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Molecular Bureaucracy: Who Controls the Delays?

Transient times in branched pathways and their control

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Analysis of metabolic control has until now been mainly confined to systems at steady state. This includes studies of the control of "transition time", which is actually a steady-state transit time that does not refer to the transient state. In this paper we examine the control of the transition state of a metabolic pathway in the approach to a stable steady state, showing that the time needed to attain it can be decreased or increased in different branches. Our analysis only applies to branched pathways, and we discuss why similar deviations cannot occur in unbranched pathways. In systems with several branches the acceleration of some branches during the transient phase, so that they reach their steady states more quickly, occurs at the expense of others, which are thus delayed. We present theorems that describe properties of the transient variables and their control.

1. Introduction

One of the best known papers of Henrik Kacser, written in collaboration with J. A. Burns, was entitled "Molecular democracy: who shares the controls?" (Kacser & Burns, 1979). It showed how the cellular world could be compared with a democratic society, because it works by the interaction of many different kinds of molecules, not all of them governed by a single molecule. This was an interesting idea, which provided a valuable context for discussing the faults in the old idea of the rate-limiting step.

We were very excited when we read that paper, which provided us with our first exposure to metabolic control theory, and remained the main text for studying this subject for some years. Subsequently some of us had the opportunity to work with Kacser on various aspects of control, first on the description of an experimental method to assay flux control coefficients *in vitro* (Torres *et al*., 1986), and later on the role of time in metabolism and its control, a very new field. We first derived the summation theorem for transit-time coefficients (Meléndez-Hevia *et al*., 1990a), and later, in a paper that was in press when Kacser died, we generalized the results to encompass any concentration of enzymes and any degree of saturation (Cascante *et al*., 1995).

The 1979 paper on molecular democracy suggested a corresponding idea for the control of time: just as metabolic government can be compared with a democracy, the approach of a metabolic pathway to a stable steady state, including the difficulties and obstacles that may delay its arrival at stable operation, can be compared with bureaucracy. Bureaucracy is not difficult to define: it is just the set of steps (including their legal forms) needed to demonstrate the right to something. From the applicant's point of view it is just a way to lose time. We are not sure that it is always necessary in any society, and it is probably more developed in less

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democratic societies. But even if a well-developed democracy should have very little, some degree of bureaucracy is likely to be necessary.

Metabolic Control Theory (or Control Analysis, as Kacser preferred to call it) was first formulated by Kacser & Burns (1973) and Heinrich & Rapoport (1974); it has been reviewed by Heinrich *et al*. (1977), Meléndez-Hevia *et al*. (1987), Fell (1992) and Schuster & Heinrich (1992). Control of transit times was first studied by Heinrich & Rapoport (1975) and later by us (Cascante *et al*., 1991, 1995; Meléndez-Hevia *et al*., 1990a), and some experimental work was also carried out (Torres *et al*., 1990; Torres & Meléndez-Hevia, 1992). The extended theory on the transition time of metabolic pathways is based on the original work of Easterby (1981, 1990), but other authors have also studied various aspects of time-dependent variables and their control, e.g. Acerenza (1990), Acerenza *et al*. (1989), Cornish-Bowden (1987), Heinrich & Reder (1991) and Torres *et al*. (1991).

Metabolic control has hitherto been analysed mainly in systems at steady state. This also includes different studies of the control of "transition time" τ, a variable defined by Easterby (1981, 1990), on the basis of mass conservation, as the total intermediate pool, σ, divided by the steady-state flux, J: this is in reality a "steady-state" transit time, whose physical meaning is the mean time in the steady state that one molecule spends traversing the pathway i.e. $\tau = \sigma/J$; it does not refer to the transient phase, and contains no direct information about transient properties. In an unbranched pathway the value of τ that can be calculated from steady-state data is the same as the lag time seen in the progress curve (see Fig. 1). In a branched pathway, however, it is possible to obain time variables that contain specific information about the transient phase, as we shall show.

