Generalization of the Theory of Transition Times in Metabolic Pathways: A Geometrical Approach

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ABSTRACT Cell metabolism is able to respond to changes in both internal parameters and boundary constraints. The time any system variable takes to make this response has relevant implications for understanding the evolutionary optimization of metabolism as well as for biotechnological applications. This work is focused on estimating the magnitude of the average time taken by any observable of the system to reach a new state when either a perturbation or a persistent variation occurs. With this aim, a new variable, called *characteristic time*, based on geometric considerations, is introduced. It is stressed that this new definition is completely general, being useful for evaluating the response time, even in complex transitions involving periodic behavior. It is shown that, in some particular situations, this magnitude coincides with previously defined transition times but differs drastically in others. Finally, to illustrate the applicability of this approach, a model of a reaction mediated by an allosteric enzyme is analyzed.

INTRODUCTION

Cell metabolism is a complex network of biochemical reactions that is continuously interacting with its environment. Thus, it can be viewed as a dynamic system that is able to adapt its behavior to changes in both the internal parameters (kinetic constants or enzyme concentrations) and the boundary constraints (input source of material or concentration of external metabolites). This adaptation occurs in a period of time that depends on the intrinsic properties of the system, mainly the design of the pathway (stoichiometric properties) and kinetic factors. Moreover, this period of time must also depend on both the current state of the system—the initial state and the boundary constraints—and the kind of perturbation it undergoes.

Getting a wide knowledge of the response time (in a general sense, the time spent to respond to a stimulus) has important implications. Within an evolutionary context, this study may allow us to obtain important clues to how cell metabolism has evolved. Response time is a key feature of living beings that is frequently critical in the struggle for life. It is decisive, for example, for predators to capture prey and for prey to escape from predators. In a more general sense, response time is a variable that determines a kind of behavior, and so it must agree with each particular ecological niche. A logical hypothesis is that every aspect of the macroscopic behavior of a species must have a closely related molecular design behind it. This includes, of course, response time. In effect, Lupiáñez et al. (1996), exploring the transition from aerobic to anaerobic glycolysis, as the metabolic support of the flight promptness in several birds, showed that long-distance flying birds—which have, however, a slow start—have a long metabolic response time, whereas the sprinters—characterized by a quick macroscopic start—showed a short metabolic response time. Thus, according to natural selection, the response time of present-day metabolic routes might be strongly adapted to its functionality, and thus macroscopic behaviors must reflect microscopic transition times.

From a biotechnological viewpoint, a suitable knowledge of the response time could allow the control and regulation of cell metabolism (for instance, by changing either the kinetic properties or the design of the pathway). If cells are considered as factories of bioproducts (Bailey, 1991), the main consequence would be the possibility of improving this function. Nevertheless, the complexity of metabolic behavior (Goldbeter, 1996) makes it difficult, in many instances, to measure the response time. There are similar considerations regarding the time for drug action in metabolism.

The theory of the response time has been developed by several researchers for the last 20 years (Heinrich and Rapoport, 1975; Easterby, 1973, 1981, 1986; Meléndez-Hevia et al., 1990, 1996; Torres et al., 1991; Cascante et al., 1995, 1996; Heinrich and Schuster, 1996; Lloréns et al., 1997, among others). It is noteworthy, however, that despite its obvious importance, this subject remains at present virtually unexplored. As far as we know, only a few direct empirical determinations of metabolic response times have been described (Torres et al., 1990; Torres and Meléndez-Hevia, 1992; Lupiáñez et al., 1996). Transition times in human erythrocyte by kinetic modeling have also been assayed (Rapoport and Heinrich, 1975; Werner and Heinrich, 1985). A possible reason for the little attention paid to this key feature could be the lack of a general agreement on the theory. In fact, the proposed definitions of a represen-

Received for publication 17 August 1998 and in final form 9 April 1999.

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0006-3495/99/07/23/14 $2.00

tative time of transition have differed from each other, depending on the initial and final state, and in general they have been defined for very restrictive boundary constraints and transitions. This aspect clearly causes uncertainty in undertaking experimental work.

Then, a question arises: Is it possible to find a physical magnitude, theoretically well supported and experimentally measurable, that is useful for the study of the time a system variable takes to achieve any transition from a state A to another state B, regardless of what they are, and independently of the boundary constraints? In this work we shall prove that the answer is positive, which leads to a completely general definition for the characteristic time of a transition.

THEORETICAL FRAMEWORK

In most models, both experimental and theoretical, time is treated as a parameter. However, to make a theory of temporal transitions (i.e., to investigate how transitions depend on system parameters as kinetic constants or enzyme concentrations), we need to deal with time as a function. The problem of evaluating the transition time and studying its properties is difficult for two main reasons: Mathematically, approaching to the final state is asymptotic, and it requires an infinite time. From an experimental point of view, it is always difficult to decide how close to the steady state the system variable is. To overcome these difficulties, historically the question of how to measure a time representative of the transition in metabolic pathways has been tried through different approaches.

Hess and Wurster (1970) analyzed experimentally an irreversible metabolic system of two reactions under saturating conditions of the first enzyme. They called the intersection point of the asymptote of the progress curve (recording the concentration of the end product with time) with the time axis the *transient time*, assuming the system was initially empty. They showed that it corresponds to the reciprocal of the eigenvalue from the theoretical model of this system.

Easterby (1973) extended this analysis to multienzyme sequences under similar constraints (irreversibility and saturating conditions of the first enzyme). He proved that each enzyme has a transient time, and that the overall transient time is given by the sum of the individual transients. Moreover, each transient time can be obtained through the ratio between the stationary concentration of the *i*th intermediate, $\bar{x}_i$, and the flux at steady state, $\bar{J}$, i.e.,

$$\tau_i^E = \frac{\bar{x}_i}{\bar{J}} \tag{1}$$

Later, Hearon (1981a) proved that in linear systems the transient time corresponds to an average time. In subsequent papers, this definition has been extended to 1) reversible reactions, even when the differential equations describing the progress curve are not readily amenable to analytical solution (Easterby, 1981); 2) transitions between steady states (Easterby, 1981); 3) systems in which the input of source material varies with time (Easterby, 1986); and 4) systems evolving under constant affinity constraints (Lloréns et al., 1997). It has also been shown that under constant input flux, the transient time corresponds to the time a molecule needs to cross the reaction chain at steady state, which has been referred to as transit time (Easterby, 1981; Hearon, 1981b; Morán et al., 1997).

An alternative way of calculating the transition time, initially formulated for the concentrations of chemical reactants, was introduced by Heinrich and Rapoport (1975). If $\delta_x(t)$ is the instantaneous deviation of a metabolite concentration, $x(t)$, from the steady-state value, $\bar{x}$, i.e., $\delta_x(t) = x(t) - \bar{x}$, then its transition time is given by

$$\tau_x^H = \frac{\int_0^\infty t\delta_x(t)dt}{\int_0^\infty \delta_x(t)dt} \tag{2}$$

As the authors pointed out, to have a well-defined magnitude, the sign of δ_x must not change during the transition. This definition takes also into account the overall features of the temporal evolution of the variable by weighting the time with $\delta_x(t)$. Although in particularly simple linear systems (e.g., $A \rightleftarrows B$) the approaches mentioned above yield the same result, in more complex situations they lead to measurements that are clearly divergent. In fact, as will be shown below, none of the referred times are representative of important transitions, and furthermore, these magnitudes are not defined in complex situations.

A third direction in evaluating transition times was suggested by Easterby (1973) and later by Storer and Cornish-Bowden (1974) and Torres et al. (1991). They defined a magnitude t_{99}, which measures the time a variable takes to reach 99% of its steady-state value. This magnitude can be used to compare transition times of different systems (regardless of their nature and constraints). However, its evaluation is prone to high experimental error because of the asymptotic shape (for long times) of the evolution profile. Moreover, t_{99} could not be really representative of the transition (in fact, it is not difficult to find similar transitions that differ appreciably in the value of t_{99}). Thus an average estimate for the response time seems more convenient.

The previous exposition points out the existence of a broad interest in studying the time biological systems take to undergo transitions. Furthermore, all of these definitions refer to particular transitions and constraints and fail when they are applied to other kinds of transitions, as will be commented on in the following sections. To solve this controversy, here we shall propose a general definition to characterize the response time of any system variable.

A POSSIBLE SOLUTION: A GEOMETRIC DEFINITION

A deep characterization of the time associated with any transition requires the definition of a magnitude that can be

handled both experimentally and theoretically. Because transitions between states can be described by the evolution profile of the variable of interest (output flux, metabolite concentration, etc.), the characteristic time of the transition must contain some average information of the overall process, also making possible the comparison between different types of transitions.

As shown in Fig. 1, in which three hypothetical transitions appear, valuable information on the time that a variable f takes to reach the stationary regime can be obtained from a quantification of the area between the evolution curve of the variable under study and its final state. However, by the simple consideration of this area, the curve c_1, which is clearly faster than curve c_3, would have a higher response time. Therefore, to get a correct measure of this time, the area must be conveniently normalized. The normalization factor in these curves is the global variation of the variable, i.e., $\bar{f} - f(0)$. This normalized area will be referred to as the *characteristic time* of the transition, T_c.

