APPENDIX

EQUATIONS, FIGURES AND TABLES

for

STEADY STATE KINETICS:
TWO-SUBSTRATE, TWO-PRODUCT REACTIONS

DIAGRAMS OF MECHANISMS

INITIAL RATE EQUATIONS

DERIVATION BY KING-ALTMAN METHOD

ANALYSIS BY PRIMARY AND SECONDARY PLOTS

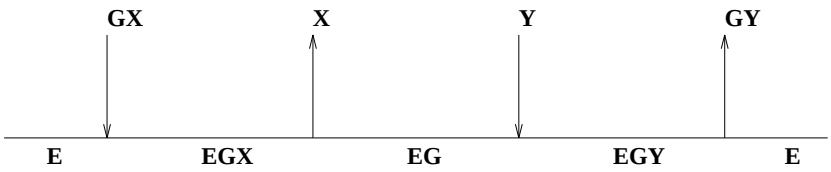
FULL RATE LAW

PRODUCT INHIBITION

TABLES

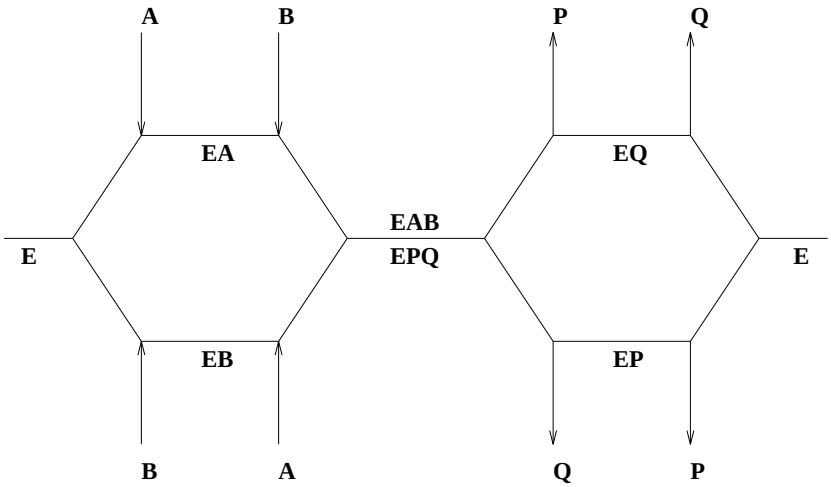
DIAGRAMS OF MECHANISMS

Substituted enzyme



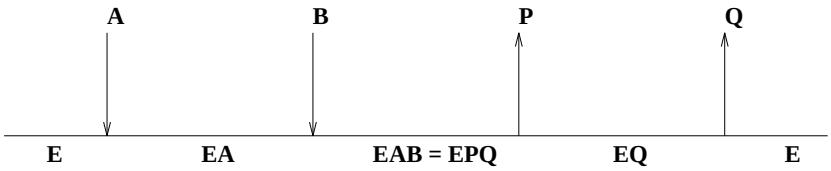
(1)

Random order



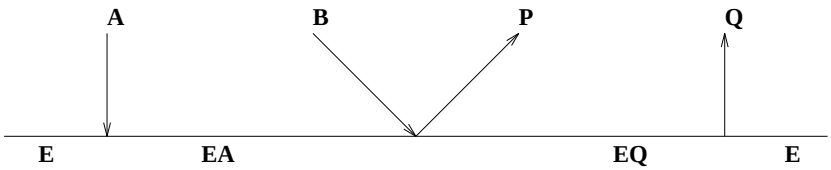
(2)

Compulsory order



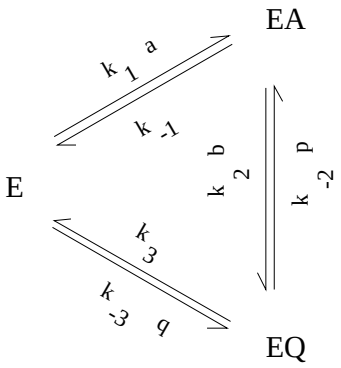
(3)

