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Control analysis of transition times in metabolic systems

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The transition time, τ, of a metabolic system is defined as the ratio of the metabolite concentrations in the system, σ, to the steady-state flux, J. Its value reflects a temporal characteristic of the system as it relaxes towards the steady state. Like other systemic properties, the value of τ will be a function of the enzyme activities in the system. The influence of a particular enzyme activity on τ can be quantified by a Control Coefficient, $C^{\tau}_{e_i}$. We show that it is possible to derive a Summation Theorem $\Sigma_{i=1}^{n} C^{\tau}_{e_i} = -1$ and a Connectivity Theorem $\Sigma_{i=1}^{n} C^{\tau}_{e_i} \cdot \epsilon^{v_i}_{S_k} = -S_k/\sigma$. We establish a 'sign rule' that predicts the order of positive and negative Control Coefficients in a sequence.

INTRODUCTION

The temporal behaviour of metabolic pathways has been subject to a number of experimental and theoretical studies. In particular, attention has been given to systems that evolve from a given state to an asymptotically stable steady state and to the time constants involved in these processes (Barwell & Hess, 1970; Hess & Wurster, 1970; Hess, 1973; Easterby, 1973, 1981, 1984, 1986; Storer & Cornish Bowden, 1974; Heinrich & Rapoport, 1975; Reich & Sel'kov, 1981). A variety of names have been used to describe certain characteristic times of the system: transit time, transient time, transition time, reaction time. Some of these have identical definitions whereas some differ from each other. We shall use the term 'transition time', τ, which will be seen to be identical with that used by Easterby (1973, 1981, 1984, 1986). We shall be concerned with establishing the influence of individual enzymes on the transition time of the system, defined by the Control Coefficient of τ with respect to one enzyme activity, e_i (which is treated as a parameter). The definition of the Transition Control Coefficient is:

$$C^{\tau}_{e_i} = \frac{e_i}{\tau} \cdot \left(\frac{\partial \tau}{\partial e_i}\right) p_1, p_2, \ldots \qquad (1)$$

with all other parameters held constant. We show its relationship to the other well-known Control Coefficients, $C^{J}_{e_i}$ (Flux Control Coefficient) and $C^{S}_{e_i}$ (Concentration Control Coefficient). We shall indicate what experimentally accessible measurements will enable us to estimate the values of these coefficients. We shall also establish a number of theorems for which some experimental correlates are available. Unlike other treatments, our approach will not involve the use of kinetic formulations for the steps in the system (and is therefore independent of particular assumptions about the mechanisms involved). We make, however, two assumptions about the enzymes in the system: (1) rates of individual reactions are proportional to enzyme concentrations ('additivity'), and (2) the enzymes in the system are independently acting catalysts, i.e. that there is no effect of one enzyme on the rate catalysed by any other ('independence') (see Kacser *et al.*, 1989; Sauro & Kacser, 1989).

We consider a steady-state system of n enzymes with constant external source, X_0, and sink, X_{m+1}. The pathway diagrammed in Scheme 1 is a representative example. We shall perform the analysis with the assumption that all metabolite transformations are monomolecular or pseudo-monomolecular and take place with a stoichiometry of 1. This makes certain relationships simpler than for the more general case when this assumption is relaxed. The case when the transformations are $S_i = n_j S_j$ (where n_j is the stoichiometric coefficient) are treated in a subsequent section. As an alternative to holding the last product X_{m+1} in Scheme 1 at constant concentration, it can be set to zero (by some external means) so that the last step, catalysed by E_m, is irreversible and the rate of production of X_{m+1} is the output of the system. Easterby (1973, 1981) considered such a pathway as a model for a coupled enzyme assay with the assumption that the first enzyme, E_0, was 'severely rate-limiting', i.e. that the rate of the first step was constant. He showed that, with this assumption and starting with an 'empty' system, the progress curve of product approached the steady state from which two extrapolated intercepts are obtainable (see Fig. 1).

J_{in} is the (constant) rate of input of X_0 and J_{out} is the (variable) rate of output of the product X_{m+1}. The concentration of X_0 having entered during an interval of time t is given by:

$$(X_0)_{in} = \int_0^t J_{in} \cdot dt$$

$$X_0 \overset{E_0}{\rightleftharpoons} S_1 \overset{E_1}{\rightleftharpoons} S_2 \ldots S_m \overset{E_m}{\rightleftharpoons} X_{m+1}$$

Scheme 1.