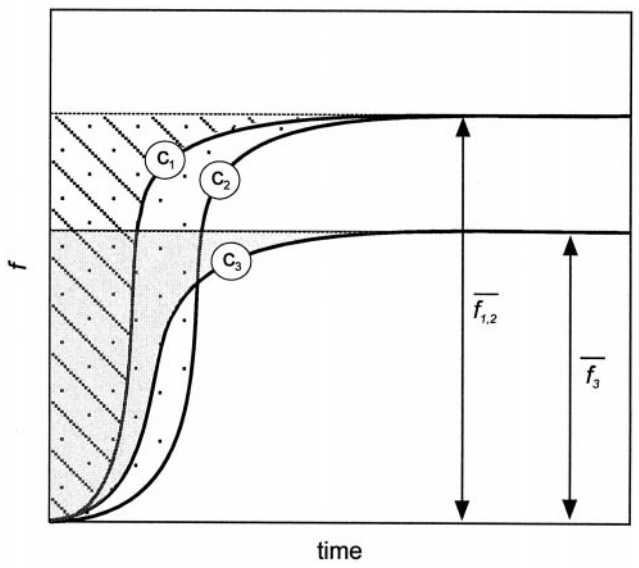

FIGURE 1 Comparison of the temporal evolution to the steady state of three hypothetical systems. Three evolution curves of a hypothetical variable, f, are shown: c_1, c_2, and c_3. A measure of the time taken by each variable to achieve a steady-state value can be obtained through the quotient of the area enclosed between the final state and the evolution curve, and the overall variation of f in each transition. It can be seen that the hatched area, which corresponds to curve c_1, is smaller than that corresponding to c_2 (*dotted curve*), whereas the two transitions lead to the same variation in f. It means that the characteristic time of c_1, $T_{c(1)}$, is lower than that corresponding to c_2, $T_{c(2)}$. With respect to c_3, although its area (*shadowed area*) is smaller than that of the other two, the quotient between this area and the change in f, $T_{c(3)}$, is lower than that corresponding to c_2 and greater than the one corresponding to c_1. This reasoning leads to the conclusion

$$T_{c(1)} < T_{c(3)} < T_{c(2)}$$

It is worth remarking that these kinds of curves are obtained in systems evolving from rest under a constant input flux, J_{in}, when the output flux, J_{out}, is analyzed. In this case the area corresponds to the mass accumulated at steady state, $\bar{\sigma}$, and the overall variation in the variable under study to $J_{in} = J_{out}(\infty) - J_{out}(0)$. Then, $T_c = \bar{\sigma}/J_{in}$, as defined by Easterby (Eq. 6). Thus the problem is reduced to determining which is the correct area to be computed in each case, as well as finding the criteria of normalization.

In the following subsections, the characteristic time T_c will be calculated for different kinds of transitions that have previously been analyzed in the literature. The variables commonly measured are the output flux, J_{out}, and the input flux of the pathway, J_{in}. It is assumed that both variables are monotonous functions, which means that the signs of their derivatives do not change during the evolution. In all of these cases the area and the normalization factor can be straightforwardly found. However, as will be discussed later, when flux is not monotonous, the relationship between the area and the normalization factor is not so obvious (see, for instance, Fig. 5).

Transition from rest under constant input flux

Many biochemical systems can be supposed to work under a constant J_{in}. In this situation, it is interesting to analyze the transition time of the output flux of the pathway, J_{out}. Under special conditions, and assuming that initially the concentration of every intermediate is null (transitions from rest) (Hess and Wurster, 1970; Easterby, 1973), the temporal evolution of J_{out} has a shape similar to those depicted in Fig. 1.

To illustrate the evaluation of the characteristic time of the transition, consider the curve labeled c_1. As commented on in the previous paragraph, the area to be taken into account for the estimation of T_c should be the hatched one, i.e.,

$$A = \lim_{t\to\infty} \left(J_{in} t - \int_0^t J_{out} dt \right) \tag{3}$$

But, at any time t from the initiation of the transition, mass conservation requires

$$J_{in} = \sum_{i=1}^{n} \frac{dx_i(t)}{dt} + J_{out}(t) \tag{4}$$

and integrating over the time,

$$J_{in} t - \int_0^t J_{out} dt = \sum_{i=1}^{n} x_i(t) \tag{5}$$

From this expression it becomes clear that, if the system achieves a stationary regime, the area A corresponds to the mass accumulated at steady state, $\bar{\sigma} = \lim_{t\to\infty} \Sigma_{i=1}^{n} x_i(t)$. If A is normalized by the variation of flux as a consequence of the transition, i.e., $J_{in} = J_{out}(\infty) - J_{out}(0)$, the characteristic time that results is

$$T_c = \frac{\bar{\sigma}}{J_{in}} = \tau^E \tag{6}$$

Therefore, in systems under this kind of constraint, the characteristic time of the output flux corresponds to the transient time defined by Easterby, τ^E. In addition, as was already pointed out by this author, the transient time (and so, the characteristic time) is given by the intersection of the asymptote to the progress curve (i.e., the integral of the output flux) and the time axis (Easterby, 1973).

Transition between steady states under constant input flux

Under physiological conditions, transitions from rest rarely occur. In practical situations, metabolism responds to variations in the environment by changing its steady state, characterized by particular values of the intermediate concentrations and fluxes, to another state (that, in the case of temporal perturbations, could be the previous one). In Fig. 2 *A*, curve 1 represents a transition between a steady state *a*, characterized by a stationary flux $\bar{J}_a$, and a state *b*, in which the final flux is $\bar{J}_b$. The area representative of the transition is the hatched one, which, as before, is given by

$$A = \lim_{t\to\infty}\left(\bar{J}_b t - \int_0^t J_{out}dt\right) \tag{7}$$

Again using mass balance equations, and assuming that at time $t = 0$ the system is at state *a*, it is possible to prove that this area coincides with the difference between the mass accumulated at the steady state *b* ($\bar{\sigma}_b$) and that corresponding to the state *a* ($\bar{\sigma}_a$). In this case, as can clearly be seen in Fig. 2 *A*, the normalization factor is the difference in flux between the two states, which yields the following expression for the characteristic time:

$$T_c = \frac{\bar{\sigma}_b - \bar{\sigma}_a}{\bar{J}_b - \bar{J}_a} \tag{8}$$

It is important to remark that now this time differs from the one proposed by Easterby for transitions between steady states (Easterby, 1981), $\tau_{ab} = (\bar{\sigma}_b - \bar{\sigma}_a)/\bar{J}_b$. A careful inspection of Fig. 2 *A* shows that, in this case, considering $\bar{J}_b$ as the height of the transition leads to an underestimation of the response time (but the result could also be an overestimation, when $\bar{J}_b < |\bar{J}_b - \bar{J}_a|$). Actually, the higher $\bar{J}_b$ is, the lower the value obtained for τ_{ab}. To illustrate this fact, let us compare transition 1 with transition 2 (Fig. 2 *A*). They represent qualitatively similar transitions, but 2 starts from rest and 1 starts from the stationary state *a*. Strikingly, the resulting τ_{0b} (i.e., τ^E for transition 2) would be much higher than τ_{ab} for transition 1, whereas their characteristic times evaluated through Eq. 8 are equal.

It should be noted that in those situations in which $\bar{J}_a = \bar{J}_b$ (as occurs with temporal perturbations), Eq. 8 is not valid, because in those cases the derivative of J_{out} changes

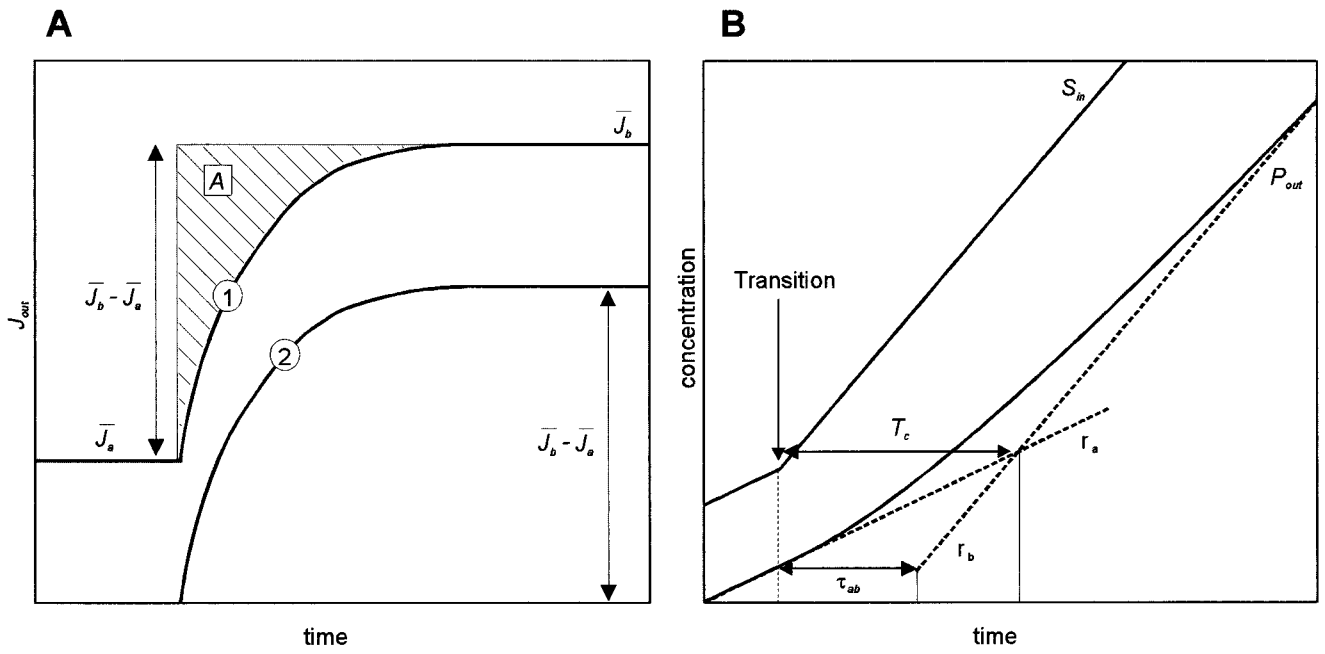

FIGURE 2 (*A*) Transition between two steady states under a constant input flux restriction. Curve 1 shows the output flux of a system that evolves from a steady state *a*, characterized by a stationary flux $\bar{J}_a$, to a steady state *b*, with a final flux $\bar{J}_b$. In this case $\bar{J}_b > \bar{J}_a$, but the calculation of T_c is equally valid for the opposite situation, i.e., when $\bar{J}_a > \bar{J}_b$. As in Fig. 1, time T_c is obtained through the ratio between the hatched area and the difference of fluxes $\bar{J}_b - \bar{J}_a$. As was discussed in the text, this time must correspond to the characteristic time of curve 2, which is qualitatively similar to curve 1. It can be seen that the transient time defined by Easterby, τ_{ab}, is higher for curve 2 than for curve 1, which seems to be contradictory. (*B*) Geometric determination of T_c from the progress curve of a transition between steady states under constant input flux. Mass that enters ($S_{in} = J_{in}t$) and leaves the system ($P_{out} = \int_0^t J_{out}dt$) is plotted versus time. T_c is obtained as the time at which the asymptote to the progress curve of state *a*, r_a: $\bar{J}_a t$, intersects that corresponding to state *b*, r_b: $\bar{J}_b t - (\bar{\sigma}_b - \bar{\sigma}_a)$. Notice the difference between T_c and Easterby's transient time, τ_{ab}.