Theorell-Chance

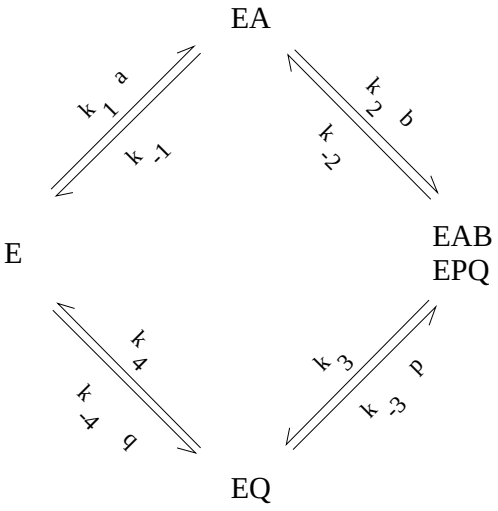


(4)

$$
\begin{aligned}
&E + A \rightleftharpoons EA \\
&EA + B \rightleftharpoons EAB \\
&EAB \rightleftharpoons EPQ \\
&EPQ \rightleftharpoons EQ + P \\
&EQ \rightleftharpoons E + Q
\end{aligned}
\qquad (5)
$$



(6a)



(6b)

INITIAL RATE EQUATIONS

The initial rate law for the forward direction (starting concentrations of products, *p* and *q*, equal to zero) for the random order (eq. 2), compulsory order (eq. 3) and Theorell-Chance (eq. 4) mechanisms is

$$v_o = \frac{V}{1 + \frac{K_m^A}{a} + \frac{K_m^B}{b} + \frac{K_m^{AB}}{ab}} \tag{7}$$

The expression of the parameters of eq. (7) in terms of the step constants differs according to mechanism.

Relationship of kinetic parameters to mechanism step constants

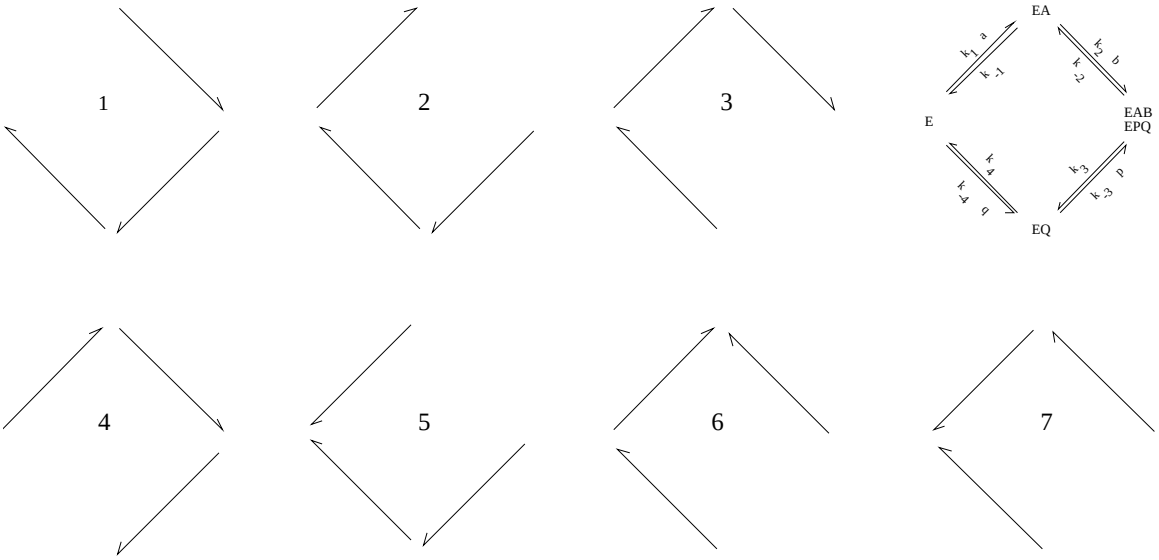
parameter	compulsory order (eq. 3)	Theorell-Chance (eq. 4)
V	$\frac{k_3 k_4 e_o}{k_3 + k_4}$	$k_3 e_o$
K_m^A	$\frac{k_3 k_4}{k_1(k_3 + k_4)}$	$\frac{k_3}{k_1}$
K_m^B	$\frac{k_4(k_{-2} + k_3)}{k_2(k_3 + k_4)}$	$\frac{k_3}{k_2}$
K_m^{AB}	$\frac{k_{-1} k_4(k_{-2} + k_3)}{k_1 k_2(k_3 + k_4)}$	$\frac{k_{-1} k_3}{k_1 k_2}$
$K_i^A = \frac{K_m^{AB}}{K_m^B}$	$\frac{k_{-1}}{k_1}$	$\frac{k_{-1}}{k_1}$
$K_i^B = \frac{K_m^{AB}}{K_m^A}$	$\frac{k_{-1}(k_{-2} + k_3)}{k_2 k_3}$	$\frac{k_{-1}}{k_2}$

