative substrate or drug as an auxiliary variable can provide a powerful tool for determining whether two different compounds are metabolized by a single enzyme, and whether two different drugs act on the same receptor. This technique may be particularly useful when other, more direct methods cannot be applied. For instance, many enzymes are bound to or are a part of a membrane and resist the usual methods of isolation and purification. In such cases, rather simple kinetics with crude preparations of the enzyme(s) may shed light on whether more than one enzyme is involved, long before physical separation and identification of the enzyme(s) becomes feasible. If a similar problem arises for drug receptors, physical isolation may be extremely difficult, if not impossible, because such receptors are often well defined physiologically but not chemically, and they may lose their identities as receptors as soon as they are isolated.

By expanding the original theory to two-substrate mechanisms, it appears that the conclusions drawn from the simplest model hold for other mechanisms as long as the basic mechanism, in the absence of the alternative substrate, follows Michaelis-Menten kinetics. When the reaction mechanism is more complicated, as indicated, for instance, by the nonlinearity of a Lineweaver-Burk plot, one must take appropriate precautions before concluding that the two compounds are acted upon by two different enzymes or receptors.

REFERENCES

1. J. B. S. Haldane, "Enzymes," p. 85. Massachusetts Institute of Technology Press, Cambridge, Mass., 1965.
2. M. B. Thorn, *Nature* **164**, 27 (1949).
3. R. J. Foster and C. Niemann. *J. Amer. Chem. Soc.* **73**, 1552 (1951).
4. H. L. Segal, in "The Enzymes" (P. D. Boyer, H. Lardy, and K. Myrbäck, eds.), Vol. 1, p. 1. Academic Press, New York, 1959.
5. E. J. Ariëns, *Arch. Int. Pharmacodyn. Ther.* **99**, 32 (1954).
6. E. J. Ariëns, J. M. van Rossum, and A. M. Simonis, *Arzneimittel-Forschung* **6**, 611 (1956).
7. L. H. Cohen and R. E. Parks, Jr., *Can. J. Biochem. Physiol.* **41**, 1495 (1963).

8. N. Mourad and R. E. Parks, Jr., *J. Biol. Chem.* **241**, 271 (1966).
9. J. F. Henderson, L. W. Brox, W. N. Kelley, F. M. Rosenbloom, and J. E. Seegmiller, *J. Biol. Chem.* **243**, 2514 (1968).
10. W. W. Cleland, *Biochim. Biophys. Acta* **67**, 104 (1963).
11. M. Dixon and E. E. Webb, "Enzymes," Ed. 2, p. 84. Academic Press, New York, 1964.
12. J. M. Reiner, "Behavior of Enzyme Systems," pp. 91, 169. Burgess, Minneapolis, 1959.
13. E. L. King and C. Altman, *J. Phys. Chem.* **60**, 1375 (1956).
14. B. K. Kim, S. Cha, and R. E. Parks, Jr., *J. Biol. Chem.* **243**, 1763 (1968).
15. S. Cha, *J. Biol. Chem.* **243**, 820 (1968).