its sign (i.e., J_{out} is not monotonous). This situation will be analyzed later.

As in the previous subsection, the characteristic time can be geometrically obtained from the progress curve shown in Fig. 2 *B*. In fact, T_c corresponds to the solution of the following equation (see Eq. 8):

$$\bar{J}_a t = \bar{J}_b t - (\bar{\sigma}_b - \bar{\sigma}_a) \tag{9}$$

where $\bar{J}_a t$ is the straight line asymptotic to the progress curve of state *a*, and $\bar{J}_b t - (\bar{\sigma}_b - \bar{\sigma}_a)$ is that corresponding to state *b*. Therefore, the characteristic time can be geometrically obtained as the intersection point of the two asymptotes.

Transition from rest under constant affinity constraints

When a metabolic pathway, with a given equilibrium constant for the conversion of the initial substrate into the final product, evolves under a constant concentration of these metabolites, is said that it is constrained to work at constant affinity. In this case, both the input and output fluxes, J_{in} and J_{out}, respectively, are reversible and variable with time. Fig. 3 *A* shows their typical profile. Because initially the system is empty, a negative local affinity appears in the last reaction and mass enters from the product, which is transduced in a negative value of J_{out} at the beginning (Lloréns et al., 1997). The evolution of the input flux has already been discussed for systems under variable input of material and irreversible output (the special case of infinite affinity) (Hearon, 1981b; Easterby, 1986; Torres et al., 1991). Again, the areas enclosed between each curve and the final state will be chosen to estimate the corresponding characteristic time:

$$A_{in} = \lim_{t\to\infty} \left(\int_0^t J_{in} dt - \bar{J}t \right)$$

$$A_{out} = \lim_{t\to\infty} \left(\bar{J}t - \int_0^t J_{out} dt \right) \tag{10}$$

where $\bar{J}$ is the steady-state flux. It has been proved that these areas, A_{in} and A_{out}, can be associated with the stationary masses accumulated because of the variable input, $\bar{\sigma}_{in}$, and the variable output, $\bar{\sigma}_{out}$, respectively (Torres et al., 1991; Cascante et al., 1995). The overall mass at steady state is given by $\bar{\sigma} = \bar{\sigma}_{in} + \bar{\sigma}_{out}$. The normalization factor corresponds to the difference between the steady-state flux and the value of the fluxes at time zero. Thus the expressions for

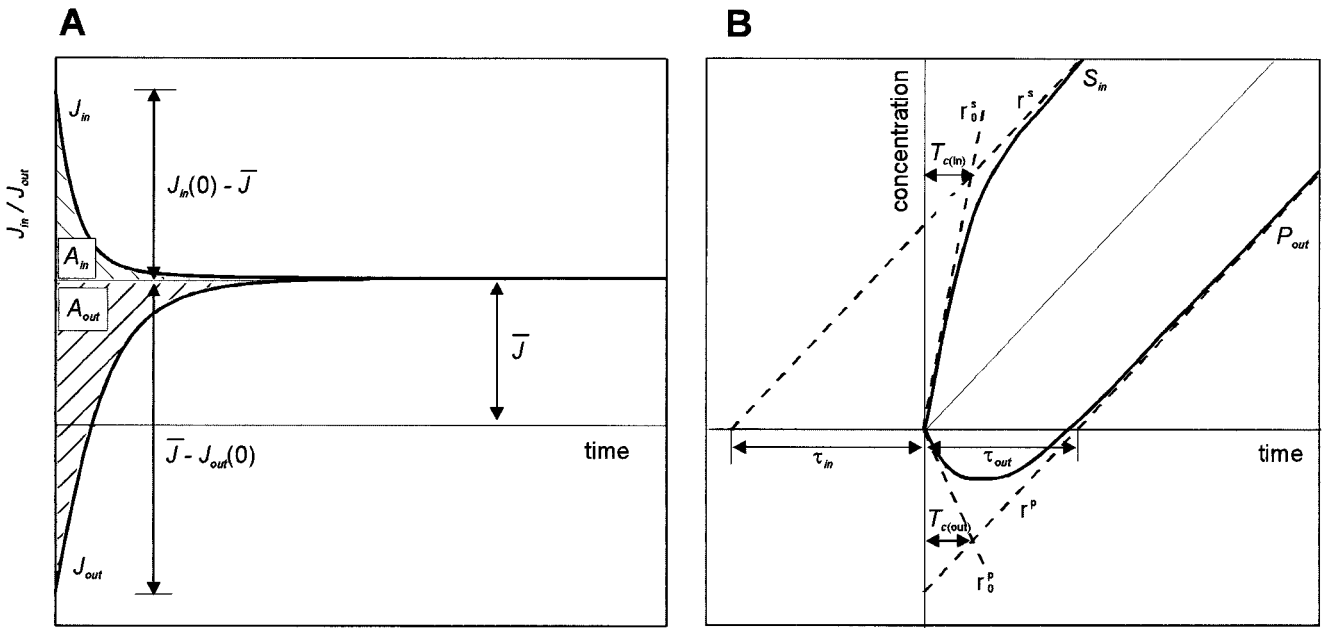

FIGURE 3 (*A*) Temporal evolution of a system evolving from rest under a constant affinity constraint. Input and output fluxes (J_{in} and J_{out}) are plotted versus time. Because both velocities are variable, it is possible to define a characteristic time for each one, $T_{c(in)}$ and $T_{c(out)}$. For the estimation of $T_{c(in)}$, the area to be considered is labeled A_{in} (which corresponds to the mass accumulated at steady state because of the variable input, $\bar{\sigma}_{in}$), and the normalization factor is $J_{in}(0) - \bar{J}$ (the overall variation in the input flux). Then, $T_{c(in)} = \bar{\sigma}_{in}/(J_{in}(0) - \bar{J})$. Similarly, $T_{c(out)}$ is obtained as the ratio between the area labeled A_{out} (which corresponds to $\bar{\sigma}_{out}$, i.e., the mass accumulated in the steady state because of the variable output) and $\bar{J} - J_{out}(0)$. Therefore, $T_{c(out)} = \bar{\sigma}_{out}/(\bar{J} - J_{out}(0))$. Notice the difference between these magnitudes and previously defined transition times, $\tau_{in} = \bar{\sigma}_{in}/\bar{J}$ and $\tau_{out} = \bar{\sigma}_{out}/\bar{J}$. (*B*) Geometric determination of T_c from the progress curve of a system evolving under a constant affinity restriction. Mass entering ($S_{in} = \int_0^t J_{in} t$) and leaving ($P_{out} = \int_0^t J_{out} dt$) the system is plotted versus time. As can be seen, $T_{c(in)}$ is the time at which the asymptote to S_{in}, r^s: $\bar{J}_{in} t + \bar{\sigma}_{in}$, intersects the straight line with a slope equal to the initial value of the input flux, r^s_0: $J_{in}(0)t$. In a similar way, $T_{c(out)}$ is given by the intersection of the asymptote to P_{out}, r^p: $\bar{J}_{out} t - \bar{\sigma}_{out}$, and the straight line r^p_0: $J_{out}(0)t$.

the characteristic times of J_{in} and J_{out} are, respectively,

$$T_{c(in)} = \frac{\bar{\sigma}_{in}}{J_{in}(0) - \bar{J}}$$
$$T_{c(out)} = \frac{\bar{\sigma}_{out}}{\bar{J} - J_{out}(0)} \quad (11)$$

It must be stressed that, in systems evolving under this kind of constraint, other transient times associated with both the input and output flux were previously defined (Easterby, 1986; Torres et al., 1991; Cascante et al., 1995), $\tau_{in} = \bar{\sigma}_{in}/\bar{J}$ and $\tau_{out} = \bar{\sigma}_{out}/\bar{J}$. However, it is clear from Fig. 3 *A* that neither τ_{in} nor τ_{out} informs us about the average time of the corresponding transitions, because the normalization factor used in both cases ($\bar{J}$) is not adequate.

Following geometric considerations, now each characteristic time can be obtained from the progress curve of the respective velocity as the time at which its asymptote intersects the straight line whose slope equals the initial value of the velocity (see Fig. 3 *B*).