DERIVATION BY KING-ALTMAN METHOD

INITIAL RATE LAW

COMPULSORY ORDER MECHANISM IN THE FORWARD DIRECTION

Construct the King-Altman patterns for the compulsory order mechanism (eq. 3 or equivalently, eq. 6b). Because for the forward direction the initial concentrations of products, *p* and *q*, are zero and steps 3 and 4 are unidirectional, there are only the following seven patterns:



From inspection of the patterns, construct expressions for the fraction of total enzyme in each enzyme form:

$$\frac{[E]}{e_o} = \frac{k_{-1}k_{-2}k_4 + k_{-1}k_3k_4 + k_2k_3k_4b}{D}$$

$$\frac{[EA]}{e_o} = \frac{k_1k_{-2}k_4a + k_1k_3k_4a}{D}$$

$$\frac{[EAB]}{e_o} = \frac{k_1k_2k_4ab}{D}$$

$$\frac{[EQ]}{e_o} = \frac{k_1k_2k_3ab}{D}$$

$$D = k_{-1}k_4(k_{-2} + k_3) + k_1k_4(k_{-2} + k_3)a + k_2k_3k_4b + k_1k_2(k_3 + k_4)ab$$

The reaction rate is equal to the rate of breakdown of the *EAB* complex

$$v_o = k_3[EAB]$$

Substituting for $[EAB]$

$$v_o = \frac{k_1k_2k_3k_4abe_o}{D}$$

Dividing numerator and denominator by $k_1k_2(k_3 + k_4)ab$, to throw the expression for v_o into the form of eq. (7)

$$v_o = \frac{\dfrac{k_3k_4e_o}{(k_3 + k_4)}}{\left[1 + \dfrac{k_3k_4}{k_1(k_3 + k_4)}\dfrac{1}{a} + \dfrac{k_4(k_{-2} + k_3)}{k_2(k_3 + k_4)}\dfrac{1}{b} + \dfrac{k_{-1}k_4(k_{-2} + k_3)}{k_1k_2(k_3 + k_4)}\dfrac{1}{ab}\right]} \qquad (7a)$$

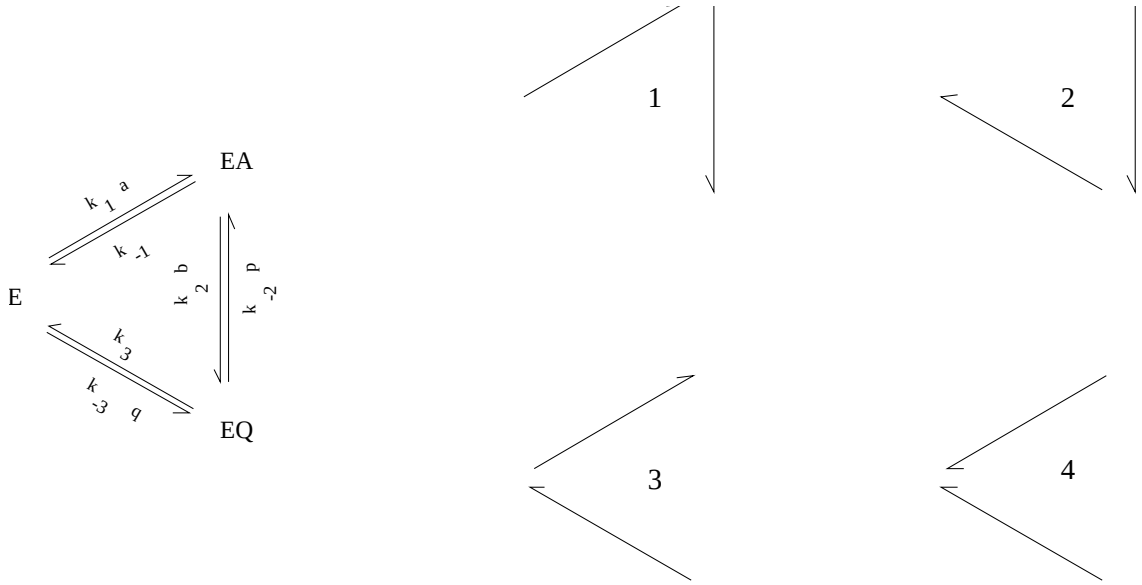
The relationship of the kinetic parameters of eq. (7) to the step constants is made clear by comparison of eqs. (7) and (7a).

