THE ROYAL SOCIETY

Received 24 October 2003
Accepted 25 February 2004
Published online 14 May 2004

Temperature effects on a whole metabolic reaction cannot be inferred from its components

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Changes in temperature affect the kinetic energy of the constituents of a system at the molecular level and have pervasive effects on the physiology of the whole organism. A mechanistic link between these levels of organization has been assumed and made explicit through the use of values of organismal Q_{10} to infer control of metabolic rate. To be valid this postulate requires linearity and independence of the isolated reaction steps, assumptions not accepted by all. We address this controversy by applying dynamic systems theory and metabolic control analysis to a metabolic pathway model. It is shown that temperature effects on isolated steps cannot rigorously be extrapolated to higher levels of organization.

Keywords: dynamic systems; flux control theory; metabolic rate; Q_{10}; temperature effects

1. INTRODUCTION

Temperature gradients, temporal or spatial, constitute the most conspicuous ecological traits affecting life on Earth. Most animal species are not fully homeothermic and, when exposed to these gradients, experience shifts in body temperature. Through effects on the energy state of molecules, these thermal fluctuations have pervasive influence on the rates of physicochemical reactions, and therefore on physiological variables (Geiser 1988; Withers 1992). Even small changes in body temperature can lead to physiological shifts that may distress, or even impair, animals. Temperature, then, has acted as a major selective factor in the evolution of life (Hochachka & Somero 2002). Understanding thermal adaptation, as well as temperature effects from cellular mechanisms to physiological function, organismal performance and community structure, has been the goal of numerous studies. The effects of temperature on energy flux have received a particular amount of attention given the interest risen by this issue in comparative, evolutionary and ecological physiology (Guppy & Withers 1999); and its practical applications in commercial animal breeding or management (Attrill & Power 2002; Karim *et al.* 2003) and human health (e.g. regarding the scope for hypothermic hypometabolism during cardiac surgery (Eisenburger *et al.* 2001; Gibbs & Loiselle 2001)).

Of fundamental concern to this field is to what extent information about temperature-induced changes at the molecular level can be used to infer temperature effects at higher levels of organization. This is not a simple question because the energy metabolism of organisms encompasses a myriad of biochemical reactions, and the theory predicting temperature-induced changes in the proportion of molecules over a given threshold of activation energy was originally coined for a single reaction in a test-tube (Prosser 1973; Hoar 1975). Whether or not the conceptual broadening from molecules to organisms is appropriate has been a matter of disagreement among students of the topic since the beginning of the twentieth century (see Prosser (1973) and Hoar (1975) for a discussion). The debate is understandable because the validity of such a conceptual broadening would allow us to propose causal relationships among temperature-induced shifts at different levels of organization. For instance, in biochemical reactions occurring at temperatures differing by 10 °C, the ratio of reaction rates (Q_{10}) usually ranges from two to three. If the above conceptual expansion is valid, departure from this range in organismal rates could be attributable to metabolic control.

Some important difficulties appear when attempting to explain temperature effects on organisms based on temperature effects at molecular levels. It was proved recently that calculations of Q_{10} for whole-organism metabolic rates, when performed using the traditional formula that applies to molecular events, result in a system containing more variables than equations (Chaui-Berlinck *et al.* 2002). The bottom line of this finding is that, if metabolic control is to be inferred from Q_{10} values, a real value of Q_{10} at the molecular level has to be known (or at least assumed) *a priori*. This finding, as well as additional supporting statements by various authors (Prosser 1973; Snyder & Nestler 1990; Heldmaier & Ruf 1992), suggests that the above conceptual broadening is meaningless in the context of the information derived from (and available for) empirical work with whole organisms. Therefore, the use of a Q_{10} between two and three as a null hypothesis for absence of metabolic control (e.g. Geiser 1988; Guppy & Withers 1999) is not appropriate; furthermore, a valid Q_{10} null hypothesis might just not exist for this problem. The main unsolved question in this topic is, what information

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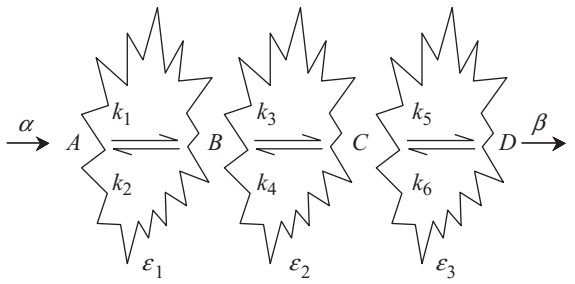