A mathematical expression for the normalized area

Although Eqs. 6, 8, and 11 for the characteristic time may look different, a careful inspection shows that all of them can be deduced from the general definition,

$$T_c = \frac{\int_0^\infty t \frac{df}{dt} dt}{\int_0^\infty \frac{df}{dt} dt} \quad (12)$$

In fact, when the function f analyzed corresponds to the output flux, J_{out}, and the system evolves from rest under a constant input, integration by parts of Eq. 12 leads to Eq. 6. Similarly, when transition starts from a state *a*, Eq. 12 reduces to Eq. 8. And finally, when the constraint imposed on the system is of constant affinity, both $T_{c(in)}$ and $T_{c(out)}$ (Eq. 11) are obtained if f is considered to be J_{in} and J_{out} in Eq. 12, respectively.

It is worth mentioning that this definition can be used to estimate the characteristic time for any variable of the system (individual reaction velocities, concentration of metabolites, etc.). The only requirement for T_c to give accurate information on the transition time is that f must be a monotonous (increasing or decreasing) function of time. In addition, df/dt must be integrable over the interval $[0, \alpha]$, for all $\alpha > 0$. The question of the convergence of the improper integrals involved in the definition of T_c can be solved by attending to the mass conservation law. For instance, when f is a reaction rate, the convergence of Eq. 12 is ensured, because its numerator is always a fraction of the mass accumulated in the system at steady state, which must be finite to accomplish the mass balance at the stationary state. On the contrary, an infinite value of T_c would indicate that the system does not reach a steady regime.

The characteristic time so defined is the subject of three complementary interpretations:

1. For systems with linear kinetics evolving from rest under constant input of substrate, Hearon proved that the transient time τ is given by a linear combination of the reciprocal of the eigenvalues of the system (all of them are strictly negative real numbers, because chemical (or biochemical) reaction models are considered; Hearon, 1981b). Under these assumptions the characteristic time coincides with the transient time, and then $T_c = -\sum_{i=1}^{n} 1/\lambda_i$.

In general, if f is a monotonous function that can be expressed by a linear combination of real exponential functions, $f(t) = \sum_{i=1}^{n} a_i e^{\lambda_i t}$, with $\lambda_k < 0$ for all k, then it can easily be shown that the characteristic time reads

$$T_c = -\frac{\sum_{i=1}^{n} a_i/\lambda_i}{\sum_{i=1}^{n} a_i} \quad (13)$$

Therefore, the characteristic time has the meaning of a preexponentially weighted average time. This magnitude has already been used to measure the features of other types of transitions, e.g., decay of excited states (Carraway et al., 1991).

2. If f is viewed as the distribution of mass of a line of infinite length, then Eq. 12 is formally identical to the expression commonly used to calculate the center of mass of the line, with a density distribution given by $\rho(x) = dm/dx$, which is

$$\langle x \rangle = \frac{\int_0^\infty x \frac{dm}{dx} dx}{\int_0^\infty \frac{dm}{dx} dx}.$$

Accordingly, the characteristic time has the meaning of the hypothetical time at which the whole transition is concentrated.

3. Complementarily, if $h(t) = f(t)/(\bar{f} - f(0))$ is considered as a distribution function, which is typical in statistics, the characteristic time

$$T_c = \langle t \rangle = \int_0^\infty t \frac{dh}{dt} dt$$

may be interpreted as the first moment or average of the probability distribution, thus sharing the meaning of mean time of the transition.

GENERALIZATION TO COMPLEX TRANSITIONS

Contrary to the profiles shown in Figs. 1–3, metabolic systems often present more complex dynamics. In fact, transitions involving critical damping, damped oscillations,

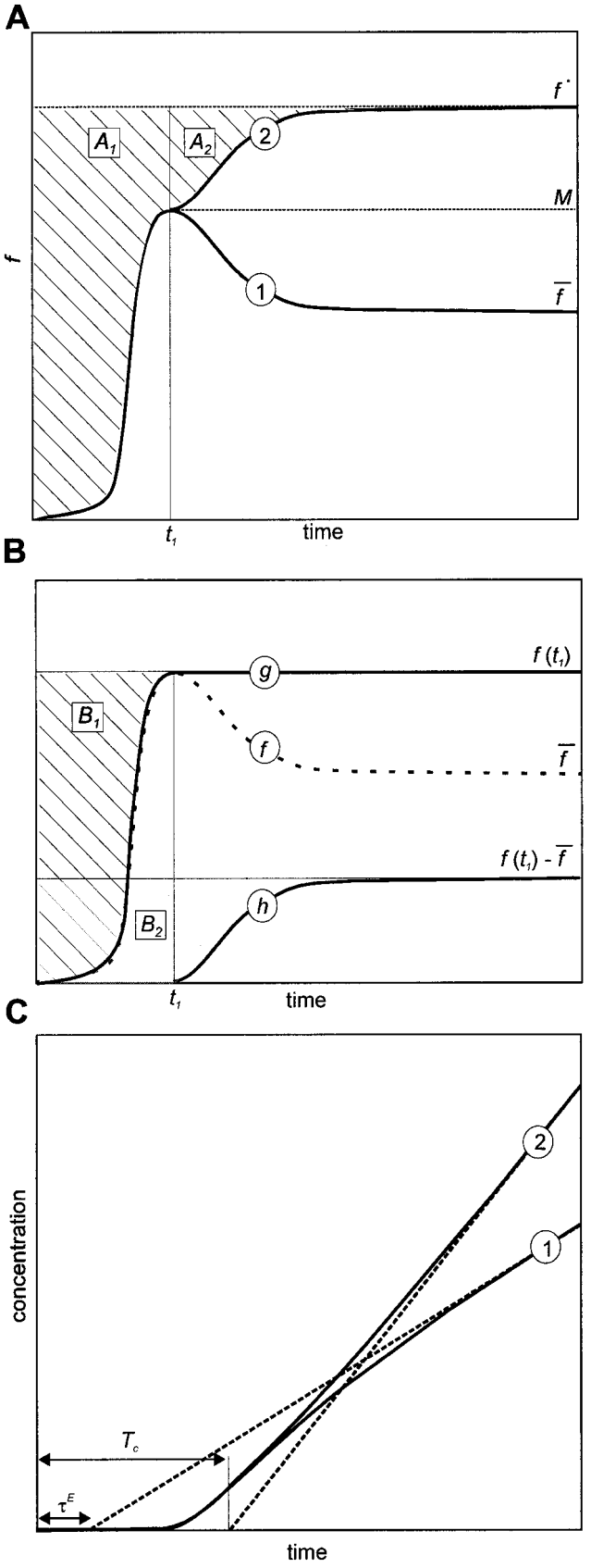

FIGURE 4 (*A*) Temporal evolution of a system variable with critical damping. Curve 1 shows the output signal (*f*) of a system that evolves toward the stationary state with critical damping. From $t = 0$ until $t = t_1$, or even sustained oscillations can be found under any kind of external constraint (Chance et al., 1964; Pye and Chance, 1966; for a review see Goldbeter, 1996). In these cases, the sign of d*f*/d*t* changes during the transition, and then the previous definition of the characteristic time is no longer valid, because negative weighted times appear. Nevertheless, as will be proved in the next subsections, there is a straightforward way of generalizing the previous definition (Eq. 12) to these complex transitions.

Critically damped transitions

The simplest complex transition involving changes in the sign of the derivative is the critically damped transition. The curve labeled 1 in Fig. 4 *A* shows the evolution of a variable with this sort of dynamics. To find an explicit expression for its characteristic time, let us consider the hypothetical transition represented by curve 2. Until $t = t_1$, its evolution profile is identical to the critically damped transition (curve 1). From this time to infinity, both transitions are mirror images with respect to the axis, *M*, parallel to the time axis. It can be assumed that if two transitions have a symmetry axis parallel to the time axis, then their characteristic times must be equal. Therefore, the evaluation of T_c for curve 2 yields a straightforward way of defining the characteristic time for critically damped transitions.

Because the sign of d*f*/d*t* does not change during the transition of curve 2, Eq. 12 can be applied to evaluate its

the derivative of *f* is positive. From $t = t_1$ to infinity, this derivative is negative. To estimate the characteristic time of this observable, curve 2 will be considered. This curve is identical to 1 until $t = t_1$. From this time to infinity, it is its mirror image with respect to the axis *M*, parallel to the time axis. Thus we have obtained a curve whose characteristic time will be equal to that corresponding to curve 1, but now the derivative of profile 2 is always positive, a necessary condition for Eq. 12 to be applied (see text). The areas to be taken into account in the calculation of T_c will be the hatched ones, $A_1 + A_2$, whereas the normalization factor will be the asymptotic state of curve 2, f^*. (*B*) Decomposition of the evolution profile depicted as curve 1 in *A*. The sum of the nondecreasing functions *g* and *h*,

$$g(t) = \begin{cases} f(t) & \text{if } 0 \le t \le t_1 \\ f(t_1) & \text{if } t \ge t_1 \end{cases}$$

and

$$h(t) = \begin{cases} 0 & \text{if } 0 \le t \le t_1 \\ f(t_1) - f(t) & \text{if } t \ge t_1 \end{cases}$$

yields function *f*. Because both *g* and *h* are monotonous functions, their respective characteristic times can be obtained through Eq. 12 as $T_c^g = B_1/f(t_1)$ and $T_c^h = B_2/(f(t_1) - \bar{f})$. Therefore, the characteristic time of *f* can be defined as the weighted average of T_c^g and T_c^h (see Eq. 20). (*C*) Geometric evaluation of T_c and τ^E for the transition described in *A*, for $f = J_{out}$. Progress curves corresponding to transitions 1 and 2 of part *A* when $f = J_{out}$ are depicted. The difference between T_c and τ^E is due to the process of inversion of those parts of the curve with negative derivative, as discussed in the text. To evaluate the characteristic time, the absolute value of d*f*/d*t* must be taken into account, which yields an asymptotic behavior with greater (as in the example) or equal slope. In general, T_c can be greater than, equal to, or lower than τ^E.