DERIVATION BY KING-ALTMAN METHOD

INITIAL RATE LAW

THEORELL-CHANCE MECHANISM IN THE FORWARD DIRECTION

Construct the King-Altman patterns for the Theorell-Chance mechanism (eq. 4 or equivalently, eq. 6a). Again, because the initial concentrations of products, *p* and *q*, are zero and steps 3 and 4 are unidirectional, there are only the following four patterns:



The expressions for the fraction of total enzyme in each enzyme form are:

$$\frac{[E]}{e_o} = \frac{k_{-1}k_3 + k_2k_3b}{D}$$

$$\frac{[EA]}{e_o} = \frac{k_1k_3a}{D}$$

$$\frac{[EQ]}{e_o} = \frac{k_1k_2ab}{D}$$

$$D = k_{-1}k_3 + k_1k_3a + k_2k_3b + k_1k_2ab$$

The reaction rate is equal to the rate of breakdown of the *EQ* complex

$$v_o = k_3[EQ]$$

Substituting for $[EQ]$

$$v_o = \frac{k_1 k_2 k_3 abe_o}{D}$$

Dividing numerator and denominator by $k_1 k_2 ab$,

$$v_o = \frac{k_3 e_o}{\left[1 + \frac{k_3}{k_1}\frac{1}{a} + \frac{k_3}{k_2}\frac{1}{b} + \frac{k_{-1} k_3}{k_1 k_2}\frac{1}{ab}\right]} \tag{7b}$$

Compare eqs. (7) and (7b).

ANALYSIS BY PRIMARY AND SECONDARY PLOTS

LACTATE DEHYDROGENASE AS AN EXAMPLE

I. FIXED $b = [pyruvate]$, VARIED $a = [NADH]$

PRIMARY PLOT:

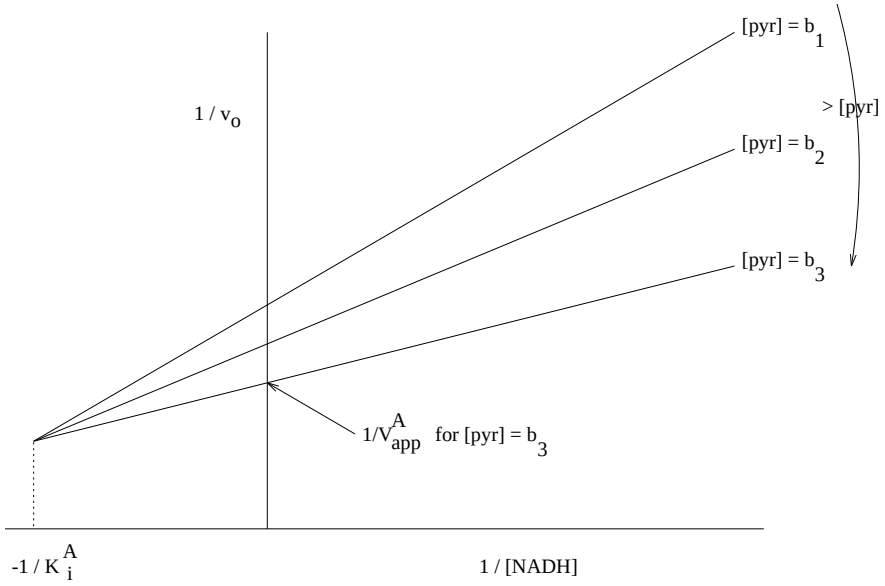
Taking the reciprocal of eq. (7)

$$\frac{1}{v_o} = \frac{1}{V} + \frac{1}{V}\frac{K_m^A}{a} + \frac{1}{V}\frac{K_m^B}{b} + \frac{1}{V}\frac{K_m^{AB}}{ab}$$

