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# A method for testing the stability of a steady-state system during the calculation of a response to large changes in regulator concentrations

B. Crabtree and E.A. Newsholme

*Rowett Research Institute, Aberdeen, Scotland and Department of Biochemistry, South Parks Rd., Oxford, UK*

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A modification of the 'finite decomposition' method (Crabtree and Newsholme (1985) Curr. Top. Cell. Regul. 25, 21–76) for calculating physiological responses from sensitivities is described, to enable the system to be tested for stability at each step of the procedure. Instability is indicated by a change of sign of the determinant of the square matrix ($N$) in the governing equation for the system. The method cannot be used to predict responses beyond any step at which instability occurs.

Metabolic control; Stability; Physiological response

## 1. INTRODUCTION

The strength of the response of a metabolic system to a given stimulus can be measured by the function, dln(response)/dln(stimulus). This function, which may be termed a 'sensitivity' [1,2], is expressed as a differential coefficient to enable the overall response of a complex system to be calculated from the responses of its components (see also [3–5]). However, since such a differential coefficient refers to infinitesimal changes and its magnitude varies continuously during a physiological transition, it cannot be used to calculate the response to a physiological stimulus directly.

For the response of steady state systems, a numerical approximation can allow such sensitivities to be used indirectly [2]. In this method the physiological (i.e. finite) stimulus is decomposed into a number of successive stimuli; each sufficiently small to be considered infinitesimal and to which the sensitivity functions can therefore be applied directly. By a successive application of the sensitivity functions, the physiological response to the stimulus is obtained cumulatively. This method of 'finite decomposition' allows a faster calculation of changes between steady states than one based on time derivatives. However, it assumes that the system encounters no unstable regions during the sequence of 'infinitesimal' changes; for example a bifurcation point which would lead to the breakdown of the steady state [6]. This paper proposes a simple extension to the method, allowing the stability to be tested at each step of the procedure.

*Correspondence address:* B. Crabtree, Rowett Research Institute, Aberdeen AB2 9SB, Scotland, UK

## 2. OUTLINE OF THE METHOD OF 'FINITE DECOMPOSITION'

To illustrate the method, let us consider a very simple system (Fig. 1) involving just one flux ($J$) and internal metabolite ($S$): which are the system variables. A physiological stimulus is provided by a change of $X$, which is an external regulator of enzyme $E1$. The general sensitivity equations for this, or any other, system may be written in matrix form as follows [7,8]:

$$Nv = p, \qquad \text{--------(1)}$$

where $N$ is a square matrix containing component sensitivities, fluxes, concentrations, $v$ is a vector containing infinitesimal changes of the variables and $p$ is a vector containing infinitesimal changes of the control parameters (e.g. enzyme activities, regulator concentrations). For the system in Fig. 1 the general equation is:

$$\begin{vmatrix} 1 & -\alpha \\ 1 & -\beta \end{vmatrix} \begin{vmatrix} J^r \\ S^r \end{vmatrix} = \begin{vmatrix} E1^r \\ E2^r \end{vmatrix} \qquad \text{------(2)}$$

$(\ )$ $(v)$ $(p)$

where the superscript $r$, denotes an infinitesimal relative change (e.g. $X = \mathrm{d}X/X$ or $\mathrm{dln}X$) and $E1$, $E2$ denote enzyme activities (not concentrations).

For the specific regulation by $X$, this equation becomes:

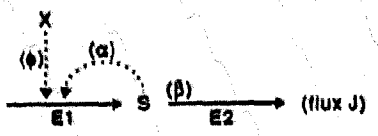


Fig. 1. Simple metabolic system with one flux, internal metabolite and regulator. Here the single internal metabolite, $S$, interacts with both enzyme-catalysed reactions ($E1$ and $E2$). The component sensitivities of these interactions ($s_S^{E1}$ and $s_S^{E2}$) are denoted as $\alpha$ and $\beta$, respectively. (Such component sensitivities are also referred to as 'elasticity coefficients' see [10]). The regulator, $X$, interacts with $E1$ with a component sensitivity ($s_X^{E1}$), $\phi$. The system is assumed to be in a steady state and not limited by any interactions upstream of $E1$. The interaction represented by $\beta$ is a feedforward effect ('information' transfer in the same direction as the flux), whereas that represented by $\alpha$ is a feedback effect ('information' transfer in the opposite direction to the flux).

$$\begin{vmatrix} 1 & -\alpha \\ 1 & -\beta \end{vmatrix} \begin{vmatrix} J' \\ S' \end{vmatrix} = \begin{vmatrix} \phi \cdot X' \\ 0' \end{vmatrix} \qquad \text{------(3)}$$

The physiological (finite) stimulus, expressed as a 'fold change', $X_f$, is decomposed into a succession of $n$ equal and near-infinitesimal fold changes, $X_\phi$, by the equation.

$$X_\phi = (X_f)^{(1/n)}$$

(For example, if $X_f$ is 2 and $n$ is 100, $X_\phi = 2^{(1/100)}$, i.e. approximately 1.007)

The corresponding 'infinitesimal' relative change, $X'$, is equal to $(X_\phi - 1)$, i.e. approx 0.007 for a 2-fold change of $X$ with $n = 100$.