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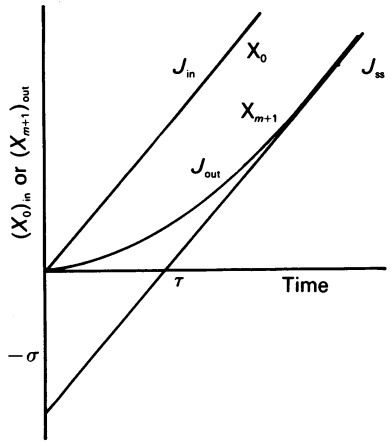


Fig. 1. Progress curves of a system with constant input

The system is as described in Scheme 1. The initial conditions are: all enzymes present, all intermediate metabolites absent. At $t = 0$ a fixed concentration of X_0 is applied and its rate of 'disappearance' (see the text) is observed as well as the rate of production of X_{m+1}. σ, the sum of all intermediate concentrations at steady state, is the intercept on the abscissa.

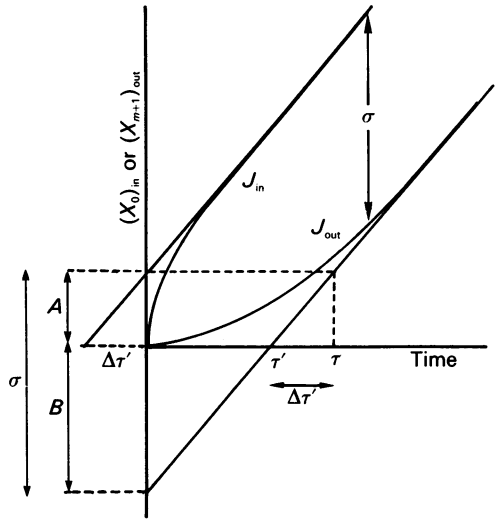


Fig. 2. Progress curves of a system with variable input

Conditions were as for Fig. 1 except that the rate of input to the system depends on the concentration(s) of some of the intermediates (see the text).

For constant J_{in}:

$$(X_0)_{in} = J_{in} \cdot t$$

Similarly, the product having left the system in the same interval is:

$$(X_{m+1})_{out} = \int_0^t J_{out} \cdot dt$$

In a system with unitary stoichiometric coefficients throughout, J_{out} will approach J_{in} as the system approaches the steady state. By mass conservation, at any time, t, the amount of X_0 having entered minus the amount of X_{m+1} having left the system is equal to the amount of S, in all forms, present in the system. With unitary stoichiometries there is also conservation of concentrations so that:

$$(X_0)_{in} - (X_{m+1})_{out} = (S)_{inside}$$

At steady state:

$$(S)_{inside} = \sum_{j=1}^{m} S_j^{ss} = \sigma$$

which symbol we use to denote the sum of all the free intermediate pools and all the enzyme-bound pools at steady state.

If we consider the progress curve of X_{m+1}, the line of the tangent to the steady state will intercept the abscissa at $-\sigma$. The intercept of the ordinate gives τ, called the transition time of the system. It also follows that τ is the sum of individual τ_j for each of the intermediate species S_j. From the intercepts we therefore have the relationship:

$$\tau = \frac{\sigma}{J} \qquad (2)$$

where J is the slope at steady state, $\sigma = \Sigma_{j=1}^{m} (S_j \text{ free} + S_j \text{ bound})$ at steady state, $\tau = \Sigma_{j=1}^{m} \tau_j$ and m is the number of metabolites in the system. It should be noted that τ is determined by two steady-state values, and hence can be estimated independently of the progress curve and is independent of the route by which the steady state was reached.

If we abandon the special assumption of constant input at the first enzyme, then we must consider the possibility of reversibility, or product inhibition or feedback (positive or negative) by some later metabolite in the pathway. The progress curves will then be as shown in Fig. 2.

Again, at steady state, the difference in the concentration of X_0 (X_0) having entered the system and the concentration of X_{m+1} (X_{m+1}) having left the system must be the sum of the concentrations of all the molecular species in the system. This time, however, the intercept τ' is not the transition time and intercept B is not σ. To these must be added the intercept values A and $\Delta\tau'$ respectively to give the correct values of σ and τ. Observation of the progress curve of product only will therefore underestimate the two values. It is experimentally difficult to establish the J_{in} curve, but it may be possible to devise a method of continuously monitoring the value of X_0 and to link this monitor to a 'topping-up' device that will keep X_0 at its initial value. The rate of topping up is then the value of J_{in} that we seek. A simpler way would probably be an actual extraction of the intermediates and summing their concentrations, $\sigma_{exp.}$. (Trichloroacetic acid precipitation would liberate both free and bound pools.) The value of $(\sigma_{exp.} - B)$ will give A and hence $\Delta\tau'$. Although $A + B = \sigma$, the sum of all the intermediates, it is not possible to assign (a) concentration(s) to either A or B separately. Both are extrapolated values from dynamical data.

[The J_{in} is not necessarily convex as shown, but, if the interaction increases the flux (positive effector) on the first enzyme, the progress curve would be concave and its intercept at steady state would have to be subtracted from B.]