Equating a with $[NADH]$ and b with $[pyruvate]$, and collecting terms in $1/[NADH]$,

$$\frac{1}{v_o} = \frac{1}{[NADH]}\frac{1}{V}\left[\frac{K_m^{AB}}{[pyruvate]} + K_m^A\right] + \frac{1}{V}\left[\frac{K_m^B}{[pyruvate]} + 1\right] \qquad (8)$$

For initial rate data (values of v_o) measured over ranges of concentrations of pyruvate and NADH, eq. (8) defines a family of lines of $1/v_o$ *vs* $1/[NADH]$.



Each line represents a different fixed concentration of pyruvate. The slopes of the lines decrease with increasing pyruvate concentration, as required by the quantity in parenthesis of the first term on the right hand side of eq. (8).

The intercept on the $1/v_o$ axis is given by the second term on the rhs of eq. (8):

$$\frac{1}{V_{app}^{A}} = \frac{1}{V}\left[\frac{K_m^B}{[pyruvate]} + 1\right] \tag{8a}$$

The intercept, like the slope and for a similar reason, decreases with increasing pyruvate concentration.

The lines of constant $[pyruvate]$ meet in quadrant II or III at the point with coordinates

$$\left[\frac{1}{v_o}, \frac{1}{[NADH]}\right]_{intersection} = \left[\frac{1}{V}\left[1 - \frac{K_m^A K_m^B}{K_m^{AB}}\right], -\frac{K_m^B}{K_m^{AB}}\right] \tag{8b}$$

(To show this, expand eq. (8), collect terms in $1/[pyruvate]$, find the value of $1/[NADH]$ that makes zero the coefficient of $1/[pyruvate]$, and then find the value of $1/v_o$ for this value of $1/[NADH]$.)

The kinetic parameter K_i^A is defined by the point of intersection

$$K_i^A = \frac{K_m^{AB}}{K_m^B} = -[NADH]_{intersection} \tag{8c}$$

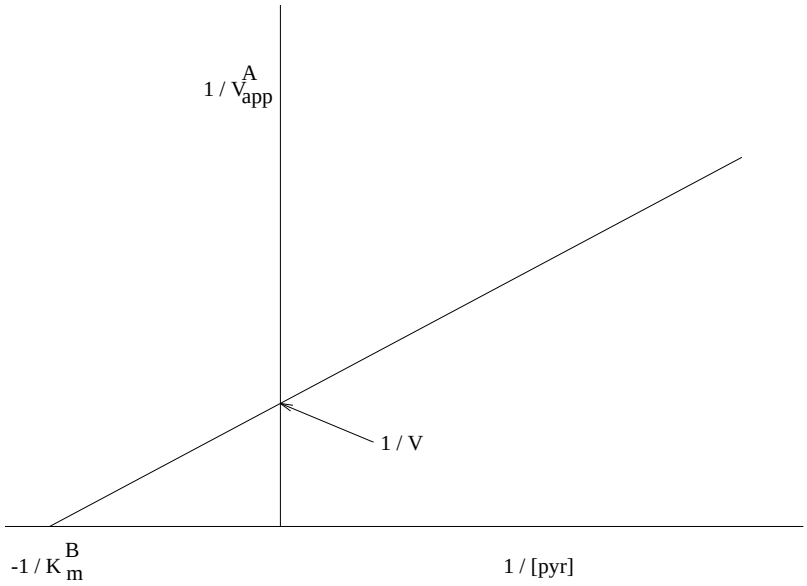
SECONDARY PLOT:

Expanding the rhs of eq. (8a)

$$\frac{1}{V_{app}^{A}} = \frac{1}{V}\frac{K_m^B}{[pyruvate]} + \frac{1}{V} \tag{9}$$

Thus a plot of the $1/V_{app}^{A}$ values determined from the primary plot *vs* $1/[pyruvate]$ defines a single line, with $1/v_o$ intercept equal to $1/V$, and $1/[pyruvate]$ intercept equal

to $-(1/K_m^B)$.