characteristic time. In this case, the area that informs us about the transition is that enclosed between the asymptotic state (which will be referred to as f^*) and the evolution of curve 2 (*hatched area* in Fig. 4 *A*). This total area has two contributions: A_1, the area enclosed from 0 to the time t_1, at which $df/dt(t) = 0$ (which corresponds with the maximum in the curve), i.e.,

$$A_1 = f^* t_1 - \int_0^{t_1} f(t)\,dt \tag{14}$$

and the rest of the area, A_2, from t_1 to infinity, which can be easily calculated as

$$A_2 = \lim_{t\to\infty}\left((t - t_1)f^* - \int_{t_1}^{t} [2f(t_1) - f(t)]dt\right) \tag{15}$$

In both expressions, $f^* = 2f(t_1) - \bar{f}$, where $\bar{f}$ is the steady-state value achieved by the function f. Then, the characteristic time of damped transitions from rest responds to the expression

$$T_c = \frac{A_1 + A_2}{f^*} \tag{16}$$

Notice that now the normalization factor is not given by the real change in the variable under study, $\bar{f}$. Using integration by parts, the numerator of this equation can be rewritten as

$$\begin{aligned} A_1 + A_2 &= \lim_{t\to\infty}\left(-\bar{f}t + 2f(t_1)t_1 - \int_0^{t_1} f(t)dt + \int_{t_1}^{t} f(t)dt\right) \\ &= \lim_{t\to\infty}\left(f(t_1)t_1 - \int_0^{t_1} f(t)dt \right. \\ &\qquad \left. - \left[\bar{f}t - f(t_1)t_1 - \int_{t_1}^{t} f(t)dt\right]\right) \\ &= \lim_{t\to\infty}\left(\int_0^{t_1} t\frac{df}{dt}dt - \int_{t_1}^{t} t\frac{df}{dt}dt\right) \\ &= \lim_{t\to\infty}\left(\int_0^{t} t\left|\frac{df}{dt}\right|dt\right) \end{aligned} \tag{17}$$

In a similar way, the denominator can be expressed as

$$f^* = \lim_{t\to\infty}\left(\int_0^{t_1}\frac{df}{dt}dt - \int_{t_1}^{t}\frac{df}{dt}dt\right) = \lim_{t\to\infty}\left(\int_0^{t}\left|\frac{df}{dt}\right|dt\right) \tag{18}$$

resulting in the following equation for the characteristic time:

$$T_c = \frac{\displaystyle\int_0^{\infty} t\left|\frac{df}{dt}\right|dt}{\displaystyle\int_0^{\infty}\left|\frac{df}{dt}\right|dt} \tag{19}$$

Therefore, a mathematical expression for the characteristic time of critically damped transitions can be found simply by considering the absolute value of the derivative of the function under study. The consideration of the absolute value is not unexpected, because the evaluation of T_c requires the estimation of all periods of time, independently of the sign of df/dt. In other words, the weight function must always be positive. The situation described here is similar to the problem of calculating the time a mechanical pendulum takes to reach the equilibrium state (independently of the direction of its movement, the time always increases). Obviously, the use of Eq. 12 would yield to an underestimated T_c.

As occurs with monotonous transitions, the convergence of the improper integrals involved in the definition of T_c (Eq. 19) is automatically ensured because of the mass conservation law. If f is a velocity, it can be shown that, because the mass accumulated in the steady state, $\bar{\sigma}$, is always finite when the system achieves a stationary regime, then the area A_2 is also finite. Therefore, T_c must be finite.

Another alternative interpretation of Eq. 19 comes from the theory of distribution functions. The variable f can be always decomposed as a sum of nondecreasing functions, i.e., $f = g - h$, where

$$g(t) = \begin{cases} f(t) & \text{if } 0 \le t \le t_1 \\ f(t_1) & \text{if } t \ge t_1 \end{cases}$$

$$h(t) = \begin{cases} 0 & \text{if } 0 \le t \le t_1 \\ f(t_1) - f(t) & \text{if } t \ge t_1 \end{cases}$$

Because both g and h are increasing functions of time, their characteristic times can be calculated by using Eq. 12. As Fig. 4 *B* shows, $T_c^g = B_1/f(t_1)$ and $T_c^h = B_2/(f(t_1) - \bar{f})$. Now, the characteristic time of f can be defined as the weighted average of the characteristic times of the increasing g and h, T_c^g and T_c^h:

$$T_c^f = \frac{f(t_1)T_c^g + (f(t_1) - \bar{f})T_c^h}{2f(t_1) - \bar{f}} \tag{20}$$

As can easily be checked, this expression coincides with Eq. 16 and, therefore, with the definition given in Eq. 19.

Fig. 4 *C* illustrates the difference between the characteristic time T_c and Easterby's definition, τ^E. In this example the value obtained for τ^E is lower than that corresponding to T_c, but the opposite situation can also be found. This fact can be understood by noting that the characteristic time

takes into account the mass *mis au jeu* during the transition, whereas τ^E only considers the mass accumulated at steady state, which is always lower than or equal to the former. Nevertheless, the normalization factor that enters the expression for τ^E is lower than the respective value for T_c (i.e., $\bar{f} \leq f^*$), which explains why the quotient between them can be lower than, equal to, or greater than the characteristic time.

It is important to remark that the same definition (Eq. 19) also applies to transitions from any state *a* to any state *b*. In particular, temporal perturbations can be treated as a particular case of critically damped transitions in which the final state is the original one, and thus T_c can be obtained from Eq. 19.

Damped oscillations

The evolution of a system variable that reaches the steady state through damped oscillations is shown by curve 1 of Fig. 5. To look for the correct expression for the characteristic time, we follow the same reasoning as that applied in the previous section. Now the curve must be inverted in every place where d*f*/d*t* is negative, that is, between each maximum and its next minimum. This yields curve 2 in Fig. 5. Because the curve oscillates infinitely approaching the steady state, the number of terms in which the overall area must be decomposed tends to infinity. As discussed in the previous subsection, both transitions 1 and 2, have identical characteristic times. Thus we define

$$T_c = \frac{A^*}{f^*} \tag{21}$$

where, again, f^* is the asymptotic state obtained after inversion of the evolution curve, and A^* is the area enclosed between this asymptotic state and the inverted curve. It can be deduced that (see Appendix)

$$A^* = \int_0^\infty t\left|\frac{df}{dt}\right|dt$$
$$f^* = \int_0^\infty \left|\frac{df}{dt}\right|dt \tag{22}$$

and then, the characteristic time responds again to Eq. 19.

For these complex situations, the problem of showing the range of convergence of Eq. 21 cannot be straightforwardly solved. Now, in the determination of T_c an infinite sum of areas (a series of real numbers) is involved. Then the improper integrals that appear in Eq. 22 converge if and only if this series converges. In general, it can be stated that to have a finite characteristic time, d*f*/d*t* must tend to zero faster than $1/t^2$ as *t* approaches infinity. This condition is always satisfied when *f* is a combination of negative exponentials, as are the solutions of linear ordinary differential equations. It is worth remarking that an infinite value of T_c would mean either that the system does not reach a steady regime or that this approximation is tremendously slow (see section Evaluation of the Characteristic Time in a Reaction Model Involving an Allosteric Enzyme, below).

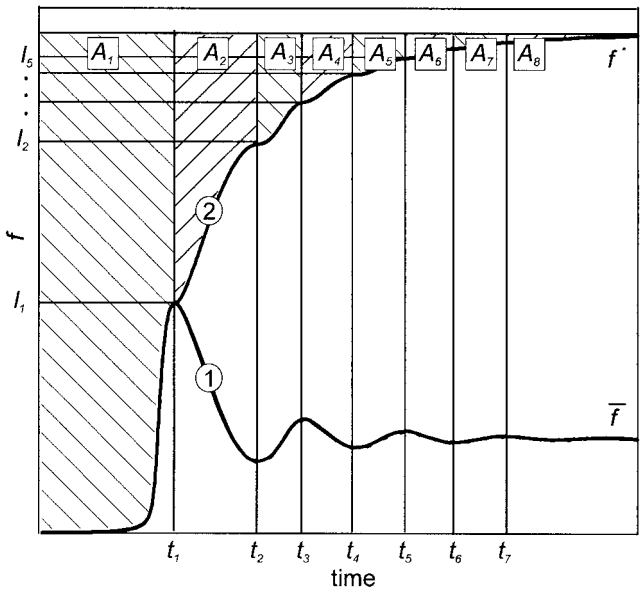

FIGURE 5 Temporal evolution of a system variable with damping. Curve 1 represents the evolution of a variable *f* toward the steady state through damped oscillations. As the estimation of T_c requires the monotonicity of *f*, its profile must be inverted in every place in which d*f*/d*t* is, for instance, negative. This process leads to curve 2. Following the same reasoning as that applied in Fig. 4, the characteristic time of this system can be obtained through the ratio between the hatched area and f^*, $T_c = A^*/f^*$ (see Appendix).

Sustained oscillations

The evolution of an observable *f* that evolves toward a limit cycle $\bar{f}$ is represented in Fig. 6 *A*. Although the final state is nonstationary ($d\bar{f}/dt \neq 0$), it is still possible to compute the characteristic time of the transition of any variable, *f*, by analyzing the evolution of $f - \bar{f}$. The resulting curve is represented in Fig. 6 *B*, and, as can be seen, it is completely analogous to the curve that evolves under damped oscillations to a steady state. Therefore, Eq. 19 can be extended to these kinds of systems only by taking into account the function $f - \bar{f}$ instead of *f*:

$$T_c = \frac{\int_0^\infty t\left|\frac{d[f-\bar{f}]}{dt}\right|dt}{\int_0^\infty \left|\frac{d[f-\bar{f}]}{dt}\right|dt} \tag{23}$$

However, contrary to Eq. 19, now the function of time $\bar{f}$ appears in the expression of T_c.