It will be noted (eqn. 2) that τ depends only on the time-invariant values at the steady state, although it

predicts a temporal aspect of some systems (such as the assay from 'empty' to 'full'. In general, however, transitions from one steady state to another are more interesting, and Easterby (1981) has shown that such a transition time from state a to state b is given by:

$$\tau_{ab} = \tau_b - (J_a/J_b)\tau_a$$

where each of the right-hand terms is determined by the values of each steady state.

THEORY

Control Coefficients and Theorems

Our analysis is based on the definition of Control Coefficients (Burns *et al.* 1985):

$$C_p^V = \frac{p}{V}\cdot\frac{\partial V}{\partial p}$$

where V stands for any system-dependent variable (such as the flux J, a metabolite concentration S_j, whole pool σ, individual transition time τ_j or whole transition time τ) and p for any independent variable (or parameter) of the system; the parameter that we consider is the concentration of the enzyme E_i, designated as e_i, which affects the isolated rate v_i proportionally.

Since most of the experimental evidence (Easterby, 1986; Torres *et al.*, 1989) is from dilute systems where the enzyme concentrations are very much smaller than those of the intermediates, we make the simplifying assumption that:

$$\sigma = \sum_{j-1}^{m}(S_j^f + S_j^b)_{ss} \approx \sum_{j-1}^{m}(S_j^f)_{ss}$$

where f stands for 'free' and b for 'bound' pools and σ is the sum of all the free pools.

Similarly:

$$\tau = \sum_{j-1}^{m}(\tau_j^f + \tau_j^b)_{ss} \approx \sum_{j-1}^{m}(\tau_j^f)_{ss}$$

This, together with eqn. (2) and definition (1), gives, after differentiation:

$$C_{e_i}^{\tau} = C_{e_i}^{\sigma} - C_{e_i}^{J} \quad (3)$$

It is easily shown that:

$$C_{e_i}^{\sigma} = C_{e_i}^{S_1}\cdot\frac{S_1}{\sigma} + C_{e_i}^{S_2}\cdot\frac{S_2}{\sigma} + \ldots + C_{e_i}^{S_m}\cdot\frac{S_m}{\sigma} \quad (4)$$

that is that the Control Coefficient of the sum of all the pools is equal to the sum of the individual metabolite Control Coefficients each multiplied by their fractional concentrations. It is therefore possible to calculate any $C_{e_i}^{\tau}$ from a knowledge of the Concentration Control Coefficients and Flux Control Coefficients.

Similarly:

$$C_{e_i}^{\tau} = C_{e_i}^{\tau_1}\cdot\frac{\tau_1}{\tau} + C_{e_i}^{\tau_2}\cdot\frac{\tau_2}{\tau} + \ldots + C_{e_i}^{\tau_m}\cdot\frac{\tau_m}{\tau} \quad (5)$$

where τ_j is the transition time of the jth pool. This is defined as

$$\tau_j = \frac{S_j}{J} \quad (6)$$

(see also Easterby, 1973, 1981). Differentiation of eqn. (6) with respect to e_i gives:

$$C_{e_i}^{\tau_j} = C_{e_i}^{S_j} - C_{e_i}^{J} \quad (7)$$

which is the equivalent relationship to eqn. (3) for individual free metabolities. We therefore have formulations quantifying the influence of individual enzymes, E_i, on the transition time of single intermediate metabolites (eqn. 7) and on the transition time of the whole system (eqn. 3).

Summation Theorems

Heinrich & Rapoport (1975) stated, using a somewhat different definition of transition times between steady states, that:

$$\sum_{i-1}^{n} C_{e_i}^{\tau_j} = -1$$

(transcribed into the now agreed nomenclature of Burns *et al.*, 1985).

Summing relationship (7) over all enzymes gives:

$$\sum_{i-1}^{n} C_{e_i}^{\tau_j} = \sum_{i-1}^{n} C_{e_i}^{S_j} - \sum_{i-1}^{n} C_{e_i}^{J} \quad (8)$$

By using the well-known relationships $\Sigma_{i-1}^{n} C_{e_i}^{S_j} = 0$ and $\Sigma_{i-1}^{n} C_{e_i}^{J} = 1$ (Kacser & Burns, 1973; Heinrich & Rapoport, 1974), it follows that:

$$\sum_{i-1}^{n} C_{e_i}^{\tau_j} = -1 \quad (9)$$

a result identical with that obtained by Heinrich & Rapoport (1975).

By using the same arguments for the transition time of the whole system (eqn. 3):

$$\sum_{i-1}^{n} C_{e_i}^{\tau} = \sum_{i-1}^{n} C_{e_i}^{\sigma} - \sum_{i-1}^{n} C_{e_i}^{J} \quad (10)$$

Since the summation of each $C_{e_i}^{S_j}$ over all enzymes = 0, it follows from eqn. (4) that:

$$\sum_{i-1}^{n} C_{e_i}^{\sigma} = 0 \quad (11)$$

and therefore:

$$\sum_{i-1}^{n} C_{e_i}^{\tau} = -1 \text{ (Transition Summation Theorem)} \quad (12)$$

This is an important relationship. It asserts that if the activities of all the enzymes in the system are increased by the same factor, the transition time of the system will be decreased by the same factor (and vice versa for decreases of enzyme activities). The Summation Theorem (12), however, is uninformative of the magnitude or sign of any individual Control Coefficients. Inspection of eqn. (3) shows that any individual Control Coefficient could be positive or negative depending on whether $C_{e_i}^{\sigma}$ is greater or less than $C_{e_i}^{J}$. We shall discuss any additional constraints on the Control Coefficients and their relationship with one another after the next section.