Although our reasoning will be principally illustrated in terms of the simple system in Scheme 1, it is easily generalized to n enzymes, and k branches, as shown. Figure 1 shows a typical pair of progress curves for such a system, with the first step subject to product inhibition (Meléndez-Hevia *et al*., 1990a; see also Cascante *et al*., 1991; Torres *et al*., 1991; Cascante *et al*., 1995). In systems where the first step can be inhibited the total steady-state pool σ can be divided in two fractions σ_{in} and σ_{out}, and the transit time τ likewise, as illustrated in Fig. 1. In the present paper we will for simplicity assume a constant input flux, and will only deal explicitly with output fluxes, and hence only with σ_{out} and τ_{out}; however, this involves no loss of generality, because the argument will be based on mass conservation and applies to the

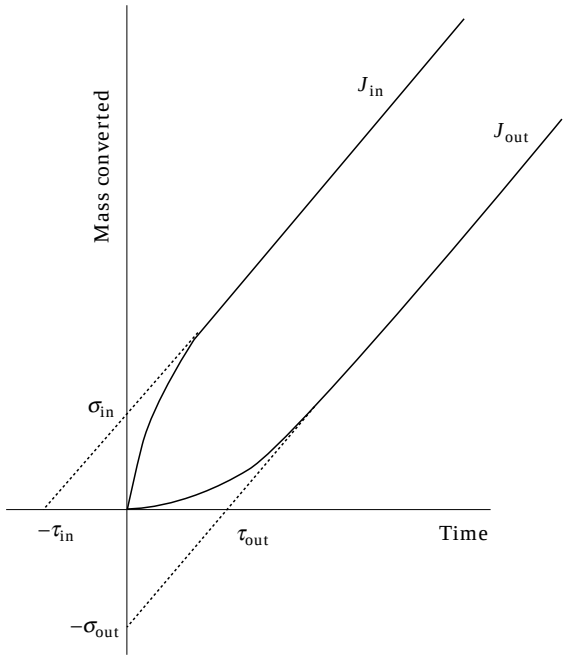
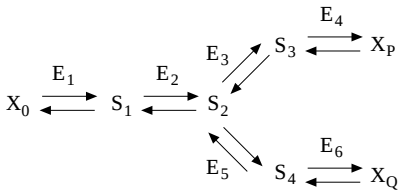


FIG. 1. Progress curves for input of starting substrate and output of final product in an unbranched metabolic system. At steady state the input flux J_{in} becomes equal to the output flux J_{out}, but they can be different during the transient state. The total mass σ of intermediate metabolites inside the system can be divided into two fractions, σ_{in} and σ_{out}, and the same applies to the transit time τ. Although σ and τ are fixed by the steady-state properties of the system the relative sizes of the input and output fractions can only be determined by studying the pre-steady-state transient phase.

total mass inside the system (see Meléndez-Hevia *et al*., 1990a).

We recently analysed the differences in control of transit time for free and enzyme-bound intermediates studied separately (Cascante *et al*., 1995). Similar analysis is also possible for the present case, but it would be unnecessarily complicated for our immediate purpose, and would not change the meaning of the conclusions. All results given here thus refer only to systems in which bound pools are negligible compared with total pools. However, we shall assume validity of the familiar hypotheses of additivity, i.e. the reaction rate of an isolated enzyme is first order with respect to total enzyme concentration, and independence of enzymes, i.e. all enzymes act as



SCHEME 1. Branched pathway in which a common starting material X_0 is converted into two different end products X_P and X_Q.

independent catalysts (see Kacser *et al.*, 1990; Meléndez-Hevia *et al.*, 1990b).

2. Control of Transit Times

Scheme 1 shows a branched pathway in which a starting substrate X_0 is converted into two alternative end products X_P and X_Q. At steady state, stoichiometry requires the total input flux J to be equal to the sum of the two branch fluxes, J_P and J_Q, i.e. $J = J_P + J_Q$.

At steady state, the total of intermediate pools $\sigma = \Sigma S_j$ can be partitioned into two fractions σ_{Pss} and σ_{Qss}, associated with the P and Q fluxes respectively, with the second subscript *ss* to denote that they are steady-state variables. As we shall show below, they are different from the corresponding values in the transient phase, which we will denote with the subscript *ts*. In partitioning σ the intermediates within any branch (such as S_3 in the P branch) are counted wholly in the fraction for that branch; common metabolites (S_1 and S_2) are considered to be shared between the two fluxes according to the magnitudes of the fluxes. Thus,

$$\sigma_{Pss} = S_3 + (S_1 + S_2)J_P/J \tag{1}$$

$$\sigma_{Qss} = S_4 + (S_1 + S_2)J_Q/J. \tag{2}$$

These two equations satisfy the requirement $\sigma_{Pss} + \sigma_{Qss} = \sigma$. If one branch of the system, for instance branch P from X_0 to X_P, is isolated by taking E_3 plus $[(E_1 + E_2)J_P/J]$, then such a pathway, working under the same conditions as the whole system, would yield the same flux J_P with the same internal pool σ_{Pss}.