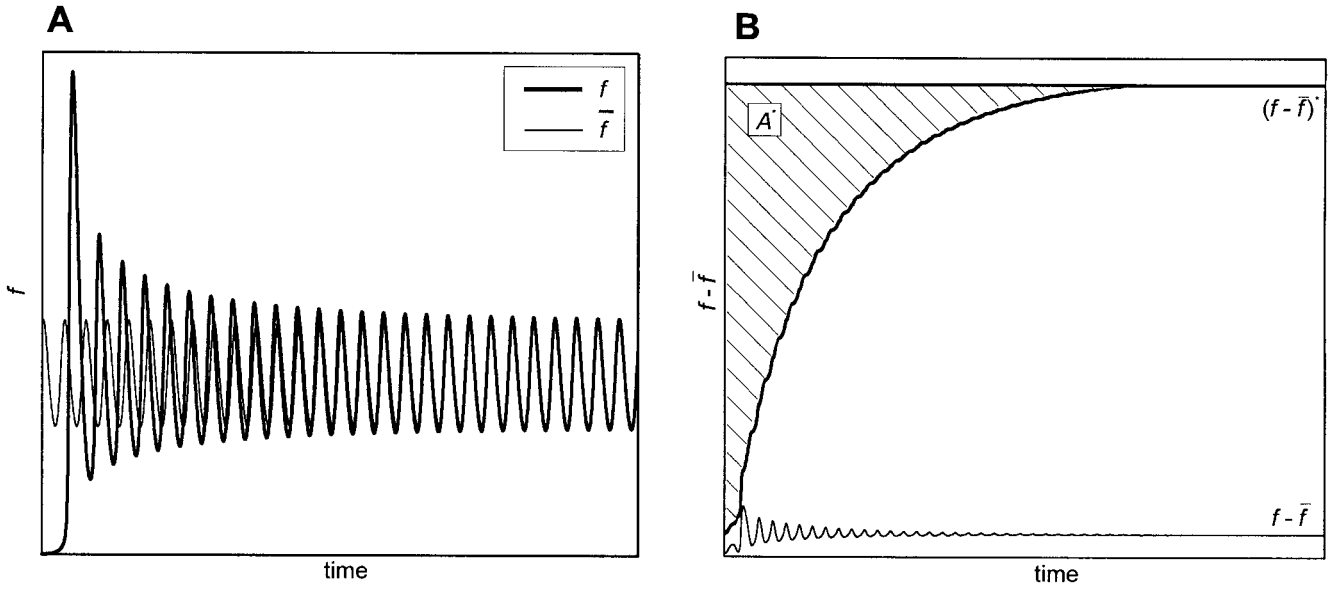

FIGURE 6 (*A*) Temporal evolution toward a limit cycle. The thicker curve shows the temporal evolution of an output signal, f, to a limit cycle, $\bar{f}$, represented by the thinner curve. (*B*) Damped convergence of the function $f - \bar{f}$. The thinner curve shows the evolution of $f - \bar{f}$ versus time. This evolution profile represents the approximation of the output signal, f, to the limit cycle, $\bar{f}$, and is qualitatively identical to that shown in Fig. 5. This makes it possible to follow the same reasoning and to obtain T_c as the ratio between the hatched area A^* and the normalization factor $(f - \bar{f})^*$.

It must be remarked that, in one-step linear reaction schemes forced with a periodic input, the characteristic time corresponds to the reciprocal of the real part of the eigenvalue. This fact shows the existence of a relationship between T_c and the eigenvalues of the system, even when the absolute value of df/dt is used.

EVALUATION OF THE CHARACTERISTIC TIME IN A REACTION MODEL INVOLVING AN ALLOSTERIC ENZYME

With the aim of analyzing the applicability of T_c to metabolic systems in which complex dynamics appear, the PFK model of Goldbeter and collaborators (Goldbeter and Lefever, 1972; Goldbeter and Nicolis, 1976, Goldbeter, 1996) is studied. This model describes the phosphorylation of fructose 6-P through the action of the enzyme phosphofructokinase in glycolysis. This reaction has been proved to be the main cause of the oscillatory behavior of this pathway.

The dimensionless equations that account for the dynamics of the system are

$$\frac{d\alpha}{dt} = \nu - \varphi\phi(\alpha, \gamma)$$
$$\frac{d\gamma}{dt} = q\varphi\phi(\alpha, \gamma) - k\gamma \tag{24}$$

where α and γ denote the normalized concentrations of substrate (ATP or fructose 6-phosphate) and product (ADP or fructose 1,6-bisphosphate), respectively; ν denotes the constant input rate of substrate, φ is related to the maximum rate of the enzyme PFK, and k is the constant output rate of product. The parameter q can be interpreted as a scale factor that appears from the relationship between the dissociation constants for the enzyme with respect to the substrate and product.

Provided a dimeric enzyme, and when the association of the substrate to its inactive form is neglected, the function ϕ is given by the following expression:

$$\phi(\alpha, \gamma) = \frac{\alpha(1 + \alpha)(1 + \gamma)^2}{L + (1 + \alpha)^2(1 + \gamma)^2} \tag{25}$$

where L is the allosteric constant of the enzyme.

As has been well illustrated by the authors (Goldbeter and Lefever, 1972; Goldbeter and Nicolis, 1976), this system presents a very rich dynamic behavior depending on the values of the parameters. As a matter of fact, all of the transitions analyzed in the previous section (monotonous, damped, oscillatory) appear in this model. In particular, the injection rate of substrate, ν, has often been used as the bifurcation parameter (keeping the rest of the parameters constant). For instance, for the setup $\varphi = 4\ s^{-1}$, $q = 1$, $L = 5 \times 10^6$, $k = 0.1\ s^{-1}$, the following bifurcation diagram has been found (Goldbeter, 1996) (see Table 1): for low values of ν, the system presents an asymptotically stable steady state. In particular, when $0 < \nu \leq 0.078$, this state is an asymptotically stable node, changing to an asymptotically stable one-tangent node for $0.078 < \nu \leq 0.082$. In the interval $0.082 < \nu \leq 0.1083$, the system presents an asymptotically stable focus. For larger values of ν the fixed point becomes unstable through a Hopf bifurcation, and a periodic orbit surrounding it appears, i.e., a limit cycle ($0.1083 < \nu \leq 1.486$). The amplitude and period of this

TABLE 1 Dynamic behavior of the PFK model as a function of the input rate *v*

Bifurcation parameter	Phase plane	Dynamic behavior starting from rest
$0 < v \leq 0.078$		Fig. 1
$0.078 < v \leq 0.082$		Fig. 4A
$0.082 < v \leq 0.1083$		Fig. 5
$0.1083 < v \leq 1.486$		Fig. 6A
$1.486 < v \leq 3.00$		Fig. 5
$3.00 < v \leq 3.85$		Fig. 4A
$3.85 < v \leq 4.00$		Fig. 1

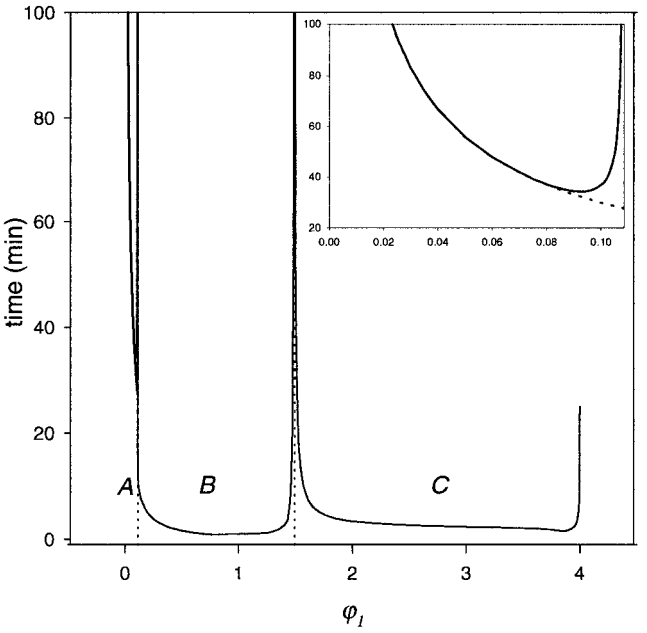

FIGURE 7 Estimation of T_c and τ^E in a reaction step mediated by an allosteric enzyme. The characteristic time is evaluated for the model described by Eq. 24. The parameters used are $\varphi = 4\ s^{-1}$, $q = 1$, $L = 5 \times 10^6$, and $k = 0.1\ s^{-1}$. Depending on the value of the input flux, v, different dynamic behaviors can be found (see Table 1). Notice how T_c tends to infinity near the Hopf bifurcation points *A–B* and *B–C*, because the real part of the eigenvalues tends to zero. The inset shows the interval $0 < v \leq 0.1083$, in which the system converges to a fixed point (node or focus) and, thus, where Easterby's definition can be applied. The qualitative difference between the two magnitudes, T_c (represented by a continuous line) and τ^E (shown by a discontinuous line), can be seen.

orbit depend on the value of v. Finally, in the interval (1.486, 4.00) the system again has a unique fixed point that is either an asymptotically stable focus ($1.486 < v \leq 3.00$) or an asymptotically stable node in the interval ($3.00 < v \leq 4.00$) (concretely, a one-tangent node for $3.00 < v \leq 3.85$ and a simple node for $3.85 < v \leq 4.00$).