We have commented on the practical difficulties in obtaining the values of τ and σ from the progress curves when a variable input rate is involved. This is relevant particularly when absolute values of τ and σ are sought. The 'false' values of τ' and B can then not be used. For the summation relationship, however, this restriction

does not apply. It is easily seen from Fig. 2 that the intercepts of J_{in} and J_{out} produce similar triangles so that $J = \sigma/\tau = B/\tau'$. When the activities of all enzymes are increased by the same factor (or when all the Control Coefficients are summed), the Summation Theorems (11) and (12) will be satisfied whether the 'true' σ and τ or the false B and τ' are used. It should, however, be noted that the individual values of each of the Control Coefficients (C_{e_i}) will not be correct if the B and the τ' intercepts are used when A is a significant fraction of σ.

Connectivity relationships

The Connectivity Theorems relate the systemic Control Coefficients, $C^S_{e_i}$ and $C^J_{e_i}$, to the 'local' Elasticity Coefficients. These represent aspects of the kinetics of the 'isolated' rate. They are defined as:

$$\epsilon^{v_i}_{S_k} = \frac{S_k}{v_i} \cdot \left(\frac{\partial v_i}{\partial S_k}\right)_{S_j S_l \ldots}$$

i.e. the response of the rate v_i to change in the concentration of a metabolite where the concentrations of all other participating molecules are constant (clamped) at their steady-state values. Two connectivity relationships have been established (Kacser & Burns, 1973; Westerhoff & Chen, 1984):

$$\sum_{i=1}^{n} C^J_{e_i} \cdot \epsilon^{v_i}_{S_k} = 0 \text{ (Flux Connectivity Theorem)} \quad (13)$$

$$\sum_{i=1}^{n} C^{S_j}_{e_i} \cdot \epsilon^{v_i}_{S_k} = -\delta_{jk} \text{ (Metabolite Connectivity Theorem)} \quad (14)$$

where δ is the Kronecker symbol, which $= 0$ for $j \neq k$ and $= 1$ for $j = k$, and S_k is the concentration of any one intermediate metabolite S_k.

Since most $\epsilon^{v_i}_{S_k}$ values are zero, i.e. the concentration S_k of a particular metabolite S_k directly affects only a small number of rates (as substrate, product or effector), the summation over all e_i values in practice means only a few non-zero terms. Thus, in a simple unbranched pathway, one metabolite affects the rates of only the two flanking enzymes. We can establish the connectivity for the transition time as follows.

Starting with eqn. (3), we can multiply all three terms by $\epsilon^{v_i}_{S_k}$. Summing over all i, we obtain:

$$\sum_{i=1}^{n} C^{\tau}_{e_i} \cdot \epsilon^{v_i}_{S_k} = \sum_{i=1}^{n} C^{\sigma}_{e_i} \cdot \epsilon^{v_i}_{S_k} - \sum_{i=1}^{n} C^J_{e_i} \cdot \epsilon^{v_i}_{S_k} \quad (15)$$

From eqn. (13) the last term of eqn. (15) is zero, and from eqn. (4) we can write for the first term of the right-hand member:

$$\sum_{i=1}^{n} C^{\sigma}_{e_i} \cdot \epsilon^{v_i}_{S_k} = \sum_{i=1}^{n} \left[\sum_{j=1}^{m} \left(C^{S_j}_{e_i} \cdot \frac{S_j}{\sigma} \right) \cdot \epsilon^{v_i}_{S_k} \right]$$

or

$$= \sum_{j=1}^{m} \left[\frac{S_j}{\sigma} \cdot \sum_{i=1}^{n} C^{S_j}_{e_i} \cdot \epsilon^{v_i}_{S_k} \right]$$

The summation over all i is, from eqn. (14), $= -\delta_{jk}$:

$$= -\sum_{j=1}^{m} \left[\frac{S_j}{\sigma} \cdot \delta_{jk} \right]$$

Since for all values of $S_j \neq S_k$, $\delta_{jk} = 0$, we are left with:

$$\sum_{i=1}^{n} C^{\sigma}_{e_i} \cdot \epsilon^{v_i}_{S_k} = -\frac{S_k}{\sigma} \text{ (Total Pool Connectivity Theorem)} \quad (16)$$

and from eqn. (15):

$$\sum_{i=1}^{n} C^{\tau}_{e_i} \cdot \epsilon^{v_i}_{S_k} = -\frac{S_k}{\sigma} \text{ (Transition Connectivity Theorem)} \quad (17)$$