We can define a specific transit time for each branch of the pathway as the mean time one molecule spends in crossing the system through such a specific sequence. These times are $\tau_{Pss} = \sigma_{Pss}/J_P$ and $\tau_{Qss} = \sigma_{Qss}/J_Q$ and the total transit time is:

$$\tau = \frac{\sigma}{J} = \frac{\sigma_{Pss} + \sigma_{Qss}}{J}$$

$$= \frac{\sigma_{Pss}}{J_P}\frac{J_P}{J} + \frac{\sigma_{Qss}}{J_Q}\frac{J_Q}{J} = \tau_{Pss}\frac{J_P}{J} + \tau_{Qss}\frac{J_Q}{J}. \tag{3}$$

For a particular branch, for example from X_0 to X_P, the transit time τ_{Pss} can be divided into two parts: the time τ_c to cross the common segment from X_0 to S_2, and the time τ_P for the branch itself, from S_2 to X_P. The transit time for the P branch is thus

$$\tau_{Pss} = \tau_c + \tau_P = \frac{S_1 + S_2}{J} + \frac{S_3}{J_P}. \tag{4}$$

Taking scaled derivatives with respect to any enzyme concentration e_i, we have:

$$\frac{\partial\tau_{Pss}}{\partial e_i}\frac{e_i}{\tau_{Pss}} = \frac{\partial\tau_c}{\partial e_i}\frac{e_i}{\tau_c}\frac{\tau_c}{\tau_{Pss}} + \frac{\partial\tau_P}{\partial e_i}\frac{e_i}{\tau_P}\frac{\tau_P}{\tau_{Pss}} \tag{5}$$

which can be written in terms of control coefficients as

$$C_{e_i}^{\tau_{Pss}} = C_{e_i}^{\tau_c}\frac{\tau_c}{\tau_{Pss}} + C_{e_i}^{\tau_P}\frac{\tau_P}{\tau_{Pss}} \tag{6}$$

in which τ_c/τ_{Pss} and τ_P/τ_{Pss} are the fractions of time spent in the common and specific parts of the branch respectively. Summing over all enzymes of the system:

$$\sum C_{e_i}^{\tau_{Pss}} = \sum C_{e_i}^{\tau_c}\frac{\tau_c}{\tau_{Pss}} + \sum C_{e_i}^{\tau_P}\frac{\tau_P}{\tau_{Pss}}. \tag{7}$$

Now $\Sigma C_{e_i}^{\tau_c} = -1$, as τ_c refers to an unbranched set of linked metabolites and to the flux through them. For the same reason $\Sigma C_{e_i}^{\tau_P}$ is also equal to -1. Therefore, as the two time fractions add up to one the following result holds:

THEOREM 1

$$\sum C_{e_i}^{\tau_{Pss}} = -1. \tag{8}$$

This is now the summation theorem for control coefficients of transit time in branched pathways, a generalization of one described previously (Meléndez-Hevia *et al.*, 1990a). The generalization to any stoichiometry shown previously also still applies, requiring no changes in eqn (8). Consequently, the summation theorem for transit-time control coefficients, like the summation theorem for flux control coefficients, applies to any metabolic system of any complexity, for any system of reactions at steady state.

3. Transient Phase of a Metabolic System Approaching a Steady State

All equations and properties given in the preceding section describe steady-state variables, and their physical meaning applies, therefore, to those conditions; for instance, $\tau_{Pss} = \sigma_{Pss}/J_P$ is the mean time that one molecule spends in crossing the pathway through the branch to X_P when the system is at steady state. As τ_{Pss} and σ_{Pss} are functions of state, they contain no information about the transient phase. This is illustrated by the dashed lines in Fig. 2, which contain no information about the transient progress