Figure 1. Scheme of the reaction pathway modelled. Catalytic steps are highlighted as 'stars'. Inflow of substrate A and outflow of product D are indicated by the unidirectional arrows (α and β, respectively). See text for nomenclature.

would validate the expansion of the Q_{10} concept from test tubes to organisms and allow for the proposal of a valid Q_{10} null hypothesis? Particularly, it is important to know whether the Q_{10} of individual steps along a chemical reaction could be used to propose a null hypothesis of expected temperature-induced changes for the whole organism. In this study, we address the above question using a minimalist model of a metabolic pathway and formally demonstrate the *non sequitur* of the rational about metabolic control underlying the transition from single reactions to an orchestrate living being.

2. THE PATHWAY MODEL

We propose a hypothetical metabolic pathway (figure 1) that is composed of three steps along which substrate A is turned into product B, B is transformed into C, and C is transformed into D. Then we present a system of differential equations that describe expected changes along time in the concentrations of A, B, C and D, based on enzyme kinetics parameters. Next, we calculate the equilibrium point of the system; that is, the concentrations of B and C that make all derivatives equal to zero and compute the net flux F at equilibrium point, which is the same at any step. This approach generates a general equation of flux change as a function of temperature that can be used to analyse temperature effects on the whole system by attributing various values of thermal sensitivity (Q_{10}) to the individual enzymes in the pathway. By comparing the sensitivities of individual steps with the resulting sensitivity of the whole pathway, we can hypothesize about the viability of using the thermal sensitivity of individual steps to postulate organismal patterns of thermal sensitivity. Last, we study the implications of metabolic control theory to the model, by developing an equation that describes the thermal sensitivity of the pathway in terms of thermal effects on its constituents. Metabolic control analysis has proved recently to be an outstanding approach to address other global questions in physiology (Darveau *et al.* 2002; Hochachka *et al.* 2003).

In the modelled pathway (figure 1), each step has forward (odd subscript) and backward (even subscript) rate constants k_i and k_j, and enzymatic activity increases reaction rates at any step. The reactions $A \leftrightarrow B$, $B \leftrightarrow C$, and $C \leftrightarrow D$ are catalysed by the enzymes ε_1, ε_2 and ε_3, respectively. The initial substrate A and the final product D are maintained at constant concentrations $\tilde{A}$ and $\tilde{D}$, as a result of the influx of A at rate α and the washout of D at rate β. Accordingly, $\mathrm{d}A/\mathrm{d}t$ and $\mathrm{d}D/\mathrm{d}t$ are set to zero in the following system of differential equations:

$$\left.\begin{aligned}
\frac{\mathrm{d}A}{\mathrm{d}t} &= \alpha + (k_2 B - k_1 \tilde{A})\varepsilon_1 = 0 \\
\frac{\mathrm{d}B}{\mathrm{d}t} &= k_1 \tilde{A}\varepsilon_1 + k_4 C\varepsilon_2 - (k_2\varepsilon_1 + k_3\varepsilon_2)B \\
\frac{\mathrm{d}C}{\mathrm{d}t} &= k_6 \tilde{D}\varepsilon_3 + k_3 B\varepsilon_2 - (k_5\varepsilon_3 + k_4\varepsilon_2)C \\
\frac{\mathrm{d}D}{\mathrm{d}t} &= (k_5 C - k_6 \tilde{D})\varepsilon_3 - \beta = 0
\end{aligned}\right\}. \qquad (2.1)$$

To simplify the equation describing flux in steady-state conditions it is convenient to define the composite variables χ, ξ and γ as follows:

$$\chi = k_2\varepsilon_1 + k_3\varepsilon_2,$$

$$\xi = \frac{k_3\varepsilon_2}{\chi},$$

$$\gamma = k_5\varepsilon_3 + k_4\varepsilon_1(1 - \xi).$$

The equilibrium point of the system is found when the concentrations of B and C render all derivatives equal to zero. These concentrations, B^* and C^*, respectively, are given by

$$B^* = \frac{k_1 \tilde{A}\varepsilon_1 + k_4 C^*\varepsilon_2}{\chi}, \qquad (2.2a)$$

and

$$C^* = \frac{\xi k_1 \tilde{A}\varepsilon_1 + k_6 \tilde{D}\varepsilon_3}{\gamma}. \qquad (2.2b)$$