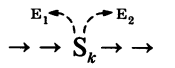
Signs of Transition Control Coefficients ($C^{\tau}_{e_i}$)

Just as it was possible (Kacser & Burns, 1973) to establish the relationship of two adjacent Flux Control Coefficients in a pathway as:

$$C^J_{e_1}/C^J_{e_2} = -\epsilon^{v_2}_{S_k}/\epsilon^{v_1}_{S_k}$$

where S_k is the concentration of the shared metabolite S_k, we can use eqn. (17) to establish the relationship of the signs of two adjacent Transition Control Coefficients.

In a pathway where S_k interacts with only two flanking enzymes



only two elasticities are involved, $\epsilon^{v_1}_{S_k}$ and $\epsilon^{v_2}_{S_k}$. From eqn. (17):

$$C^{\tau}_{e_1} \cdot \epsilon^{v_1}_{S_k} + C^{\tau}_{e_2} \cdot \epsilon^{v_2}_{S_k} = -\frac{S_k}{\sigma} \quad (18)$$

Re-arranging:

$$C^{\tau}_{e_1} = C^{\tau}_{e_2}\left(-\frac{\epsilon^{v_2}_{S_k}}{\epsilon^{v_1}_{S_k}}\right) - \frac{S_k/\sigma}{\epsilon^{v_1}_{S_k}} \quad (19)$$

Assuming 'normal' kinetics (i.e. substrates 'activate' the rate, products 'inhibit' the rate), $\epsilon^{v_1}_{S_k} < 0$ (negative) and $\epsilon^{v_2}_{S_k} > 0$ (positive). Since $S_k/\sigma > 0$ (a positive number less than 1), it follows that:

$$-\frac{S_k/\sigma}{\epsilon^{v_1}_{S_k}} > 0$$

and

$$-\epsilon^{v_2}_{S_k}/\epsilon^{v_1}_{S_k} > 0$$

If therefore $C^{\tau}_{e_2}$ is, say, positive, then $C^{\tau}_{e_1}$ is also positive. Extending this argument to a sequence of any number of enzymes, we can conclude the following.

(1) In a simple unbranched metabolic pathway, where all enzymes have 'normal' kinetics, a positive Transition Control Coefficient $C^{\tau}_{e_i}$ for enzyme E_i implies that all enzymes proximal to i have Transition Control Coefficients that are also positive.

By similar arguments the following can be shown.

(2) If $C^{\tau}_{e_i}$ is negative then all Transition Control Coefficients for enzymes distal to i will also be negative.

The conclusions (1) and (2), together with the Summation Theorem (eqn. 12), lead to the following.

(3) The Transition Control Coefficient of the last enzyme will always be negative.

Should any of these predictions be found to be violated (and possible experimental errors in the methods cannot account for it), the form of the kinetics (or the structure of the pathway) should be re-investigated.

Simulation of a pathway

Our conclusions are well illustrated by the simulation of a three-step pathway as shown:

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

All steps were taken to have 'normal' kinetics and to

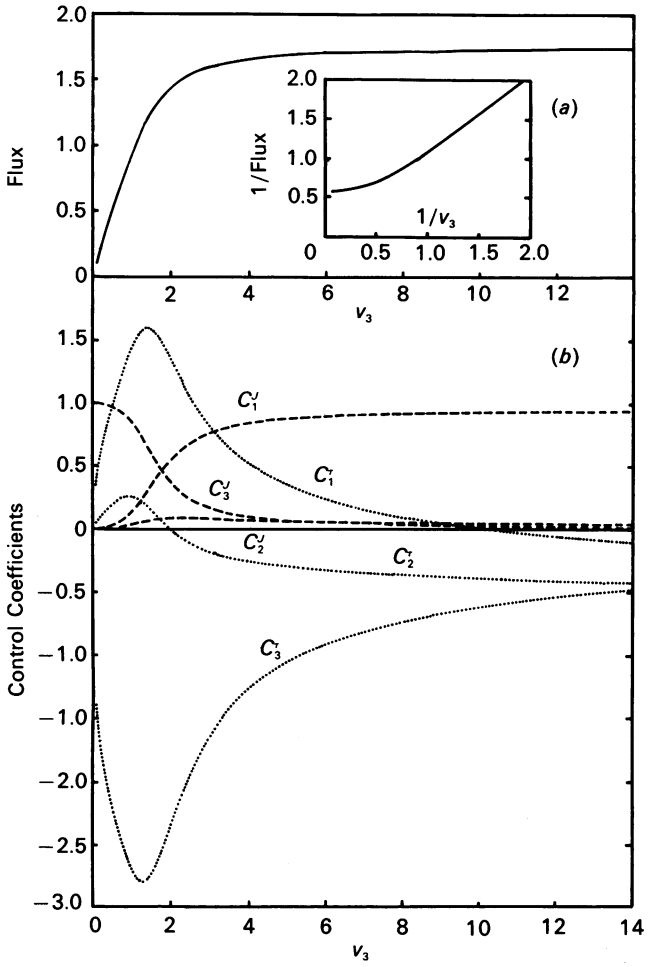


Fig. 3. Results of computer simulation of a metabolic system with three enzymes

All enzymes obeyed reversible Michaelis–Menten kinetics with the following parameters: for E_1, $V_{max.} = 2.00$, $K_m^S = 0.50$, $K_m^P = 0.60$ and $K_{eq.} = 10.0$; for E_2, $V_{max.} = 6.00$, $K_m^S = 0.20$, $K_m^P = 0.40$ and $K_{eq.} = 5.0$; for E_3, $V_{max.} = 0.10$ to 14.0, $K_m^S = 0.25$, $K_m^P = 0.30$ and $K_{eq.} = 5.0$. The concentrations of initial and final metabolites were constant at $X_0 = 5.00$ and $X_3 = 1.0$. Panel (*a*) shows the flux as a function of varying $V_{max.(3)}$. The inset gives the double-reciprocal plot. Panel (*b*) shows the values of the Control Coefficients as a function of V_3. ------, Flux Control Coefficients; ·····, Transition Control Coefficients.

obey simple reversible Michaelis–Menten laws. For each step this is of the form:

$$v = \frac{V_{max.}/K_m^S(S - P/K_{eq.})}{1 + S/K_m^S + P/K_m^P}$$

where K_m^S and K_m^P are the Michaelis constants for S and P respectively, S and P are their respective concentrations and $K_{eq.}$ is the equilibrium constant.

Kinetic parameters and equilibrium constants used in the simulation are given in Fig. 3; all of them were fixed with the exception of V_3 of the last enzyme (E_3), which was varied from 0.1 to 14, in order to see the control distribution at different steady states. The behaviour of the system in these conditions at steady state was simulated by computer, using the program SCAMP (Sauro, 1986). This program gives automatically Flux Control Coefficients and individual Concentration Control Coefficients. From these data, Control Coefficients of whole transit time were calculated by eqn. (20). This is derived from the relationship of the Control Coefficient of whole pool and the Control coefficients for each individual metabolite, which is given by eqn. (4).

Eqns. (3) and (4) lead to:

$$C_{e_i}^\tau = \sum_{j=1}^{m} \left(C_{e_i}^{S_j} \cdot \frac{S_j}{\sigma} \right) - C_{e_i}^J \qquad (20)$$

which allows calculation of Transition Control Coefficients.

Continuous recordings of these results are shown in Fig. 3, where re-arrangement of control among the three enzymes for flux and transition time can be seen, while the activity of the third enzyme is altered. Control of flux ($C_{e_i}^J$) is in all situations mainly shared between E_1 and E_3. It can be seen that whereas Flux Control Coefficients always have positive values (and sum to +1), the sign of the Transition Control Coefficients depends on the particular distribution of enzyme activities. The Transition Control Coefficient of the last enzyme, $C_{e_3}^\tau$, is always negative. The values of the Transition Control Coefficients of the first and second enzyme change from positive to negative, but the 'switch-over' occurs at different values of V_3.

In Table 1 we show three sets of values, extracted from the simulation at convenient points, which show the only three permissible sign patterns for $C_{e_i}^\tau$. These are (+ + −), (+ − −) and (− − −). Furthermore all three summation relationships are satisfied within rounding errors.

Table 1. Steady state

	(a)			(b)			(c)		
$V_{max.}$ for E_3	1.00			2.50			12.00		
Flux J	0.92			1.53			1.73		
	C^J	C^τ	C^σ	C^J	C^τ	C^σ	C^J	C^τ	C^σ
C_{e_1}	0.12	1.44	1.56	0.68	1.05	1.73	0.94	−0.06	0.88
C_{e_2}	0.03	0.25	0.28	0.08	−0.11	−0.03	0.04	−0.40	−0.35
C_{e_3}	0.85	−2.70	−1.85	0.24	−1.95	−1.71	0.02	−0.54	−0.52
ΣC_{e_i}	1.00	−1.00	−0.01	1.00	−1.01	−0.01	1.00	−1.00	−0.01

Pathways with non-unitary stoichiometries

Modification of the analysis is required if some steps in the system have stoichiometric coefficients $n_j \neq 1$. If, for example, at step $j-1$ one molecule of S_{j-1} produces two molecules of S_j ($n_j = 2$):

$$X_0 \rightarrow S_1 \rightarrow \rightarrow \ldots S_{j-1} \xrightarrow{E_{j-1}} 2S_j \xrightarrow{E_j} \rightarrow X_{m+1}$$

then the flux at j and at all subsequent steps will (in concentration units, dS/dt) be twice that of the flux before j. At steady state the input and output fluxes will therefore not approach each other, but:

$$J_{out}^{ss} = 2 \times J_{in}^{ss}$$

This is shown diagramatically in Fig. 4. As before (see Fig. 1), the abscissa intercept is $\tau = \Sigma_{j=1}^{m} \tau_j$, but the ordinate intercept is no longer the arithmetic sum of the steady-state concentrations of the intermediates, i.e. $\sigma' \neq \Sigma_{j=1}^{m} S_j$. The meaning of σ' is obtained from the analysis below.

