Integration of temporal analysis and control analysis of metabolic systems

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A theory is developed that integrates approaches to the analysis of pathway transient response and metabolic control analysis. A Temporal Control Coefficient is defined that is a measure of the system's transient response to modulation of enzyme activity or concentration. The approach allows for the analysis of the establishment of a steady state from rest, of the system's 'agility' of response to minor perturbations of a pre-existing steady state and of the macroscopic transition between steady states. In the last-mentioned case it is shown that, like the transient time itself, the control of transient response retains the property of independence from the mechanism of the transition. In consequence, the Temporal Control Coefficient can be defined in terms of the control properties of the initial and final states alone without reference to the mechanism of transition. A summation property is shown to apply to the Temporal Control Coefficients in each case. Connectivity relationships between elasticities and Temporal Control Coefficients are also established.

INTRODUCTION

Kacser & Burns (1973) developed a theory of metabolic control now generally referred to as metabolic control analysis. This theory allowed the division of the control of flux between enzymes in a metabolic system to be quantified in terms of Flux Control Coefficients. Heinrich & Rapoport (1975) developed a less general approach to the same problem applied to linear enzymic chains and later described a Control Coefficient that measured the transient response of individual metabolites of such systems. More recently a general theory of the transient or transition time of metabolic sequences has been developed (Easterby, 1981, 1986), and the general form of this theory deals in the same quantities as metabolic control analysis, namely metabolite pool sizes and fluxes. It is therefore now possible to integrate the two approaches and ask not only what are the determinants of flux in a metabolic system but also what determines the time required for the system response. One fundamental difference between temporal analysis and metabolic control analysis is that the former, by nature, deals with transitions between steady states whereas the latter is concerned with essentially homeostatic mechanisms for the maintenance of steady states. In other words temporal analysis is often concerned with large deviations and control analysis often with small perturbations to the system. These differences are addressed by the present theory.

The approach of Heinrich & Rapoport (1975) dealt with control analysis applied to transition times associated with individual intermediates and established a summation property for the Temporal Control Coefficients of a linear enzymic chain, similar to that common in the analysis of Flux or Concentration Control Coefficients. Torres *et al.* (1989) have demonstrated the validity of this summation property experimentally. Acerenza *et al.* (1989) have adopted a somewhat different approach to temporal variability of a system, and have defined a Time Coefficient that has similarities to Control Coefficients but is a time-variable property of the system. Their Time Coefficient gives a measure of local stability of the system. They have also established summation and connectivity relationships involving the Time Coefficients. Their analysis describes the time-dependence of Control Coefficients in a developing system.

The present analysis takes the simple and general definition of the pathway transient, transition or transit time proposed previously (Easterby, 1981) and establishes relationships between

the Temporal Control Coefficients and the Concentration and Flux Control Coefficients. Three types of transition are considered. Firstly the establishment of a steady state from rest, secondly the transition between two macroscopically distinct steady states and thirdly microscopic perturbations of a pre-existing steady state. The last-mentioned approach is the most closely related to classical metabolic control analysis. For all types of transition the same summation property of the Temporal Control Coefficients is shown to apply, and connectivity properties have been established. The aim of this type of analysis is to identify those enzymes that determine the responsiveness of the system to change. The time delay in the transmission of substrate through to end product is taken as a measure of this response and sets the time scale on which metabolism operates. A preliminary report of part of this work has been presented elsewhere (Easterby, 1990).

RESULTS

Definition of the transient time and the Temporal Control Coefficient

The definition of transient time for a steady state established from rest (zero flux and zero metabolite pools) is that given by Easterby (1981), namely:

$$\tau = \sum_{j=1}^m s_j / J \quad (1)$$

where s_j is the concentration of the j th of m metabolites s_1 to s_m in the system and J is the steady-state flux to product. S_0 is the buffered substrate for the pathway. τ represents the sum of the lifetimes of the m metabolite pools and can be regarded as the system transient time. It is equally possible to write expressions for the 'local' transient times of which the system transient is constituted:

$$\tau_j = s_j / J \quad (2)$$

For the purposes of this analysis it is assumed that enzyme-bound metabolite pools do not contribute significantly to the temporal response of the pathway. It has been shown previously that this is generally the case (Easterby, 1981). Similarly the present analysis is assumed to apply to reaction schemes where all the stoichiometries are unitary.