Note that three of the four parameters of eq. (7) are determined by one pair of primary and secondary plots, in this case, K_m^B and V from the secondary plot and K_m^{AB} from K_m^B and the value of K_i^A from the primary plot.

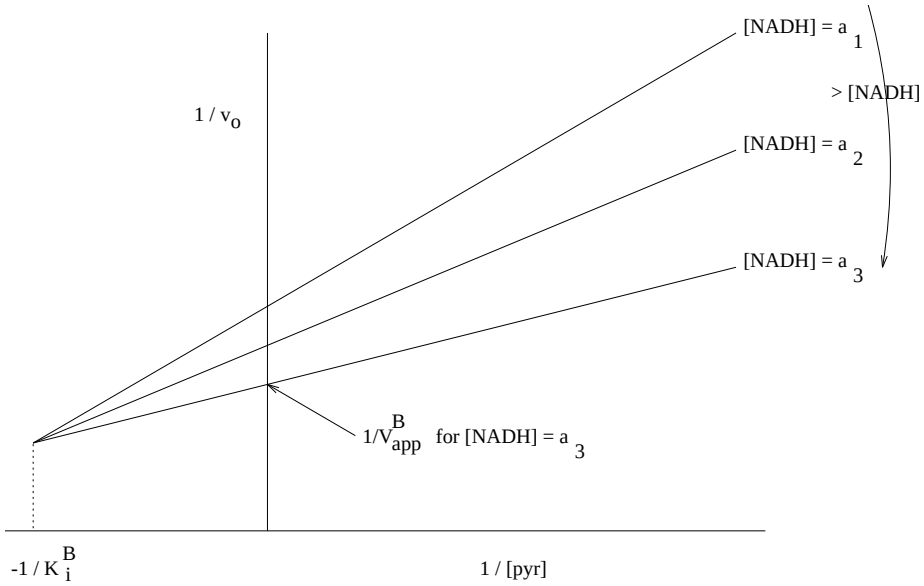
II. VARIED $b = [pyruvate]$, FIXED $a = [NADH]$

PRIMARY PLOT:

As with eq. (8), but collecting terms in $1/[pyruvate]$,

$$\frac{1}{v_o} = \frac{1}{[pyruvate]} \frac{1}{V} \left[\frac{K_m^{AB}}{[NADH]} + K_m^B \right] + \frac{1}{V} \left[\frac{K_m^A}{[NADH]} + 1 \right] \tag{10}$$

Similar to eq. (8), eq. (10) defines a family of lines of $1/v_o$ *vs* $1/[pyruvate]$, each line for a fixed concentration of NADH.



The intercepts on the $1/v_o$ axis, the coordinates of the point of intersection, and the kinetic parameter K_i^B are given by eqs. (10a-c).

$$\frac{1}{V_{app}^B} = \frac{1}{V} \left[\frac{K_m^A}{[NADH]} + 1 \right] \tag{10a}$$

$$\left[\frac{1}{v_o}, \frac{1}{[pyruvate]} \right]_{intersection} = \left[\frac{1}{V} \left[1 - \frac{K_m^A K_m^B}{K_m^{AB}} \right], -\frac{K_m^A}{K_m^{AB}} \right] \tag{10b}$$