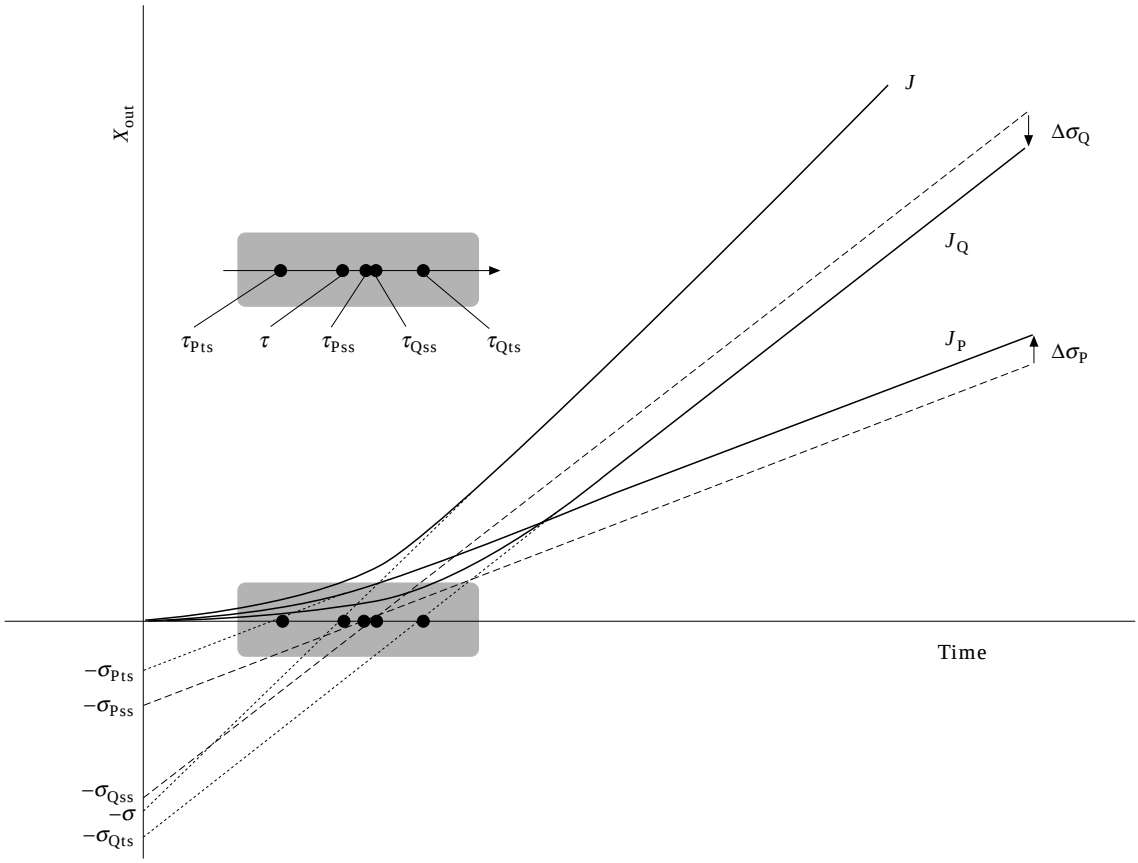


FIG. 2. Transient properties of the branched system shown in Scheme 1. The continuous lines are progress curves for output from the two branches, J_P and J_Q, together with the total output flux J. These curves may approach asymptotes (dotted lines) that are parallel to the straight lines (dashed lines) that represent the steady-state behaviour, but displaced from them by amounts $\Delta\sigma_P$ and $\Delta\sigma_Q$. As a result the values of σ and τ that characterize the transient-state behaviour of the two branches (σ_{Pts}, σ_{Qts}, τ_{Pts}, τ_{Qts}) may be different from the corresponding quantities for the steady state (σ_{Pts}, σ_{Qts}, τ_{Pts}, τ_{Qss}). Note that no corresponding displacement is possible for the progress curve for the total reaction (J) and thus no displacement of the total σ and τ values. For greater clarity the five abscissa intercepts are labelled with the appropriate τ values in the redrawn shaded region away from the axis.

curve, as they can be drawn with steady-state data for each flux and pool alone. Their slopes are the fluxes, and their ordinates are calculated by applying eqns (4) and (5).