Because only one solution is possible for equations (2.2), there is only one equilibrium point in the system. Such a point is asymptotically stable, i.e. once perturbed, the system tends to the pair (B^*,C^*) indicated above (e.g. Strogatz 1994; Monteiro 2002; stability analysis not shown). When the system is in steady-state conditions (i.e. at the equilibrium point), the net flux F is the same at each stage. Because $F = \alpha = \beta$, the first equation in equation system (2.1) can be chosen for further analysis and we obtain $F = (k_1\tilde{A} - k_2 B^*)\varepsilon_1$. Then, inserting equations (2.2) into the preceding equality, the flux F can be obtained as

$$F = \left\{ k_1 \tilde{A}\left[1 - \frac{k_2\varepsilon_1}{\chi}\left(1 + \frac{\xi k_4\varepsilon_2}{\gamma} \right) \right] - \frac{k_2 k_4 k_6 \varepsilon_2 \varepsilon_3 \tilde{D}}{\chi\gamma} \right\} \varepsilon_1. \qquad (2.3)$$

3. TEMPERATURE EFFECTS ON FLUX

Equation (2.3) can be employed to analyse temperature effects on the net flux of the pathway. First, a reference flux F_0 is computed at a reference temperature $T_0 = 0$ (see figure 2*a*). Then, a new flux F_i is computed at a new temperature T_i, considering a putative thermal sensitivity for each enzyme ($Q_{10}^{\varepsilon_i}$). For simplicity, thermal effects on rate constants were not taken into account. Next, we performed three sets of *in machina* experiments. In each set, two enzymes had the same thermal sensitivity, which

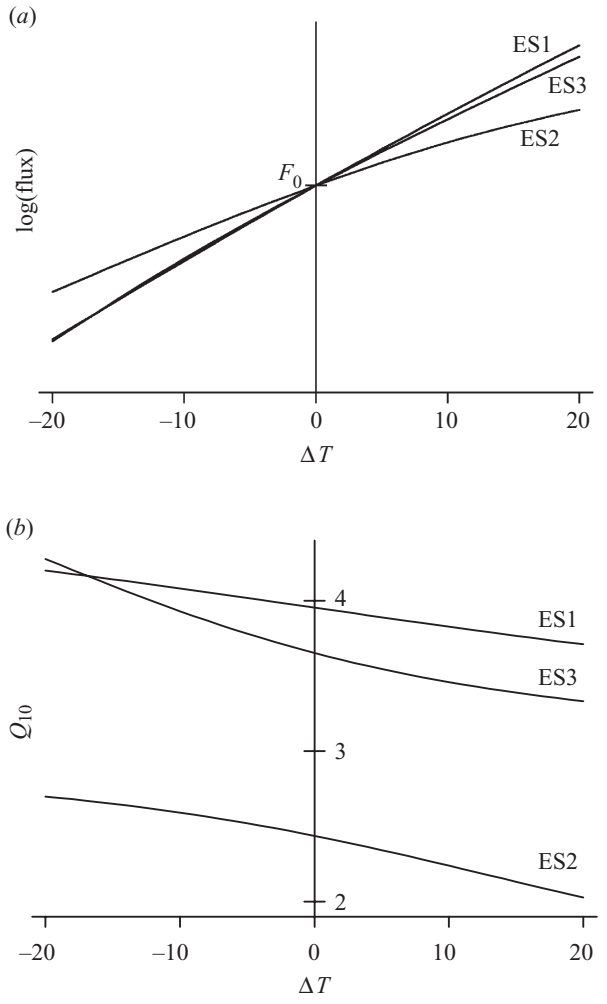

Figure 2. Graphical representation of the results from the three *in machina* experiments as functions of the temperature difference in relation to the reference temperature T_0 (arbitrary units). (*a*) Log of flux F as a function of ΔT. (*b*) Typical Q_{10}, calculated from equation (3.1), as a function of ΔT. In the first experimental set (ES1), enzyme ε_1 had a thermal sensitivity of five whereas ε_2 and ε_3 had thermal sensitivities both equal to three. In the second experimental set (ES2), enzyme ε_2 had a thermal sensitivity of 1.2 whereas ε_1 and ε_3 had thermal sensitivities both equal to three. In the third experimental set (ES3), enzyme ε_3 had a thermal sensitivity of seven whereas ε_1 and ε_2 had thermal sensitivities both equal to three. Simulations performed in MATLAB v. 6.1 (The MathWorks Inc., see http://www.mathworks.com/). Note that in neither case does the Q_{10}^F calculated approach three.