When a transition occurs between two steady states A and B, the transition time τ is given by (Easterby, 1981):

$$\tau = \tau_B - (J_A/J_B) \cdot \tau_A \quad (3)$$

where τ_A and τ_B are the transient times associated with the formation of each steady state from rest and J_A and J_B are the associated steady-state fluxes.

A convention has been adopted in referring to τ values. When a steady state is established from rest, τ is termed the transient time. When a transition between steady states occurs, τ is called a transition time. When microscopic perturbations are applied to a pre-existing steady state, τ is referred to as a transit time for the steady state and represents the summed lifetimes of the metabolite pools.

The Temporal Control Coefficient is defined as:

$$C_{e_i}^{\tau} = (\partial\tau/\partial e_i) \cdot (e_i/\tau) \quad (4)$$

and for microscopic perturbations of the steady state represents the fractional change in transit time accompanying a fractional change in activity or concentration (e_i) of the i th enzyme, E_i . It should be noted that the Temporal Control Coefficient is defined with reference to the steady state of the system, and it is assumed that all independent variables other than e_i (usually the other enzyme concentrations) are fixed for the purpose of forming the partial derivative. The nomenclature/notation adopted for Control Coefficients conforms to that of Burns *et al.* (1985) and Kacser & Porteous (1987). However, the term Temporal Control Coefficient has been adopted instead of Transition Time Control Coefficient as used by Torres *et al.* (1989). The reason for this is that, although algebraically the transit, transition and transient times are identical, their physical significances are subtly different. Use of the term Temporal Control Coefficient allows eqn. (4) to be used in each case, without redefinition, and encompasses all circumstances. The Temporal Control Coefficients represent a group of properties relating both to the system transient time and the lifetimes of individual metabolite pools.

Establishment of a steady state from rest

In the case of the establishment of a steady state from a state of rest in which there are no metabolite pools and no flux to product, the system transient time is given by eqn. (1). Partial differentiation of τ with respect to enzyme concentration e_i results in:

$$(\partial\tau/\partial e_i) = (\partial\Sigma s/\partial e_i) \cdot (1/J) - (\partial J/\partial e_i) \cdot (\Sigma s/J^2) \quad (5)$$

where Σs represents the sum of the concentrations of all metabolite pools s_1 to s_m . Multiplication by e_i/τ gives the Temporal Control Coefficient:

$$C_{e_i}^{\tau} = (\partial\Sigma s/\partial e_i) \cdot (e_i/\Sigma s) - (\partial J/\partial e_i) \cdot (e_i/J) \quad (6)$$

$$C_{e_i}^{\tau} = C_{e_i}^{\Sigma s} - C_{e_i}^J \quad (7)$$

From this it will be clear that an enzyme's effects on the pool size and flux are in opposition in determining the temporal response. The first term on the right of eqn. (7) represents the control coefficient associated with the sum of the metabolite pools and may be referred to as the Pool Control Coefficient. The Pool Control Coefficient may be expressed in terms of the Concentration Control Coefficients as follows:

$$C_{e_i}^{\Sigma s} = (\partial\Sigma s/\partial e_i) \cdot (e_i/\Sigma s) = (1/\Sigma s) \cdot \sum_{j=1}^m (s_j \cdot C_{e_i}^{s_j}) \quad (8)$$

It is therefore the weighted sum of the Concentration Control Coefficients.

A summation property applies to the Temporal Control Coefficients:

$$\sum_{i=1}^n C_{e_i}^{\tau} = \sum_{i=1}^n C_{e_i}^{\Sigma s} - \sum_{i=1}^n C_{e_i}^J \quad (9)$$

where n is the number of independent enzymes. This, according to the well-known summation property for Flux Control Coefficients for linear systems, yields:

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

The Pool Control Coefficients, like the Concentration Control Coefficients, may be shown by the method first proposed by Kacser & Burns (1973) to sum to zero. If each of the enzymes has its concentration modulated by an infinitesimal fraction α , then, assuming linear dependence of flux on enzyme concentration, the pool sizes will remain unaltered while the pathway flux increases by fraction α . Consequently the change in the summed pool sizes is given by:

$$\alpha \sum_{i=1}^n C_{e_i}^{\Sigma s} = 0 \quad (11)$$

from which it follows:

$$\sum_{i=1}^n C_{e_i}^{\Sigma s} = 0 \quad (12)$$

and from eqn. (10):