However, if we follow the progress curves by monitoring the output fluxes we will usually obtain graphs similar to the continuous lines in Fig. 2, which are the actual progress curves, the dotted lines being their asymptotes. The asymptote of the progress curve for each branch is displaced with respect to the corresponding steady-state line; the slopes J_P and J_Q are unchanged, but the intercepts, σ_{Pts} and σ_{Qts} on the ordinate and τ_{Pts} and τ_{Qts} on the abscissa, are different. As the pair of lines corresponding to any branch flux are parallel, the difference of fluxes between steady state and transient phase can be described by the difference in the ordinate. There is no difference in the curve for the total flux, because its behaviour is qualitatively the same as in an unbranched pathway, and competition between different branches for the common mass is only possible in branched pathways. The relationship between the displacements of the two pairs of curves is described by the following theorem:

THEOREM 2

If the transient pool of a given branch σ_{Pts} in a branched pathway with two alternative end products is displaced from its steady-state value by some amount $\Delta\sigma_P = \sigma_{Pts} - \sigma_{Pss}$, then the other transient pool σ_{Qts} is displaced by the same amount in the opposite direction, i.e., $\Delta\sigma_Q = \sigma_{Qts} - \sigma_{Qss} = -\Delta\sigma_P$.

PROOF

In Fig. 2, the steady-state and transient-state asymptotes corresponding to the same branch are parallel, because their slopes, the steady-state fluxes, must be equal. But mass conservation requires that $\sigma_{Pts} + \sigma_{Qts} = \sigma_{Pss} + \sigma_{Qss} = \sigma$, and so if one is displaced

the other must be equally displaced in the opposite direction; the extension of such displacements is given by $\Delta\sigma$ values, since one asymptote is always displaced without changing its slope, and therefore:

$$\Delta\sigma_{\mathrm{Q}} = -\Delta\sigma_{\mathrm{P}}. \tag{9}$$

This can be generalized in an obvious way to a system with k branches P_{j}, to give

$$\sum \Delta\sigma_{\mathrm{P}j} = 0 \tag{10}$$

where the summation is over all branches.

For the same reason, the transient times will be different from the steady-state transit times, and the relationship is described by the following theorem:

THEOREM 3

In the same conditions as Theorem 2, if the transient time $\tau_{\mathrm{P}ts}$ of a branch differs from the corresponding steady-state transit time $\tau_{\mathrm{P}ss}$ by $\Delta\tau_{\mathrm{P}} = \tau_{\mathrm{P}ts} - \tau_{\mathrm{P}ss}$ then the transient time $\tau_{\mathrm{Q}ts}$ of the other branch is displaced in the opposite direction by $\Delta\tau_{\mathrm{Q}} = \tau_{\mathrm{Q}ts} - \tau_{\mathrm{Q}ss}$, these displacements being related by $\Delta\tau_{\mathrm{P}} = -\Delta\tau_{\mathrm{Q}}J_{\mathrm{Q}}/J_{\mathrm{P}}$, which follows from the displacement of τ_{P}, which is

$$\Delta\tau_{\mathrm{P}} = \frac{\Delta\sigma_{\mathrm{P}}}{J_{\mathrm{P}}} = \frac{-\Delta\sigma_{\mathrm{P}}}{J_{\mathrm{Q}}}\frac{J_{\mathrm{Q}}}{J_{\mathrm{P}}}. \tag{11}$$

Like Theorem 2, this can be generalized in an obvious way to a system with k branches P_j, to give

$$\sum J_{\mathrm{P}_j} \cdot \Delta\tau_{\mathrm{P}_j} = 0 \tag{12}$$

where the summation is again over all branches.

Why are transient pools and times not equal to the steady-state pools and times? In the steady state, the total flux that passes through the common segment is shared between the different branches in a fixed ratio determined by the kinetic properties of the enzymes of each branch, including their interactions with intermediates. Under steady-state conditions, the activity of each enzyme is stable, as all substrates and products are at constant concentrations and the interactions are fixed, giving stable fluxes. However, these conditions are not satisfied in the transient phase, and in general, in a branched pathway with several output fluxes, the proportion of the total flux that passes transiently through some branches will be greater than it is at steady state, and correspondingly lower than in other branches.