was set to three, while the third enzyme had a different Q_{10} (i.e. $Q_{10}^{\varepsilon_h}=Q_{10}^{\varepsilon_j}=3\neq Q_{10}^{\varepsilon_m}$, $(h,j,m)\in(1,2,3)$ and $h\neq j\neq m$). Temperature ranged within ±20 arbitrary temperature units around T_0. At each temperature T_i, the Q_{10} of the flux in the pathway ($Q_{10}^{F_{T_i}}$), in relation to the reference temperature, can be obtained from the most popular equation (Chaui-Berlinck *et al.* 2002):

$$Q_{10}^{F_{T_i}}=\left(\frac{F_i}{F_0}\right)^{\frac{10}{T_i-T_0}}. \tag{3.1}$$

The results of $Q_{10}i$ computations are graphically shown in figure 2*b*.

4. METABOLIC CONTROL THEORY AND THE TEMPERATURE EFFECTS ON A PATHWAY

A final step in the analysis is to use metabolic control theory (see Visser & Heijnen (2002) for a review of the subject) for further investigation of thermal effects on the pathway. Control coefficients (c_j) specify how sensitive the flux is to a change in the activity of a given enzyme in a pathway and are defined as the relative change in flux derived from the relative change in enzymatic activity:

$$c_j=\frac{\varepsilon_j}{F}\Theta_j, \tag{4.1a}$$

where Θ_j is the change in flux because of a change in activity of the enzyme j:

$$\Theta_j=\frac{\partial F}{\partial \varepsilon_j}. \tag{4.1b}$$

Figure 3 shows the flux-control coefficients of each enzyme at each temperature in the range studied. Because the flux-control coefficients vary with temperature, the influence of each enzyme on the net flux also changes with temperature; this is true even if the thermal sensitivity of each enzyme remains the same.

Additional insights into the above discussion emerge from the analytical study of the relationship between flux and temperature changes. Noticing that the sum of the control coefficients of a given pathway must add up to one, $\Sigma c_j=1$, and inserting equation (4.1*a*) in the previous equality, rearranging and deriving in temperature, we obtain (see Appendix A):

$$\frac{\partial F}{\partial T}=\sum\frac{\partial \Theta_j}{\partial T}\varepsilon_j+\sum\frac{\partial \varepsilon_j}{\partial T}\Theta_j, \tag{4.2}$$

that describes the thermal sensitivity of the net flux of the pathway. The three components of equation (4.2), highlighted below for heuristic purposes are:

(i) $\partial F/\partial T$: the Q_{10} of the pathway;
(ii) $\partial \Theta_j/\partial T$: the Q_{10} of the change in flux owing to a change in the activity of enzyme j; and
(iii) $\partial \varepsilon_j/\partial T$: the Q_{10} of the enzyme j, i.e. $Q_{10}^{\varepsilon_j}$.

These three components of equation (4.2) allow for a clear distinction between temperature effects on enzyme activity and temperature effects on changes in the flux of a system.

5. DISCUSSION

The most important finding of this study is that the alleged Q_{10} of the individual enzymes cannot be used to determine the Q_{10} of the simulated pathway, even if the proposed pathway is very simple, the thermal sensitivities of the enzymes remain constant, and no allosteric effects are allowed. Not only is the Q_{10} of the whole pathway unpredictable from individual steps, it also changes with temperature (see figure 2*b*). This phenomenon is explained because the Q_{10} values of enzymatic activities along a metabolic pathway are not the only factors responsible for changes in the flux of the pathway. The observed Q_{10} of the pathway results from an interplay between the thermal sensitivities of the enzymatic activities and the