$$\sum_{i=1}^n C_{e_i}^{\tau} = -1 \quad (13)$$

This establishes a definition of the Temporal Control Coefficient (eqn. 7) and its related summation property (eqn. 13). Eqn. (12) may also be derived from eqn. (8) and the Summation Theorem for Concentration Control Coefficients by changing the order of summations. It has been derived here in order to emphasize its physical significance. A Control Coefficient may also be written for the local transient times associated with the individual metabolite pools:

$$C_{e_i}^{\tau_j} = C_{e_i}^{s_j} - C_{e_i}^J \quad (14)$$

It represents the difference between the Concentration and Flux Control Coefficients and exhibits the same summation property as the system Temporal Control Coefficient:

$$\sum_{i=1}^n C_{e_i}^{\tau_j} = -1 \quad (15)$$

It should be noted that the Temporal Control Coefficient differs from other Control Coefficients in one important respect. It is a retrospective property of the system and indicates what would have happened in the approach to the steady state, although modulation of enzyme activity to establish the Flux and Pool Control Coefficients took place in the steady state. The problem of how to assess the effects of enzyme modulation on the 'agility' or 'temporal responsiveness' of the steady state itself is addressed in a later section below.

At this point it is worth demonstrating the application of these concepts to a model system. For this purpose the simple linear enzymic chain of Scheme 1 is chosen because a complete

Scheme 1.

analytical solution of the rate equations describing it exists and its properties are well documented (Easterby, 1973). In this system an initial, severely rate-limiting, enzyme feeds the pathway at rate v_0 , which also becomes the steady-state rate of product (P) formation. The n Michaelis–Menten coupling enzymes obey pseudo-first-order kinetics with respect to the intermediates and the i th enzyme has first-order rate constant V_i/K_i , where V_i and K_i represent maximum velocity and Michaelis constant respectively. One surprising conclusion concerning this system was that the initial enzyme controlled the flux but had no effect on the transient time for the establishment of the steady state (Easterby, 1973). For this system the following relationships hold:

$$\tau = \sum_{i=1}^n s_i/v_0 = \sum_{i=1}^n (K_i/V_i) \quad (16)$$

$$\tau_i = s_i/v_0 = K_i/V_i \quad (17)$$

where all summations are carried out over the n coupling enzymes and intermediates. The initial substrate S_0 is considered to be buffered.

The Temporal Control Coefficient is given by:

$$C_{e_i}^r = (\partial\tau/\partial V_i) \cdot (V_i/\tau) \quad (18)$$

For the initial, rate-limiting, enzyme we have according to eqns. (6) and (7):

$$C_{e_0}^r = [\partial(v_0 \sum \tau_j)/\partial v_0] \cdot (v_0/v_0 \sum \tau_j) - (\partial v_0/\partial v_0) \cdot (v_0/v_0) \quad (19)$$

$$C_{e_0}^r = 1 - 1 = 0 \quad (20)$$

The Pool Control Coefficient is unity, but so also is the Flux Control Coefficient. Therefore the Temporal Control Coefficient is zero. If the activity of the initial enzyme is increased the flux increases but the pool sizes increase by the same factor, and therefore the transient time remains unaltered. For the remaining n enzymes:

$$C_{e_i}^r = \left[\partial \left(v_0 \cdot \sum_{j=1}^n \tau_j \right) / \partial V_i \right] \cdot \left((V_i/v_0) \cdot \sum_{j=1}^n \tau_j \right) - (\partial v_0 / \partial V_i) \cdot (V_i/v_0) \quad (21)$$

$$C_{e_i}^r = -\tau_i / \sum_{j=1}^n \tau_j \quad (22)$$

The Temporal Control Coefficients for enzymes E_1 to E_n therefore sum to -1 , and the whole of the temporal control resides in them and is distributed in proportion to the lifetimes of their substrate pools, but they do not contribute to flux control. In this instance the moduli of the Control Coefficients are all in the range 0–1.

A useful extension to Scheme 1 is to lift the restriction of severe rate-limitation of the first enzyme. Under such circumstances the coupling enzymes respond hyperbolically to substrate concentration and the transient times are defined as follows (Easterby, 1981):

$$\tau_i = K_i/(V_i - v_0) \quad (23)$$

$$\tau = \sum_{i=1}^n K_i/(V_i - v_0) \quad (24)$$

The Temporal Control Coefficients are defined as:

$$C_{e_i}^r = \{\partial/\partial V_i [K_i/(V_i - v_0)]\} \cdot V_i/\tau \quad (25)$$

which yields:

$$C_{e_i}^r = -(\tau_i/\tau) \cdot V_i/(V_i - v_0) \quad i = 1, 2, 3, \dots, n \quad (26)$$

and for the initial enzyme:

$$C_{e_0}^r = \sum_{i=1}^n (\tau_i/\tau) \cdot v_0/(V_i - v_0) \quad (27)$$

The Temporal Control Coefficients sum to -1 , but now the initial enzyme contributes to the temporal control. In the limit

that $v_0 \ll V_i$ eqn. (26) reduces to eqn. (22) and eqn. (27) to eqn. (20).