We can now explore the physical meanings of the transient pools and times: the reason for the transient behaviour is a nonlinear distribution of mass for each branch, $\Delta\sigma_{\mathrm{P}}$ being the extent of the deviation for the P branch. For example, in Fig. 2 J_{P} is initially accelerated during the transient phase, reaching its steady state before it would reach it if the kinetic interactions during the transient were linear. A negative value of $\Delta\sigma_{\mathrm{P}}$, i.e., $\sigma_{\mathrm{P}ts} < \sigma_{\mathrm{P}ss}$ means an accelerated transient; if such a situation remained at steady state, then the system would be able to have the same flux with less mass inside ($\sigma_{\mathrm{P}ts}$ instead of $\sigma_{\mathrm{P}ss}$), and then, also with a lower transit time. As mentioned above, it is in principle possible to isolate the pathway from X_0 to X_{P} (including its corresponding fraction of the common enzymes) and to make it work under the same conditions; it would then give the same steady-state flux, and σ would be the same as $\sigma_{\mathrm{P}ss}$. However, it is not possible to isolate a branch so that it yields the same flux at steady state with a total internal pool of $\sigma = \sigma_{\mathrm{P}ts} \neq \sigma_{\mathrm{Ps}s}$, even if it had a different amount of the common enzymes. In fact, the deviation of flux distribution occurs in this way during the transient phase because in the common segment the fraction of enzymes which will supply the other branch in the steady state are transiently supplying some part of the intermediates that it uses. The system, however, reaches the same steady state, as these nonlinear interactions only exist while the pool concentrations are changing; however, they allow some branches to reach steady state earlier and others later. When an unbranched system evolves to an asymptotically stable steady state from the empty state (without intermediates), it has to fill all its intermediate pools. The acceleration of a given branch in the transient is due to the fact that it does not contribute to fill the system intermediate pools in the same proportion as it will use that mass invested there. In other words the branch that has been accelerated during the transient has not contributed to the common pool in the proportion that it does in the steady state. So $\sigma_{\mathrm{P}ts}$ is different from $\sigma_{\mathrm{P}ss}$, $\sigma_{\mathrm{P}ts}$ being the contribution that the branch has made to the system during the transient phase, and $\tau_{\mathrm{P}ts}$ the mean time that one molecule would spend in crossing the system at steady state through that chain if it could operate at the same flux with a total internal pool concentration of $\sigma_{\mathrm{P}ts}$ instead of $\sigma_{\mathrm{P}ss}$. It is interesting to see that the events that cause the transient phase to deviate from linear behaviour concern differences in mass production and use, and thus they can be well described by the variables described here. $\Delta\sigma_{\mathrm{P}}$ and $\Delta\sigma_{\mathrm{Q}}$ describe that difference of mass due to the deviation of linearity, carried out by each chain during the transient.

4. Control of Transient Pools and Times

Consider the branched pathway in Scheme 1. From any state it can be modulated by multiplying all enzyme concentrations by the same factor α (assumed to be greater than one) so that any enzyme concentration e_i is replaced by αe_i. At steady state all interactions are stabilized in both the original and the modulated system. During the transient phase, the interactions change with time, but as we have changed proportionally all enzymes by the same factor α, only the magnitudes of such interactions will have been changed, but not the ratios between them, assuming no direct interactions between them (hypotheses of independence). So the modulated system differs from the original one only in operating on a shorter timescale. The effects of the modulation are known from familiar theorems of metabolic control analysis: according to the hypothesis of additivity, the flux J increases by a factor α, the transit time τ decreases by the same factor α, and the pool size σ is unchanged, because J, τ and σ are homogeneous function of e_i of degrees 1, -1 and 0, respectively. Everything depending on enzyme rates happen in the modulated system in the same sequence as before, but on a shorter timescale; any event that occurs in the unmodulated system at time t will happen in the modulated system at time t/α. So, as $\Delta\sigma_P = \sigma_{Pts} - \sigma_{Pss}$ is a result of the total transient (see Fig. 3) its value will be the same as without the modulation. This provides the following theorem:

THEOREM 4

$$\sum C_{e_i}^{\sigma_{Pts}} = 0. \tag{13}$$

This is the summation theorem for the control of transient pools. It applies to any branch of the system. In an unbranched system it is the same as the familiar theorem described by Heinrich & Rapoport (1974) (see also Meléndez-Hevia *et al*., 1990a), but in branched systems it is different, because then the control coefficients of σ_{Pts} do not have to be the same as those of σ_{Pts}. The real control of the transient phase is the control of the transient-phase variables, such as σ_{Pts} and τ_{Pts}.