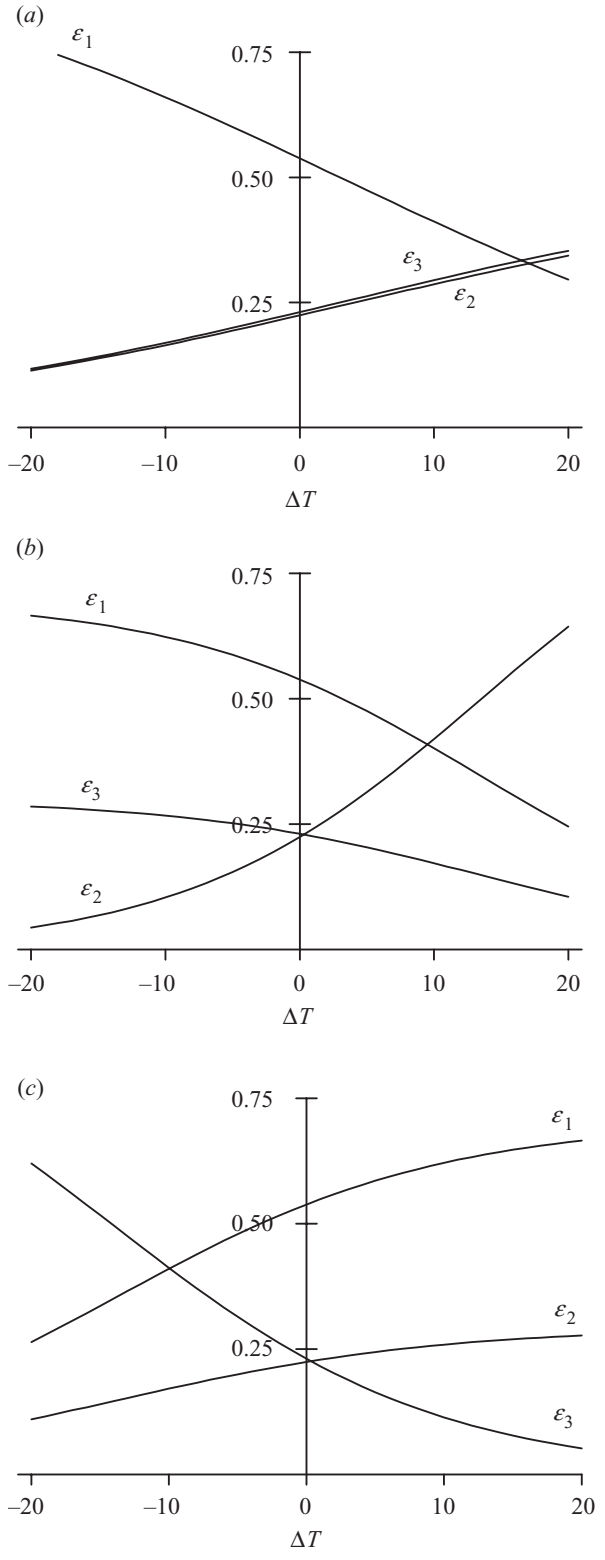

Figure 3. Distributions of the flux control coefficients of each enzyme (ε_1, ε_2, ε_3) in the pathway as a function of ΔT in (*a*) experimental set 1, (*b*) experimental set 2, and (*c*) experimental set 3 (see figure 2 for details in experimental sets).

thermal sensitivities of the absolute coefficients of change in flux.

The only situation in which the Q_{10} of the pathway can be directly derived from the Q_{10} of individual enzymes is when all the steps in the pathway have the same thermal sensitivity; that is, when all the enzymes have the same Q^{ε}_{10} (see Appendix A). Only under this very special circumstance, might an extrapolation from the enzymatic to the organismal level be appropriate. Then, if a researcher is to accept 'such a conceptual expansion' s/he should do this at his or her own risk. Because this restricted circumstance is so unlikely to occur in nature, it seems to be much more a theoretical curiosity than an empirical problem.

Q_{10} values are often calculated for metabolic rates and other types of organismal variable. One important conclusion from this work is that 'the Q_{10} has no theoretical pretense' (McNab & Brown 2002, p. 47). In other words, Q_{10} values applied to organismal rates cannot be used to propose subjacent mechanisms or events at a biochemical level; they just inform about the thermal sensitivity of a certain organismal flux under given conditions. Furthermore, empirically calculated changes in Q_{10} with temperature could be either attributable to metabolic control, to temperature-induced changes in the control coefficients of enzymes (which encompass the thermal sensitivities of enzyme activities, see Appendix A), or to other factors not accounted for in our minimalist model.

We thank the continuous support to our research by FAPESP (State of São Paulo Science Foundation) and CNPQ (Brazilian National Science Foundation). This study is part of a thesis by J.G.C.-B.