It is constructive to consider a special case of this system, namely the coupling of two enzymes in which there is an initial rate-determining enzyme and a single auxiliary enzyme obeying Michaelis–Menten kinetics with respect to the single intermediate. This system has been extensively studied both from the kinetic aspect (Storer & Cornish-Bowden, 1974) and from the point of view of transient behaviour (Easterby, 1981). It is of importance owing to its common use in the laboratory for the coupled assay of enzyme activity. In this system the two Temporal Control Coefficients become:

$$C_{e_0}^r = v_0/(V_1 - v_0) = 1/(r - 1) \quad (28)$$

$$C_{e_1}^r = -V_1/(V_1 - v_0) = -r/(r - 1) \quad (29)$$

where r is the ratio of activities:

$$r = V_1/v_0 \quad (30)$$

The Temporal Control Coefficients depend only on the ratio of enzyme activities, not on the other kinetic properties of the enzymes. Moreover, the ratio of Temporal Control Coefficients is the same as the ratio of activities:

$$|C_{e_1}^r/C_{e_0}^r| = r \quad (31)$$

In this example the moduli of the Control Coefficients are generally greater than unity.

This analysis has the advantage of describing the relative effect of modulation of the two enzyme activities on the time required to reach the steady state, without the need to solve differential equations. What is also clear, but not entirely obvious previously, is that the enzymes work in opposition in determining the temporal response. An increase in the activity of E_0 always increases τ whereas an increase in the activity of E_1 will always reduce it.

Transition between macroscopically distinct steady states

So far what has been considered is the establishment of steady states from rest. This is unrealistic in a cell context, as most changes will involve a transition between distinguishable steady states. Eqn. (3) describes the transition time for such events and differentiation of it with respect to the activity of E_i leads to the following most general relationship:

$$C_{e_i}^r = [1/(J_B \tau_B - J_A \tau_A)] \cdot [J_B \tau_B C_{e_i}^{rB} - J_A \tau_A C_{e_i}^{rA} + J_A \tau_A (C_{e_i}^{rB} - C_{e_i}^{rA})] \quad (32)$$

which may also be expressed with less generality (see the Discussion section) as:

$$C_{e_i}^r = [1/(\sum s_B - \sum s_A)] \cdot [(\sum s_B) C_{e_i}^{rB} - (\sum s_A) C_{e_i}^{rA} + (\sum s_A)(C_{e_i}^{rB} - C_{e_i}^{rA})] \quad (33)$$

or:

$$C_{e_i}^r = [1/(\sum s_B - \sum s_A)] \cdot [(\sum s_B) C_{e_i}^{rB} - (\sum s_A) C_{e_i}^{rA}] - C_{e_i}^{rB} \quad (34)$$

or:

$$C_{e_i}^r = [1/(\sum s_B - \sum s_A)] \cdot [(\sum s_B \cdot C_{e_i}^{rB} - \sum s_A \cdot C_{e_i}^{rA})] - C_{e_i}^{rB} \quad (35)$$

where summations are carried out over all m intermediates in each of the steady states A and B. Summation of the Control Coefficients of eqn. (33) over all enzymes E_i and use of the relationship of eqn. (13) demonstrates the summation relationship once more. The Temporal Control Coefficient for the transition between steady states is therefore independent of the mechanism of the transition and depends only on the Flux and Concentration Control Coefficients of each steady state.

Microscopic perturbations of a pre-existing steady state

It is now possible to ask how an enzyme affects the responsiveness of an existing steady state to change. Starting with eqn. (32), eqn. (33), eqn. (34) or eqn. (35) the limiting value can be found as the steady states A and B become coincident:

$$\lim_{\substack{J_A \rightarrow J_B = J \\ \tau_A \rightarrow \tau_B = \tau \\ \Sigma s_A \rightarrow \Sigma s_B = \Sigma s}} C_{e_i}^{\tau} = C_{e_i}^{\Sigma s} - C_{e_i}^J \quad (36)$$

Thus the Temporal Control Coefficient related to the transit time for a particular steady state is identical with the Control Coefficient associated with the formation of the steady state from rest (Eqn. 7), and the normal summation property applies (eqn. 13). Therefore the temporal control for microscopic perturbations to a particular steady state may be expressed in terms of the Concentration and Flux Control Coefficients.