Although this model has been deeply analyzed and its application to the glycolytic pathway broadly admitted, as far as we know, there is no work devoted to the study of the response time of this enzymatic reaction scheme. In this section, the characteristic time will be estimated for different values of the bifurcation parameter. It is assumed that at initial time, when an injection of substrate is applied, the system is completely empty, i.e., the system undergoes a transition from rest. Fig. 7 shows the resulting values for T_c. As can be seen, the characteristic time can be obtained under any kind of dynamic behavior. Moreover, this magnitude is a continuous function of the injection velocity, v, although bifurcations node-focus and focus-node exist. However, it tends to infinity near the Hopf bifurcation points, *A–B* and *B–C*. This fact is a direct consequence of the appearance of centers at these bifurcation points (so that the real parts of the eigenvalues approach zero). The infinite increase of the characteristic time for values of v near $4\ s^{-1}$ is due to the existence of a limit in the capacity of the system. As a matter of fact, when $v > 4$, the system does not achieve a stationary regime because more matter enters than can be processed by the enzymes.

Finally, it is worth comparing the values obtained for the transient time defined by Easterby (τ^E) and the characteristic time (T_c). As was stated in previous sections, in those ranges where the system presents a monotonous convergence to a node, which occurs in the intervals (0, 0.078) and (3.85, 4.00), the two definitions coincide. However, in the intervals (0.078, 0.082) and (3.00, 3.85), they differ, although the attractor is still a node (critically damped approximation to the fixed point). As can be seen in Fig. 7, in the ranges in which the global attractor of the system is a focus ((0.082, 0.1083) and (1.486, 3.00)), τ^E is lower than T_c. For the limit cycle regime (0.1083, 1.486), Easterby's definition is no longer valid. However, for large values of t, an average transit time can be defined as $\langle\tau\rangle = (1/T)\int_t^{t+T} \tau(t)dt$, where $\tau(t) = \sigma(t)/v$. The comparison with T_c shows an important difference between the two magnitudes, as the average of Easterby's definition decreases monotonically, even in the bifurcation point *A–B*.

DISCUSSION

In this paper we have studied the response time of general metabolic systems. With this aim, an average time called

characteristic time, denoted as T_c, has been defined. When the variable of interest, f, is monotonous, this magnitude takes into account the area between the evolution curve of the variable f and its value at the steady state, $\bar{f}$. Nonetheless, in more complex situations (in which the sign of df/dt changes with time) the characteristic time is related to an area defined by the global trajectory of the observable and a hypothetical state, f^*. Such an area must be normalized by a factor that depends on the final state and the initial conditions. In this way, T_c is dependent on the characteristics of the system dynamics and must be considered a function instead of a parameter. It must be pointed out that, from an intuitive geometric definition, a general mathematical expression has been obtained. Thus, although a different expression for T_c can be deduced in each kind of transition (see Eqs. 6, 8, 11, 12, and 19), all of them correspond to the general Eq. 23, valid for any variable (fluxes, metabolite concentrations, etc.) and for systems evolving under any type of boundary constraint (constant input flux, constant affinity, etc.), undergoing any kind of transition (from rest, from any state a to any state b, relaxation after a perturbation, etc.) and achieving any kind of final regime, either stationary or oscillatory.

To show the applicability of this definition to calculation of the characteristic time of biochemical systems, a theoretical model of a reaction step mediated by an allosteric enzyme (Goldbeter and Lefever, 1972; Goldbeter and Nicolis, 1976) has been studied. It has been shown that, depending on the rate of injection of substrate, v, the model displays a large variety of behaviors, from stable focus to limit cycles (see Table 1). Despite this complexity, Eq. 23 has been used to compute the characteristic time for all values of v (see Fig. 7). Two major comments are worth mentioning again. First, except at those points where a Hopf bifurcation occurs, the characteristic time depends continuously on the input rate v. Second, the results present a clear divergence of the characteristic time with respect to other magnitudes previously defined. In particular, in those situations in which the system variable is not monotonous, the difference with Easterby's definition comes from the fact that the definition of the characteristic time takes into account the relationship between the total mass processed by the system and the total trajectory covered by the variable during the transition.

The characteristic time, T_c, can be obtained as a limit of partial characteristic times:

$$T_c = \lim_{\alpha \to \infty} T_c(\alpha) \tag{26}$$

where

$$T_c(\alpha) = \frac{\int_0^\alpha t \left| \frac{d}{dt}[f - \bar{f}] \right| dt}{\int_0^\alpha \left| \frac{d}{dt}[f - \bar{f}] \right| dt} \tag{27}$$

As can be seen, the normalization factor changes, depending on the interval of f analyzed ([0, α] in this case). This fact leads us to conclude that the characteristic time is not, strictly speaking, an additive function. On the contrary, the contribution to T_c of any part of the curve must be weighted with the contribution of this partial transition with respect to the whole transition (see subsection Damped Oscillations). In practice, when a partial time $T_c(\alpha)$ is measured, the information contained in the global curve for times higher than α is being neglected, and then the contribution of this partial curve to the transition is different from that when the whole profile is considered. This means that, as occurs with some magnitudes in statistics, the partial characteristic time changes as more data are taken into account.

The definition of the characteristic time must be seen as an extension of the transient time introduced by Easterby for systems under constant input flux (Easterby, 1973), which, together with the transition time defined by Heinrich and Rapoport (1975), has been commonly used to measure the response time of metabolic reactions. This work has shown that they have a similar physical meaning, as both magnitudes are weighting the time with a function representative of the variable under study (implicitly, df/dt in Easterby's time, and, explicitly, $f - \bar{f}$ in the definition proposed by Heinrich and Rapoport). This aspect was already pointed out for linear systems in the early 1980s by Hearon (1981a).

The question of which function (either df/dt or $f - \bar{f}$) must be chosen for evaluating T_c has been solved by attending to two main criteria: first, it is well known from statistics that when information about a certain probability distribution is required, the weighting function is always its derivative, i.e., the density function. Thus the first moment of the distribution, the analog to T_c, as well as higher moments are calculated. Second, by using df/dt as the density function, we are weighting the time accordingly with the rate of variation during the transition, thus stressing the temporal range where most of the transition occurs. On the contrary, averaging with $f - \bar{f}$ as the density function mainly weights the initiation period of the transition.

It is reasonable to expect that response times must be under enzymatic control. As is usually done, a control coefficient that measures the fractional change in T_c due to a fractional change in one enzyme, E_i, can be defined as

$$C_{E_i}^{T_c} = \frac{\partial T_c}{\partial E_i}\frac{E_i}{T_c} = \frac{\partial \ln T_c}{\partial \ln E_i} \tag{28}$$

As was proved by Acerenza et al. (Acerenza and Kacser, 1990), assuming a linear relationship between reaction rates and total enzyme concentrations, a simultaneous change in all of the enzymatic concentrations by the same factor will only change the time scale of the system (see also Melén-dez-Hevia et al., 1996). Thus it can be concluded that T_c is a homogeneous function of degree -1 with respect to the enzyme concentration, and then the summation theorem, $\Sigma_i C_{E_i}^{T_c} = -1$, holds. A practical consequence of this fact is that, under the above assumptions, the characteristic time of

any variable is scalable. Therefore, it can be used to compare experimental profiles obtained under different conditions, such as different enzyme concentrations.

It is important to note that the summation theorem has been stated for control coefficients of Easterby's transient time (Meléndez-Hevia et al., 1990) as well as of the transition time of Heinrich and Rapoports (1975). However, in light of the results presented in this article, the study of the distribution of control coefficients in metabolic pathways working under constraints different from constant input flux (Meléndez-Hevia et al., 1990, 1996; Cascante et al., 1996) should be revised.

The interpretation of T_c as the average of a function makes it possible to define another interesting magnitude, the standard deviation around T_c, referred to as s. This parameter is given by

$$s = \left(\frac{\int_0^\infty (t - T_c)^2 \left| \frac{d[f - \bar{f}]}{dt} \right| dt}{\int_0^\infty \left| \frac{d[f - \bar{f}]}{dt} \right| dt} \right)^{1/2} \tag{29}$$

and yields a certain measure of the width of the density function $|d[f - \bar{f}]/dt|$. Therefore, it gives information about the shape of the evolution profile of the variable $|f - \bar{f}|$. A value of s near zero would indicate that the response is of the type once for all, whereas a high value of s would correspond to a smooth transition. Obviously, it is also possible to define higher order moments. Among them, perhaps the most interesting is the third moment, also called skewness, K_w, which informs us about the asymmetry of the progress curve, that is, about the way in which the processes of initiation and asymptotic relaxation of the transition occur.

At a macroscopic level, the response time of an organism can be an important aspect of its adaptation to the surroundings and therefore of its survival. Also from a biotechnological viewpoint, it could be desirable to minimize the response time (i.e., after administration of drugs, or to obtain cellular products). But even the shape of the response may be of interest. Thus different values of the triplet (T_c, s, K_w) would imply different strategies of response. This study automatically leads to a problem of multicriteria optimization.

Because the evaluation of T_c only needs the knowledge of the "output" of the system, this magnitude can be used to evaluate the temporal transition of any device, experimental or theoretical, without knowing its structure. In other words, this means that the system can be considered a black box that responds to either external or internal perturbations by changing its output. A characteristic time exists not only for linear reaction chains, but also for pathways of any complexity (i.e., metabolic networks). In this sense, it must be remarked that, by analyzing output signals, some important clues about the structure of metabolism can be obtained (Arkin et al., 1997). Work in this direction is currently being done.

APPENDIX

This appendix is devoted to proving that the quotient between A^* and f^*, given in Eq. 21, is equivalent to the general definition of T_c (Eq. 19), even for transitions with damped oscillations (see Fig. 5).