Let us take a pathway where each step has an arbitrary stoichiometric coefficient, n_j. For a splitting reaction $n_j > 1$ (e.g. as above = 2), whereas for a condensation reaction $n_j < 1$ (e.g. two molecules → one molecule, $n_j = \frac{1}{2}$) and so on (see Scheme 2).

Each individual τ_j will be given by:

$$\tau_j = S_j/J_j \tag{21}$$

In general, each J_j will be different from every other (unless one of the $n_j = 1$). We wish to refer each J_j to a reference flux, say the output flux J_{out}, the rate of production, X_{m+1}, of the last metabolite X_{m+1}.

We can write eqn. (21) as:

$$\tau_j = \frac{S_j \times J_{out}/J_j}{J_{out}}, \tag{21a}$$

It is easily shown that the ratio J_{out}/J_j can be obtained from the stoichiometric coefficients by 'walking backwards' from X_{m+1}. Thus $J_{out}/J_m = n_{m+1}$, $J_{out}/J_{m-1} = n_{m+1} \times n_m$ etc., so that for any J_j:

$$J_{out}/J_j = \prod_{l=j+1}^{m+1} n_l \tag{22}$$

Therefore from eqns. (21) and (22):

$$\tau_j = \left(S_j \times \prod_{l=j+1}^{m+1} n_l\right) \Big/ J_{out} \tag{23}$$

The term in parentheses is the product of the metabolite concentration scaled by the appropriate product of the stoichiometric coefficients distal to the metabolite. We can symbolize this by S'_j, so that:

$$\tau_j = S'_j/J_{out} \tag{23a}$$

It is often convenient to couple step m to some other reaction so that the step is effectively irreversible (e.g. coupling with NAD^+ or NADH). If the changes in the coupled metabolites are measured, then the n_{m+1} will refer to the stoichiometry of that reaction.

$$X_0 \xrightarrow{J_0} n_1 S_1 \xrightarrow{J_1} n_2 S_2 \rightarrow \ldots \rightarrow n_m S_m \xrightarrow{J_m} n_{m+1} X_{m+1}$$

Scheme 2.

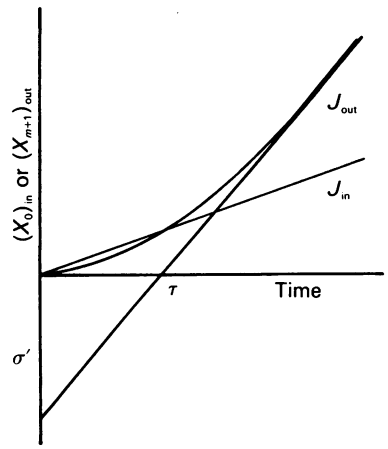


Fig. 4. Progress curves of a system with non-unitary stoichiometric coefficients and constant input

The system is as described in Scheme 2. As an example, it is assumed that one coefficient has a value, $n_j = 2$. The input rate is therefore one-half the steady-state output rate. σ' is given by eqn. (24) of the text.

Summing over all τ_j values gives τ, the transition time of the whole system:

$$\tau = \sum_{j=1}^{m} S'_j/J_{out} = \sigma'/J_{out} \tag{24}$$

The intercept σ' is therefore not the simple sum of the concentrations of all intermediate steady-state metabolites but the sum of each scaled by the appropriate product of stoichiometric coefficients. This meaning of σ' is not important for the graphical determination of τ in the case of a constant input (Fig. 4). It becomes relevant to the variable-input case where a direct extraction is probably the more realistic way of obtaining the value of σ'. Fig. 5 shows this case. If we assume that it is

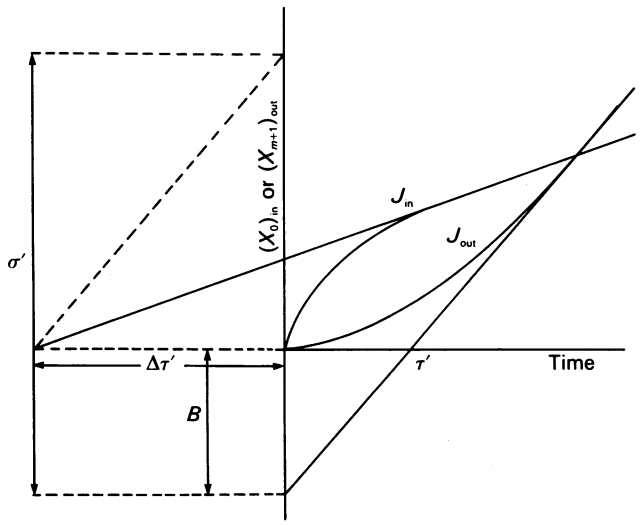


Fig. 5. Progress curves of a system with non-unitary stoichiometric coefficients and variable input