Connectivity relationships

The way in which Flux and Concentration Control Coefficients are related through the elasticities has been demonstrated by Kacser & Burns (1973) and by Westerhoff & Chen (1984) respectively. The same approach may be applied to Temporal Control Coefficients. From eqns. (7) and (36) it follows that:

$$\sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = \sum_{i=1}^n C_{e_i}^{\Sigma s} \cdot e_{s_j}^i - \sum_{i=1}^n C_{e_i}^J \cdot e_{s_j}^i \quad (37)$$

Here $e_{s_j}^i$ represents the elasticity of the velocity of E_i with respect to the concentration of s_j and Σs represents the sum of the concentrations of all m metabolite pools. Applying eqn. (8):

$$\sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = \sum_{i=1}^n (1/\Sigma s) \cdot \sum_{k=1}^m s_k \cdot C_{e_i}^{s_k} \cdot e_{s_j}^i - \sum_{i=1}^n C_{e_i}^J \cdot e_{s_j}^i \quad (38)$$

$$\sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = \sum_{k=1}^m (1/\Sigma s) \cdot \sum_{i=1}^n s_k \cdot C_{e_i}^{s_k} \cdot e_{s_j}^i - \sum_{i=1}^n C_{e_i}^J \cdot e_{s_j}^i \quad (39)$$

Applying the concentration connectivity relationship (Westerhoff & Chen, 1984) and the flux connectivity (Kacser & Burns, 1973) results in:

$$\sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = -s_j / \sum_{k=1}^m s_k \quad (40)$$

In the case of the transition between steady states, eqn. (35) yields:

$$\sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = -(s_{j,B} - s_{j,A}) / (\Sigma s_B - \Sigma s_A) \quad (41)$$

where $s_{j,A}$ and $s_{j,B}$ are the concentrations of S_j in states A and B respectively. This is only true for those elasticities that are constant between the two states and no general connectivity is possible. However, in all cases the Control Coefficients can still be expressed in terms of the elasticities obtaining in the two states.

Consideration of the 'local' transients results in a connectivity relationship for the lifetimes of the individual metabolite pools:

$$\begin{aligned} \sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i &= -1 \quad j = k \\ &= 0 \quad j \neq k \end{aligned} \quad (42)$$

and in all cases:

$$\sum_{j=1}^m \sum_{i=1}^n C_{e_i}^{\tau} \cdot e_{s_j}^i = -1 \quad (43)$$

The connectivity property of eqn. (40) may be used in conjunction with the summation property of eqn. (13) to express the Temporal Control Coefficients in terms of the elasticities and pool sizes. Again a specific example is useful. Taking the very simple system of two enzymes E_0 and E_1 separated by a single intermediate S_1 , we have from the summation property (eqn. 13):

$$C_0^{\tau} + C_1^{\tau} = -1 \quad (44)$$

and from the connectivity property (eqn. 40):

$$C_0^{\tau} \cdot e_1^0 + C_1^{\tau} \cdot e_1^1 = -s_1/s_1 = -1 \quad (45)$$

This results in:

$$C_0^{\tau} = (e_1^1 - 1)/(e_1^0 - e_1^1) \quad (46)$$

$$C_1^{\tau} = (1 - e_1^0)/(e_1^0 - e_1^1) \quad (47)$$

In the system described by eqns. (28)–(30), differentiation of the Michaelis–Menten equation with respect to the concentration of S_1 yields:

$$e_1^1 = (V_1 - v_0)/V_1 \quad \text{and} \quad e_1^0 = 0 \quad (48)$$

Combining eqns. (46)–(48) produces the relationships of eqns. (28) and (29), which may also be expressed as:

$$C_0^{\tau} = (1 - e_1^1)/e_1^1 \quad \text{and} \quad C_1^{\tau} = -1/e_1^1 \quad (49)$$

In this particular example, with a single intermediate, the Control Coefficients may be expressed as functions of the elasticities alone. In general, however, the pool sizes also enter into the relationships, with the exception of the Control Coefficients relating to the lifetimes of individual metabolite pools.