Theorem 1 states that modulation of enzyme activities by a factor α decreases the transit times by

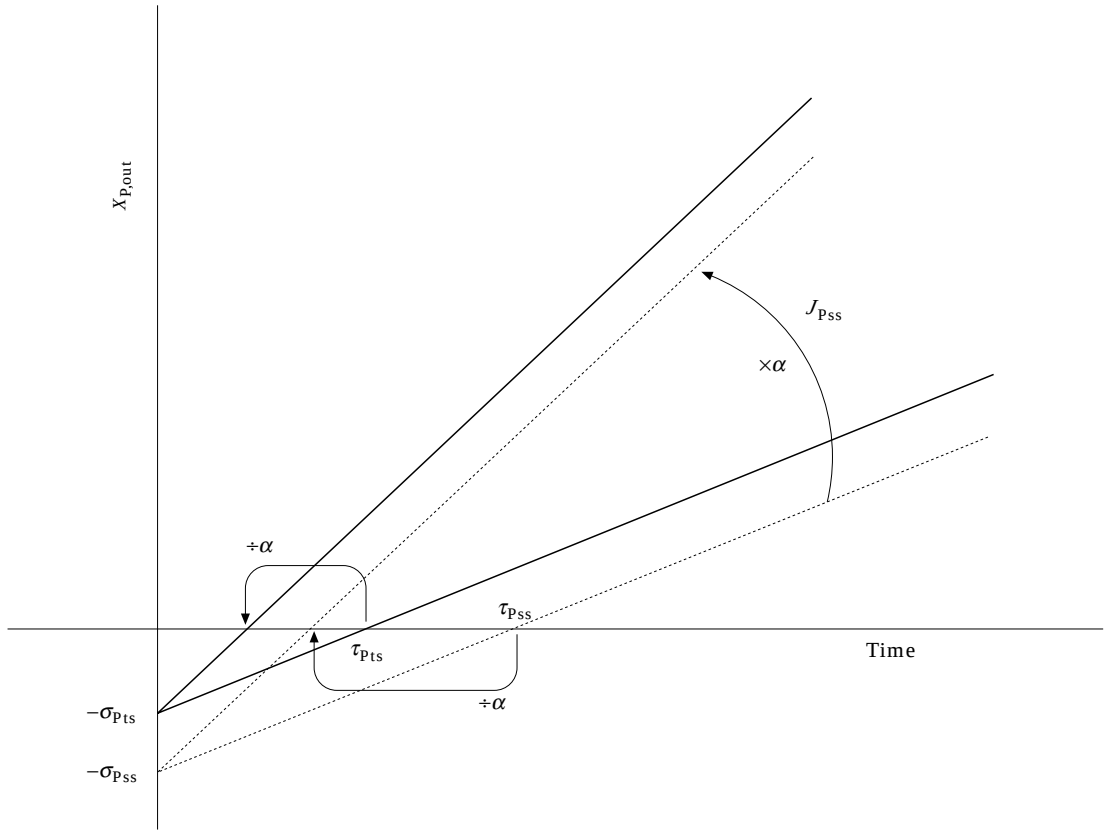


FIG. 3. Transient behaviour required by Theorems 1, 4 and 5. Modulation of any branched system by increasing all enzyme activities by a factor α causes all fluxes to increase by α, all times to be decreased by a factor α, and all masses to be unchanged. In effect the modulated system behaves exactly like the original one but on a shorter timescale.

the same factor α. On the other hand, in the modulated system all functions strictly dependent on the enzyme rate arise to the same extent but with a timescale decreased by the factor α. Transient times must therefore also be related in the same way, providing the following theorem:

THEOREM 5

$$\sum C_{e_i}^{\tau_{Pts}} = -1 \qquad (14)$$

which is the summation theorem for the control of transient times.

Theorem 3 states that any acceleration of one branch during the transient phase produces delays in others; any stimulus that decreases τ_{Pts} simultaneously increases τ_{Qts} elsewhere. The sluggishness of some metabolic pathways is a consequence of the promptness of others.

To summarize the relationships between transient and transit times shown in this paper we end with a sentence reminiscent of the concluding words of Kacser & Burns (1979). In the Molecular Bureaucracy the struggle for time in a pathway is governed by the following principle: a branch can be accelerated by using the time of other branches; the more branches a pathway has the more of the others' time any one of them can use, and the more it can be accelerated in the transient phase. According to Darwin's principle of natural selection, the struggle for life is based on competition between genetically different individuals of the same species; in the Molecular Bureaucracy, control of promptness is based on competition between different branches of the same pathway. This similarity of the competition between individuals in the struggle for life and the competition between branches of metabolic pathways for quicker responses suggests to us the possibility that branched pathways have played a role in the evolution of response times, and we plan to study this in more depth in the future.

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