$$K_i^B = \frac{K_m^{AB}}{K_m^A} = -[pyruvate]_{intersection} \tag{10c}$$

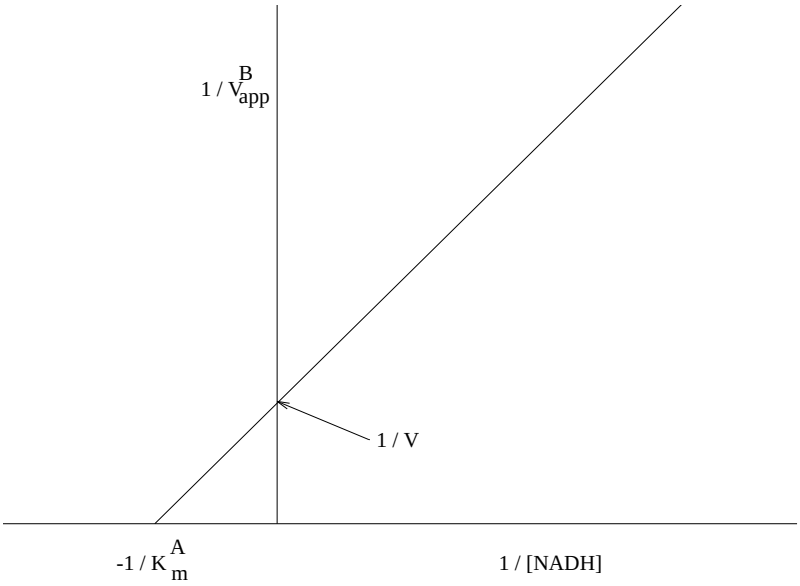
Note that the $1/v_o$ coordinate of the intersection point is the same for cases I and II, i.e., regardless of which substrate is fixed. This requirement of eqs. (8) and (10) must be taken into account when drawing the lines of the primary plots.

SECONDARY PLOT:

The values of $1/V^B_{app}$ determined from the primary plot are described by the equation

$$\frac{1}{V^B_{app}} = \frac{1}{V}\frac{K^A_m}{[NADH]} + \frac{1}{V} \qquad (11)$$

A plot of $1/V^B_{app}$ *vs* $1/[NADH]$ gives a line with $1/v_o$ intercept equal to $1/V$, and $1/[NADH]$ intercept equal to $-(1/K^A_m)$.



Values of K^A_m and V are determined from the secondary plot and K^{AB}_m from K^A_m and the value of K^B_i from the primary plot. Note that the graphical procedures of case I and case II each determine values for V and K^{AB}_m. This duplication can be used as a check on the analysis or can be taken into account in construction of the graphs.

FULL RATE LAW

FOR MECHANISMS OF EQ. (1-4)

A King-Altman analysis for the two-substrate, two-product mechanisms, without the restriction that the starting concentrations of products, *p* and *q*, be equal to zero, gives the following initial rate laws.

For compulsory order:

$$v_o = \left[\frac{V^f ab}{K_m^{AB}} - \frac{V^r pq}{K_m^{PQ}}\right] \Big/ \left[1 + \frac{K_m^B}{K_m^{AB}}a + \frac{K_m^A}{K_m^{AB}}b + \frac{1}{K_m^{AB}}ab + \frac{K_m^P}{K_m^{PQ}}q + \frac{K_m^Q}{K_m^{PQ}}p + \frac{1}{K_m^{PQ}}pq + \frac{K_m^A K_m^P}{K_m^{AB}K_m^{PQ}}bq + \frac{K_m^B K_m^Q}{K_m^{AB}K_m^{PQ}}ap + \frac{K_m^Q}{K_m^{AB}K_m^{PQ}}abp + \frac{K_m^A}{K_m^{AB}K_m^{PQ}}bpq\right] \quad (12)$$

For random order, omit terms in: bq, ap, abp, bpq.

For Theorell-Chance, omit terms in: abp, bpq.

For substituted enzyme, omit terms in: abp, bpq, and the constant term, 1.

Note the symmetry between reactant and product contributions. Note also that eq. (7) is obtained from eq. (12) by setting *p* and *q* at zero and multiplying numerator and denominator by K_m^{AB}/ab.

PRODUCT INHIBITION

I. PRODUCT INHIBITION BY PRODUCT *P*, WITH $q = 0$.

Product inhibition by be described for a two-substrate, two-product mechanism by setting the initial concentration of one product at zero in eq. (12). Here, set $q = 0$ in eq. (12), in order to describe inhibition by the first product, *P*. Reciprocal relationships are given below.