APPENDIX A

Equation (4.2) is obtained from equation (4.1*a*) as follows:

$$\sum \frac{\varepsilon_j}{F}\Theta_j = \frac{1}{F}\sum \varepsilon_j \Theta_j = 1 = \sum c_j, \tag{A 1}$$

$\Leftrightarrow$

$$F = \sum \varepsilon_j \Theta_j \Rightarrow \frac{\partial F}{\partial T} = \sum \left(\frac{\partial \Theta_j}{\partial T}\varepsilon_j + \frac{\partial \varepsilon_j}{\partial T}\Theta_j \right). \tag{A 2}$$

A ground state (indicated by $\widehat{\ }$) can be generalized in terms of enzymatic activity and flux such that $\widehat{\varepsilon}_j = 1 \forall j$ and $\widehat{F} = 1$. In the ground state, control coefficients are

$$\widehat{c}_j = \frac{\widehat{\varepsilon}_j}{\widehat{F}}\widehat{\Theta}_j = \frac{1}{1}\widehat{\Theta}_j = \widehat{\Theta}_j. \tag{A 3}$$

Thus

$$\sum \widehat{c}_j = \sum \widehat{\Theta}_j = 1. \tag{A 4}$$

Then, in a non-ground state, enzymatic activity and flux are represented by changes in activity and flux from the ground state as $\varepsilon_j = r_j \widehat{\varepsilon}_j$ and $F_j = s\widehat{F}$. Control coefficients in the non-ground state become

$$c_j = \frac{r_j \widehat{\varepsilon}_j}{s\widehat{F}}\Theta_j = \frac{r_j}{s}\Theta_j. \tag{A 5}$$

Imposing the condition that all the enzymes are equally affected in a non-ground state caused by changes in a state-parameter '*x*' (i.e. $\partial \varepsilon_j / \partial x = r \forall j$), it follows that

$$\frac{\partial F}{\partial x} = \sum \frac{\partial \varepsilon_j}{\partial x}\Theta_j + \sum \frac{\partial \Theta_j}{\partial x}\varepsilon_j = r\sum \Theta_j + \sum \frac{\partial \Theta_j}{\partial x}\varepsilon_j, \tag{A 6}$$

and

$$\sum c_j = \sum_s^r \Theta_j = 1 \Leftrightarrow \sum \Theta_j = \frac{s}{r}. \quad \text{(A 7)}$$

Inserting this result in the preceding equation,

$$\frac{\partial F}{\partial x} = r\frac{s}{r} + \sum \frac{\partial \Theta_j}{\partial x}\varepsilon_j. \quad \text{(A 8)}$$

Because, by imposition, $\partial F/\partial x = s$, it follows that, in the general case,

$$\sum \frac{\partial \Theta_j}{\partial x}\varepsilon_j = 0 \Leftrightarrow \frac{\partial \Theta_j}{\partial x} = 0 \forall j. \quad \text{(A 9)}$$

This means that Θ_j is a constant for each enzyme along the variation of x, i.e. change in flux as a result of change in enzymatic activity is constant. The changing in control coefficient as a result of x is

$$\frac{\partial c_j}{\partial x} = \left(\frac{\partial \varepsilon_j}{\partial x}\Theta_j F + \frac{\partial \Theta_j}{\partial x}\varepsilon_j F - \frac{\partial F}{\partial x}\varepsilon_j \Theta_j\right) \Big/ F^2 \quad \text{(A 10)}$$

$$= \frac{r\Theta_j s\widehat{F} + 0 - sr\widehat{\varepsilon}_j\Theta_j}{s^2\widehat{F}^2}.$$

Thus

$$\frac{\partial c_j}{\partial x} = r\Theta_j\left(\frac{1}{s\widehat{F}} - \frac{\widehat{\varepsilon}_j}{s\widehat{F}^2}\right) = r\Theta_j\left(\frac{1}{s} - \frac{1}{s}\right) = 0. \quad \text{(A 11)}$$

Notice that this last equality comes from the values of flux and enzyme activities in the ground state. Therefore, the control coefficients do not change along x as well as the Θ_js (see above). This implies that $s \equiv r$. A corollary is that if all the enzymes experience the same variation as a result of some parameter 'x' (e.g. temperature), the flow would experience the very same variation (and there would be no changes in control coefficients and values of Θ_j along the parameter 'x').

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