The numerator of Eq. 19 is given by

$$\int_0^\infty t \left| \frac{df}{dt} \right| dt = \sum_{n=0}^{\infty} (-1)^n \int_{t_n}^{t_{n+1}} t \frac{df}{dt} dt \tag{A1}$$

with $t_0 = 0$. Integrating by parts, each of the integrals of the right-hand summation can be expressed as

$$\int_{t_n}^{t_{n+1}} t \frac{df}{dt} dt = f(t_{n+1})t_{n+1} - f(t_n)t_n - \int_{t_n}^{t_{n+1}} f(t)dt \tag{A2}$$

resulting in

$$\int_0^\infty t \left| \frac{df}{dt} \right| dt = \lim_{N\to\infty} \left\{ 2 \sum_{n=1}^{N-1} (-1)^{n+1} f(t_n)t_n + (-1)^{N+1} f(t_N)t_N + \sum_{n=0}^{N-1} (-1)^{n+1} \int_{t_n}^{t_{n+1}} f(t)dt \right\} \tag{A3}$$

The asymptotic value of f obtained after inversion of the evolution curve in those intervals in which df/dt is negative reads $f^* = \lim_{N\to\infty} f_N^* = \lim_{N\to\infty} \Sigma_{k=1}^{N} l_k$, where $l_k = (-1)^{k+1}[(f(t_k) - f(t_{k-1})]$, and t_k are the times such that $df/dt(t_k) = 0$. Thus, $f_N^* = -f(0) + (-1)^{N+1} f(t_N) + 2\,\Sigma_{k=1}^{N-1} (-1)^{k+1} f(t_k)$. The overall area contained between the straight-line f^*t and the inverted curve is

$$A^* = \lim_{N\to\infty} A_N^* = \lim_{N\to\infty} \sum_{k=1}^{N} A_k \tag{A4}$$

where A_k is the kth area. It can be proved by induction that

$$A_k = [f(0) + f^*](t_k - t_{k-1}) - 2 \sum_{n=1}^{k-1} (-1)^{n+1} f(t_n)(t_k - t_{k-1}) + (-1)^k \int_{t_{k-1}}^{t_k} f(t)dt \tag{A5}$$

for all $k = 1, 2, \ldots$. Therefore, the Nth area can be written as

$$A_N^* = (f^* - f_N^*)t_N + 2 \sum_{n=1}^{N-1} (-1)^{n+1} f(t_n)t_n + (-1)^N f(t_{N-1})t_N + \sum_{n=1}^{N-1} (-1)^n \int_{t_{n-1}}^{t_n} f(t)dt \tag{A6}$$

Comparing Eq. A6 and Eq. A3,

$$\left| A_N^* - \sum_{n=0}^{N-1} (-1)^n \int_{t_n}^{t_{n+1}} t \frac{df}{dt} dt \right| = (f^* - f_N^*) t_N \quad \text{(A7)}$$

Because in practice $f_N^* \to f^*$ faster than $t_N \to \infty$ as N tends to infinity, then

$$\lim_{N \to \infty} (f^* - f_N^*) t_N = 0 \quad \text{(A8)}$$

Therefore,

$$A^* = \int_0^\infty t \left| \frac{df}{dt} \right| dt \quad \text{(A9)}$$

In a similar way, it can be seen that

$$\int_0^\infty \left| \frac{df}{dt} \right| dt = \lim_{N \to \infty} \left\{ \sum_{n=1}^{N-1} (-1)^{n+1} \int_{t_{n-1}}^{t_n} \frac{df}{dt} dt \right\}$$

$$= \lim_{N \to \infty} \left\{ \sum_{n=1}^{N-1} (-1)^{n+1} [f(t_n) - f(t_{n-1})] \right\} = f^* \quad \text{(A10)}$$

Therefore, the quotient between A^* and f^* leads to Eq. 19.

This work was partially supported by grants BIO96-0895 from Programa Nacional de Biotecnología (Comisión Internacional de Ciencia y Tecnología) and DGICYT PB95-0406. M. Lloréns is a recipient of a fellowship from the FPI program of the Ministerio de Educación y Ciencia of Spain, and Y. Rodríguez is a recipient of a fellowship from the ICI program of the Agencia Española de Cooperación Internacional, through the Universidad de La Habana, Cuba.

REFERENCES

Acerenza, L., and H. Kacser. 1990. Enzyme kinetics and metabolic control. A method to test and quantify the effect of enzymic properties on metabolic variables. *Biochem. J.* 269:697–707.

Arkin, A., P. Shen, and J. Ross. 1997. A test case of correlation metric construction of a reaction pathway from measurements. *Science.* 277: 1275–1279.

Bailey, J. E. 1991. Toward a science of metabolic engineering. *Science.* 252:1668–1675.

Carraway, E. R., J. N. Demas, and B. A. DeGraff. 1991. Luminiscence quenching mechanism for microheterogeneous systems. *Anal. Chem.* 63:332–336.

Cascante, M., M. Lloréns, E. Meléndez-Hevia, J. Puigjaner, F. Montero, and E. Martí. 1996. The metabolic productivity of the cell factory. *J. Theor. Biol.* 182:317–325.

Cascante, M., E. Meléndez-Hevia, B. N. Kholodenko, J. Sicilia, and H. Kacser. 1995. Control analysis of transit time for free and enzyme-bound metabolites: physiological and evolutionary significance of metabolic response times. *Biochem. J.* 308:895–899.

Chance, B., B. Hess, and A. Betz. 1964. DPNH oscillations in a cell-free extract of *S. carlsbergensis. Biochem. Biophys. Res. Commun.* 16: 182–187.

Easterby, J. S. 1973. Coupled enzyme assays: a general expression for the transient. *Biochim. Biophys. Acta.* 293:552–558.

Easterby, J. S. 1981. A generalized theory of the transition time for sequential enzyme reactions. *Biochem. J.* 199:155–161.

Easterby, J. S. 1986. The effect of feedback on pathway transient response. *Biochem. J.* 233:871–875.

Goldbeter, A. 1996. Biochemical Oscillations and Cellular Rhythms. Cambridge University Press, Cambridge.

Goldbeter, A., and R. Lefever. 1972. Dissipative structures for an allosteric model. Application to glycolytic oscillations. *Biophys. J.* 12:1302–1315.

Goldbeter, A., and G. Nicolis. 1976. An allosteric enzyme model with positive feedback applied to glycolytic oscillations. *In* Progress in Theoretical Biology, Vol. 4. F. Snell and R. Rosen, editors. Academic Press, New York. 65–160.

Hearon, J. Z. 1981a. Asymptotic output of compartmental systems. *Math. Biosci.* 55:259–264.

Hearon, J. Z. 1981b. Transient times in enzyme and coupled enzyme systems. *Math. Biosci.* 56:129–140.

Heinrich, R., and T. A. Rapoport. 1975. Mathematical analysis of multienzyme systems. II. Steady state and transient control. *BioSystems.* 7:130–136.

Heinrich, R., and S. Schuster. 1996. The Regulation of Cellular Systems. Chapman Hall, New York.

Hess, B., and B. Wurster. 1970. Transient time of the pyruvate kinase-lactate dehydrogenase system of rabbit muscle in vitro. *FEBS Lett.* 9:73–77.

Lloréns, M., J. C. Nuño, and F. Montero. 1997. Transient times in linear metabolic pathways under constant affinity constraints. *Biochem. J.* 327:493–498.

Lupiáñez, J. A., L. García-Salguero, N. V. Torres, J. Peragón, and E. Meléndez-Hevia. 1996. Metabolic support of the flight promptness of birds. *Comp. Biochem. Physiol.* 113B:439–443.

Meléndez-Hevia, E., J. Sicilia, M. T. Ramos, E. I. Canela, and M. Cascante. 1996. Molecular bureaucracy: who controls the delays? Transient time in branched pathways and their control. *J. Theor. Biol.* 182: 333–339.

Meléndez-Hevia, E., N. V. Torres, J. Sicilia, and H. Kacser. 1990. Control analysis of transition times in metabolic systems. *Biochem. J.* 265: 195–202.

Morán, F., M. O. Vlad, and J. Ross. 1997. Transition and transit time distributions for time dependent reactions with application to biochemical networks. *J. Phys. Chem. B.* 101:9410–9419.

Pye, E. K., and B. Chance. 1966. Sustained sinusoidal oscillations of reduced pyridine nucleotide in a cell-free extract of *S. carlsbergensis. Proc. Natl. Acad. Sci. USA.* 55:888–894.

Rapoport, T. A., and R. Heinrich. 1975. Mathematical analysis of multienzyme systems. I. Modelling of the glycolysis of human erythrocytes. *BioSystems.* 7:120–129.

Storer, A. C., and A. Cornish-Bowden. 1974. The kinetics of coupled enzyme reactions. *Biochem. J.* 141:205–209.

Torres, N. V., F. Mateo, J. M. Riol-Cimas, and E. Meléndez-Hevia. 1990. Control of glycolysis in rat liver by glucokinase and phosphofructokinase: influence of glucose concentration. *Mol. Cell. Biochem.* 93:21–26.

Torres, N. V., and E. Meléndez-Hevia. 1992. Transition time control analysis of a glycolytic system under different glucose concentrations. Control of transition time versus control of flux. *Mol. Cell. Biochem.* 112:109–115.

Torres, N. V., J. Sicilia, and E. Meléndez-Hevia. 1991. Analysis and characterization of transition states in metabolic systems. *Biochem. J.* 276:231–236.

Werner, A., and R. Heinrich. 1985. A kinetic model for the interaction of energy metabolism and osmotic state of human erythrocyte. Analysis of the stationary "in vivo" state and of time dependent variation under blood preservation conditions. *Biomed. Biochim. Acta.* 44:185–212.