The relationship between the intercepts τ' and B, observable by monitoring the output rate J_{out} only, and the true values τ ($= \Delta\tau' + \tau'$) and σ' are shown.

experimentally not practicable to obtain the J_{in} progress curve but that the stoichiometries in our pathway are known, we can proceed as follows. Determine J^{ss}_{out} and extract all intermediates, determining their individual concentrations. By means of eqn. (24) we can obtain the value of σ' and hence τ.

It should be noted that, with appropriate combinations of n_j values, it is possible to have, fortuitously, $J^{ss}_{in} = J^{ss}_{out}$. Nevertheless, σ' values will still have to be calculated by eqn. (24) to obtain the value of τ.

It remains to determine how far the above considerations affect the control analysis. Differentiating eqn. (24) with respect to e_i will give the more general form of eqn. (3):

$$C^{\tau}_{e_i} = C^{\sigma'}_{e_i} - C^{J_{out}}_{e_i} \quad (25)$$

Similarly, eqn. (4) will now be modified by replacing each S_j by S'_j. Eqn. (5) remains unaffected. The summation relationships (9) and (12) are also unaffected, since every $\Sigma^{m}_{i=1} C^{S'_j}_{e_i} = 0$ and therefore $\Sigma^{n}_{i=1} C^{\sigma'}_{e_i} = 0$. It follows that:

$$\sum_{i=1}^{n} C^{\tau}_{e_i} = -1$$

For the connectivity relationships, the terms containing σ and S_j/σ will be replaced by σ' and S'_j/σ'. All the theorems therefore remain identical, with 'prime' values replacing those for the unscaled values.

Importantly, the Transition Summation Theorem is generally true, independent of the stoichiometries.

DISCUSSION

It will be seen that the magnitudes of the Flux Control Coefficients are dissociated from the magnitudes (and signs) of the Transition Control Coefficients (although they are not independent of one another, both being determined by the matrix of Elasticity Coefficients). An enzyme that is 'important' in controlling flux may have a very small effect in controlling transition time (Table 1, columns c) or vice versa (columns a).

Although we have discussed the control of transition time with reference to an unbranched system of steps, the Summation and Connectivity Theorems apply to systems of any structural complexity and size (as do the Flux and Concentration Summation and Connectivity Theorems).

The only experimental investigation of any depth is that by Torres *et al.* (1989). We give some of the results obtained (Table 2). It will be noted that, although they are in reasonable conformity with the Summation Theorems, the pattern of the signs of the $C^{\tau}_{e_i}$ values is not as expected. This could be due to the 'abnormal' kinetics of some of the steps. Both product activations and substrate inhibitions may play a part in generating this pattern. Alternatively, the intercepts may not be τ and σ (as discussed in the text), so that, although the ΣC^{τ} and ΣC^{σ} values are correct, the individual values may not be.

Apart from their application to coupled enzyme assays, the relationships established here may be relevant to the biological significance of transition times. Enzyme activities are subject to evolutionary change. Natural selection acts on all aspects of the phenotype, and hence only indirectly on the enzymes, to maximize fitness. All phenotypic characters are systemic variables, and hence components of fitness, of which fluxes and pools are examples. Transition time can, in certain situations, also be an important component of fitness insofar as the speed of response to changing external conditions may contribute to the survival of the organism. The magnitudes of the Control Coefficients for such responses, the $C^{\tau}_{e_i}$ values, however, reflect the 'solution' that natural selection has found to satisfy all the competing factors that, jointly, contribute to the fitness of the organism. No simple-minded interpretation of the experimentally found τ values is therefore possible.

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Table 2. Experimental estimates of Flux Control Coefficients and Transition Control Coefficients in a reconstructed glycolytic system from rabbit muscle (from Torres *et al.*, 1989)

Enzyme	$C^{J}_{e_i}$	$C^{\tau}_{e_i}$
Hexokinase	+0.20±0.03	−0.46±0.025
Phosphoglucose isomerase	+0.01±0.005	−0.11±0.09
Phosphofructokinase	+0.65±0.1	−0.71±0.15
Aldolase	+0.05±0.003	+0.05±0.007
Triose-phosphatase isomerase	+0.06±0.006	+0.22±0.08
Glycerol-3-phosphate dehydrogenase	+0.02±0.001	−0.08±0.004
$\Sigma^{6}_{i=1} C_{e_i}$	+0.99±0.09	−1.08±0.15

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