The value of $X'$ is used in eqn 3, which is solved $n$ successive times for $J$ and $S$; and hence for $J$ and $S$. At the end of each step the values of the component sensitivities $\alpha$, $\beta$ and $\phi$ (which are usually functions of $J$, $S$ and $X$) are recalculated. The values of $J$ and $S$ after $n$ successive applications of eqn 3 are then those resulting from the physiological change of regulator $X$; provided that the system did not encounter any unstable region during the procedure.

## 3. MONITORING THE STABILITY OF THE STEADY STATE

The general solution of eqn 1 is,

$$v = N^{-1} \cdot p,$$

and, by a standard result of matrix algebra [11], all the elements of $N^{-1} \cdot p$ (and hence the overall sensitivities of the variables to the regulators [7,8]) have a common denominator, equal to the determinant of matrix $N$ (det $N$). Thus, for the system in Fig. 1 by solving eqn 3,

$$\det N = (\alpha - \beta) \qquad \text{------(4)}$$

$$\text{sensitivity of } J \text{ to } X\ (s_X^J) = -\beta.\phi/\det N \qquad \text{-----(5)}$$

$$\text{sensitivity of } S \text{ to } X\ (s_X^S) = -\phi/\det N \qquad \text{-----(6)}$$

If the feedback effect ($\alpha$) is negative and the feedforward effect ($\beta$) is positive, changes of $S$ produced by changes of the rate of either $E1$ or $E2$ will be opposed by the effect of $S$ on the other reaction. Consequently, under these conditions, the steady state is stable. A negative feedback can be obtained by replacing $\alpha$ by $-\bar{\alpha}$ (where $\bar{\alpha}$ is positive) in eqn 4. Det $N$ then becomes $(\bar{\alpha} + \beta)$, and since $\beta$ and $\bar{\alpha}$ are now both positive, its value is negative for stability.

This condition for stability may be illustrated by considering the sensitivities in eqn 6. This equations shows that, when det $N$ is negative and $\phi$ is positive, the sensitivity of $S$ to $X$ is positive; so that an increased $X$ increases the concentration of $S$, in agreement with a qualitative examination of this system. However, if det $N$ were positive, the sensitivity of $S$ to $X$ would be negative. This is impossible because, in this system, an increase of $X$ must increase $S$: it can never decrease it. In other words, if det $N$ ever becomes positive (e.g. due to changes in the relative values of $\alpha$ and $\beta$ when both are positive, i.e. with a feedback activation of $E1$ by $S$), the system becomes unstable and a steady state cannot exist.

This effect of a changed sign for det $N$ is a general result and provides a simple test of stability during the 'finite decomposition' method. The value of det $N$ is calculated at the end of each of the $n$ steps and, if its sign changes, a region of instability has been reached. The method is then terminated at that step.

Three further comments need to be made. Firstly, it must be emphasised that the sign of det $N$ giving stability depends on the order of the equations in the matrix equation. Thus, if the component equations for the system in Fig. 1 were interchanged and written as:

$$\begin{vmatrix} 1 & -\beta \\ 1 & -\alpha \end{vmatrix} \begin{vmatrix} J' \\ S' \end{vmatrix} = \begin{vmatrix} 0 \\ \phi \cdot X' \end{vmatrix} \qquad \text{------(7),}$$

$$\det N = (\beta - \alpha)$$

$$= -\det N \text{ for eqn 3}$$

Consequently, when the matrix equation is written in this way, stability is indicated by a positive value for det $N$. As stated above, with any matrix representation, the sign giving stability can be deduced by making all the

feedback interactions negative and all the feedforward ones positive. However, for finite decomposition, the initial steady state is taken to be stable; so that only a change of sign need be monitored.

Secondly, for systems with extensive branching, matrix $N$ may be quite large. However, its size can be reduced, often considerably, by partitioning it into the following submatrices [8]:

$$\begin{vmatrix} I & A \\ B & C \end{vmatrix}$$

As a result, the determinant of the matrix $[C - BA]$, which is a smaller matrix than $N$, is also the common denominator of the net sensitivities of the system. Consequently, det $N$ is equal to det $[C - BA]$ and can usually be calculated more easily from the latter matrix than from $N$ itself.

Thirdly, in our analysis the variables (in $v$) and the control parameters (in $p$) are kept separate throughout. In contrast, a recent variant of our general approach [9] combines the elements of $v$ and $p$ into net sensitivities of 'control coefficients' before solving the matrix equation (eqn 1). However, this prior combination is not recommended when the equation is used (as above) to calculate changes in the variables directly from the stimulus.

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