DISCUSSION

Eqn. (1) represents the transit time for a pathway and is the summed lifetimes of the metabolite pools. It also represents the transient or lag time for the formation of the pathway steady state from rest when the rate of input to the pathway is constant. Under some circumstances the rate of input may vary during the transient, and these instances have been documented previously (Easterby, 1986). This variation leads to an additional term in the description of the transient, and in many instances this is insignificant compared with the transit time of eqn. (1). Where it is significant, eqns. (3) and (32) still apply for the transition between steady states. Similarly the Temporal Control Coefficient for the microscopic perturbation of a steady state remains identical with that associated with formation of the steady state from rest. However, in both cases the summation property no longer holds. Despite this complication, the transit time defined in eqn. (1) remains the most general property determining the temporal behaviour of the system. Before a change may be transmitted through a pathway, the metabolite pools must turn over. The control analysis of this turnover provides an important indication of the response of the system in time. This may be summarized as follows. (a) A Temporal Control Coefficient may be defined for both the system and local transit times (eqn. 4). (b) This is expressible in terms of the Concentration and Flux Control Coefficients (eqns. 7, 8, 14 and 32–36). It demonstrates that an enzyme's effect on the control of a pathway response comprises opposing components. Its effect on pool size is generally in conflict with its effect on pathway flux. The Temporal Control Coefficients can be positive or negative, and there will generally be no restriction on their individual magnitudes. (c) The Temporal Control Coefficients do, however, exhibit a summation property. They always sum to -1 (eqns. 13 and 15). (d) Connectivity relationships also hold for the transit time, the transition between steady states and the individual pool lifetimes

(eqns. 40–43) and lead to expressions for the Temporal Control Coefficients in terms of elasticities and pool sizes.

This analysis provides a firm theoretical basis for the summation property that Torres *et al.* (1989) have demonstrated experimentally. The control of the time scale of metabolism can be expressed in terms of the concentration-control and flux-control properties of the system, and much of the information required by the approach will already be available.

Note on the derivation of Temporal Control Coefficients

The derivations of the Temporal Control Coefficients are all specific examples of more general properties of Control Coefficients. If x and y are continuous functions of z , then it may readily be shown that the following generally hold:

$$C_z^{x/y} = C_z^x - C_z^y \quad (50)$$

$$C_z^{xy} = C_z^x + C_z^y \quad (51)$$

$$C_z^{x+y} = [x/(x+y)] \cdot C_z^x + [y/(x+y)] \cdot C_z^y \quad (52)$$

$$C_z^{x-y} = [x/(x-y)] \cdot C_z^x - [y/(x-y)] \cdot C_z^y \quad (53)$$

$$C_z^{-x} = C_z^x \quad (54)$$

$$C_z^{1/x} = -C_z^x \quad (55)$$

All of the properties of the Temporal Control Coefficients may be written down directly by using these relationships. Multiplication of these equations by an appropriate elasticity and summation over all z values shows that exactly analogous relationships apply to the connectivities (eqns. 37–43).

REFERENCES

- Acerenza, L., Sauro, H. M. & Kacser, H. (1989) *J. Theor. Biol.* **137**, 423–444
- Burns, J. A., Cornish-Bowden, A., Groen, A. K., Heinrich, R., Kacser, H., Porteous, J. W., Rapoport, S. M., Rapoport, T. A., Stucki, J. W., Tager, J. M., Wanders, R. J. A. & Westerhoff, H. V. (1985) *Trends Biochem. Sci.* **10**, 16
- Easterby, J. S. (1973) *Biochim. Biophys. Acta* **293**, 552–558
- Easterby, J. S. (1981) *Biochem. J.* **199**, 155–161
- Easterby, J. S. (1986) *Biochem. J.* **233**, 871–875
- Easterby, J. S. (1990) in *Control of Metabolic Processes* (Cornish-Bowden, A. & Cardenas, M. L., eds.), pp. 281–290, Plenum Press, New York
- Heinrich, R. & Rapoport, T. A. (1975) *BioSystems* **7**, 130–136
- Kacser, H. & Burns, J. A. (1973) *Symp. Soc. Exp. Biol.* **27**, 65–104
- Kacser, H. & Porteous, J. W. (1987) *Trends Biochem. Sci.* **12**, 5–14
- Storer, A. C. & Cornish-Bowden, A. (1974) *Biochem. J.* **141**, 205–209
- Torres, N. V., Souto, R. & Meléndez-Hevia, E. (1989) *Biochem. J.* **260**, 763–769
- Westerhoff, H. V. & Chen, Y.-D. (1984) *Eur. J. Biochem.* **142**, 425–430

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