For compulsory order:

$$\frac{1}{v_o} = \frac{1}{V}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p + \frac{K_m^A}{a} + \frac{K_m^B}{b}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p\right] + \frac{K_m^{AB}}{ab}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p\right]\right] \tag{13a}$$

For random order:

$$\frac{1}{v_o} = \frac{1}{V}\left[1 + \frac{K_m^A}{a} + \frac{K_m^B}{b} + \frac{K_m^{AB}}{ab}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p\right]\right] \tag{13b}$$

For Theorell-Chance:

$$\frac{1}{v_o} = \frac{1}{V}\left[1 + \frac{K_m^A}{a} + \frac{K_m^B}{b}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p\right] + \frac{K_m^{AB}}{ab}\left[1 + \frac{K_m^Q}{K_m^{PQ}}p\right]\right] \tag{13c}$$

Inspection of eq. (13a-c) verifies line 4 of Table I, for *a* fixed, *b* varied, *q* zero, and *p* non-zero. For the random order and Theorell-Chance mechanisms, a sufficiently high concentration of *B* will make negligible any contribution from *P*, because every term in eq. (13b-c) containing *p* in the numerator has *b* in the denominator. Eq. (13b-c) show that for fixed *a* and varied *b*, the presence of product *P* as inhibitor (*p* non-zero) has no effect on the $1/v_o$ intercept and the inhibition is competitive (entries of "C" in columns 3 and 5 of line 4 of Table I). In contrast, for the compulsory order mechanism, there is a term with *p* in the numerator that does not contain *b* in the denominator (the second term within the rhs brackets of eq. 13a). Thus for the compulsory order mechanism, for fixed *a* and varied *b*, product *P* is a non-competitive inhibitor (entry of "NC" in column 4 of line 4 of Table I). Product inhibition by *P* with *B* the varied substrate distinguishes the

compulsory order mechanism from the random order and Theorell-Chance mechanisms. In the absence of product inhibitor the three mechanisms are indistinguishable through steady-state kinetic measurements.

Inspection of terms with *p* in eq. (13a-c) shows that inhibition by *P* with *a* varied shows noncompetitive behavior (terms without *a* in the denominator) for the compulsory order and Theorell-Chance mechanisms and competitive behavior for the random order mechanism (in agreement with line 3, columns 3-5 of Table I).

II. PRODUCT INHIBITION BY PRODUCT *Q*, WITH $p = 0$.

A similar analysis with $p = 0$ describes inhibition by *Q*, the second product.

For compulsory order:

$$\frac{1}{v_o} = \frac{1}{V}\left[1 + \frac{K_m^A}{a}\left[1 + \frac{K_m^P}{K_m^{PQ}}q\right] + \frac{K_m^B}{b} + \frac{K_m^{AB}}{ab}\left[1 + \frac{K_m^P}{K_m^{PQ}}q\right]\right] \qquad (14a)$$

For random order:

$$\frac{1}{v_o} = \frac{1}{V}\left[1 + \frac{K_m^A}{a} + \frac{K_m^B}{b} + \frac{K_m^{AB}}{ab}\left[1 + \frac{K_m^P}{K_m^{PQ}}q\right]\right] \qquad (14b)$$

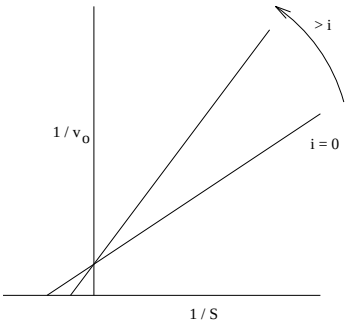
For Theorell-Chance: as for compulsory order.

Lines 1 and 2 of Table I can be verified by inspection of eq. (14a-b).

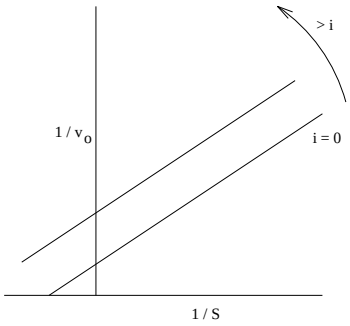
Table I. Product Inhibition Patterns

variable substrate	product inhibitor	Inhibition Pattern		
		random order (eq. 2)	compulsory order (eq. 3)	Theorell-Chance (eq.4)
A	Q	C	C	C
B	Q	C	NC	NC
A	P	C	NC	NC
B	P	C	NC	C

Competitive (C)



Uncompetitive (UC)



Noncompetitive (NC)