Generalization of the double-modulation method for *in situ* determination of elasticities

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The double-modulation method [Kacser and Burns (1979) Biochem. Soc. Trans. **7**, 1149–1160] was the first method proposed for determining elasticities *in situ*. It is based on measuring changes in steady-state metabolite concentrations and fluxes induced by parameter modulations. It has the important advantage that it is not necessary to know the values of the changes in the parameters. Here we develop a matrix formulation of the double-modulation method that allows it to be applied to metabolic systems of any structure and size. It also shows which parameters need to be modulated and which variables need to be measured in order to calculate the elasticities that correspond to particular rates. Some suggestions for the practical implementation of the method are given, including various ways of testing the reliability of the results.

INTRODUCTION

Since the first development of metabolic control analysis from the pioneering work of Kacser and Burns [1] and Heinrich and Rapoport [2], much effort has been devoted to delineating the relationships between the properties of metabolic systems and the kinetic properties of their components. Instead of the familiar kinetic constants used in mechanistic studies, metabolic control analysis expresses the latter in terms of elasticities, which define the sensitivities of the rates of individual reactions to the concentrations of metabolites. Although in principle elasticities can be measured on an isolated enzyme, such measurements are always open to doubts as to whether the artificial system truly reproduces the properties of the same enzyme *in situ*, because metabolites that interact with it *in situ* may be omitted, or present but quantitatively different. These are probably more important than the more obvious difficulty that the enzyme may be altered during isolation, because this is more easily checked. There has accordingly been much interest in developing experimental methods for measuring both control coefficients and elasticities in intact systems.

The simplest type of such method is one involving a single modulation, in which the activity of one enzyme is perturbed, for example by addition of an inhibitor that is specific for one enzyme. Such a perturbation can yield a value for the flux control coefficient of the perturbed step, and values for other steps can be estimated if the elasticities of neighbouring enzymes with respect to common intermediates are known. For example, in a study of the urea cycle in rat hepatocytes, Wanders and co-workers [3] used norvaline to inhibit ornithine carboxylase, and estimated the flux control coefficient for this enzyme; from this they calculated the flux control coefficient of carbamoyl-phosphate synthase by taking account of the elasticities of the two enzymes with respect to their common intermediate, carbamoyl phosphate.

The double-modulation method of Kacser and Burns [4] represented a major step forward. It offered the hope that modulation of two steps would allow analysis of a pathway segment without prior knowledge of the intermediate elasticities, which could, instead, be obtained from the analysis itself, and it avoided any assumption that enzyme elasticities are unity, i.e. that rates are necessarily proportional to enzyme concentrations. For several years after it was proposed this method was little used, but subsequently a number of groups [5–7] applied it to various systems. Fell [8,9] has reviewed these and other applications.

Giersch [10,11] has generalized the double-modulation method into the multiple-modulation method, and a somewhat different but related procedure known as co-response analysis has also been described [12–14]: this involves modulating all of the enzymes in the system and measuring all of the fluxes and metabolite concentrations. Although one can in this way obtain the complete set of control coefficients and elasticities that characterize the system, it demands more experimental effort than is strictly necessary, as in many systems one can obtain all of the information from a much smaller number of modulations. For this reason, Giersch and Cornish-Bowden [15] have recently explored ways of defining the precise sets of reactions that need to be modulated in order to allow determination of particular elasticities. In the present paper we also address this question, as part of a more general formulation of the multiple-modulation method. The matrix method that we propose is completely general, as it can be applied to systems of any structure and size.

A somewhat different but related approach to the control analysis of larger systems is found in the top-down method [16], recently reviewed by one of its originators [17], and in the conceptually similar modular analysis [18]. These were motivated by a desire to determine what information could be obtained from limited experiments in which groups of enzymes are considered together as blocks, in systems too large and complicated to allow a complete analysis. Such methods have recently been extended to systems with more than one intermediate connecting the blocks [19] or more than one flux connecting the intermediates [20]. We believe that the combination of top-down or modular approaches and the general principles developed in the present paper should give a more solid ground to the design of modulation experiments.

STRUCTURAL PROPERTIES

In this section we review the structural properties of steady-state metabolic systems, i.e. those properties that depend only on the stoichiometry of the network. We focus on the properties that

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will be used later; a fuller and mathematically rigorous account of the topic has been provided by Reder [21] (see also [22,23]).

In any system the steady-state fluxes are constrained by mass conservation. The number of dependent fluxes, i.e. fluxes whose values are fixed by other fluxes, is equal to the number of flux-conservation equations and to the number of independently variable metabolite concentrations. For instance, in an unbranched chain of r steps there are $r-1$ independently variable metabolite concentrations and, therefore, $r-1$ dependent fluxes. Any one of the fluxes can be designated as independent, i.e. it can, in principle, take any arbitrary value. But once this choice is made all the other fluxes are dependent as they must take the same value as the independent flux. A simple branch point involves one metabolite concentration and three fluxes: two of these, which can be designated at will, are independent, but the third is dependent because it is a linear combination of the two independent fluxes. In a general scheme we can arbitrarily choose a flux and designate it as independent, but for the second and subsequent fluxes we may not be able to make such a choice, and the last characterization is never arbitrary.

Some metabolite concentrations may also be constrained by conservation relationships, the number of dependent concentrations being equal to the number of concentration-conservation relationships. Once the values of the independent concentrations are specified the values of the dependent concentrations follow automatically.

The flux-conservation relationships may be expressed in a matrix form in which the vector of dependent fluxes $\mathbf{j_D}$ is calculated from the vector of independent fluxes $\mathbf{j_I}$ and the stoichiometry of the network; similarly, for the concentration–conservation relationships the vector of dependent concentrations, $\mathbf{s_D}$, is calculated from the vector of independent concentrations, $\mathbf{s_I}$. This is described in [24], of which eqns. (8), (48) and (54) are especially pertinent.

The control coefficients of any system satisfy structural properties, with constraints of two types. The first of these, the conservation constraints on the control coefficients, follows from the conservation relationships between the variables. In essence, these properties mean that the control coefficients $\mathbf{C}^{\mathbf{j}_D}_{\mathbf{v}}$ of a dependent flux with respect to any rate v depends on the control coefficients $\mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}}$ of the independent fluxes with respect to the same rate, and similar considerations apply to the control coefficients $\mathbf{C}^{\mathbf{s}_D}_{\mathbf{v}}$ and $\mathbf{C}^{\mathbf{s}_I}_{\mathbf{v}}$ of dependent and independent concentrations respectively, as in eqns. (13) and (56) of [24].

The second type of structural constraint satisfied by control coefficients is expressed by the summation relationships [1,2], according to which the control coefficient $\mathbf{C}^{\mathbf{j}}_{\mathbf{v}_I}$ or $\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_I}$ of any flux or concentration with respect to an independent rate can be calculated from the control coefficients $\mathbf{C}^{\mathbf{j}}_{\mathbf{v}_D}$ or $\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D}$ of the same flux or concentration with respect to the dependent rates.

Combining the two types of constraints allows us to conclude that it is possible to assign arbitrary values to $r_D(m_I+r_I)$ control coefficients, where r_D is the number of dependent rates, and m_I and r_I are the numbers of independent metabolite concentrations and rates respectively. It is important to note that, as the number of independent metabolite concentrations is equal to the number of dependent rates, i.e. $m_I = r_D$, the $[m_I \times r_D]$ matrix $\mathbf{C}^{\mathbf{s}_I}_{\mathbf{v}_D}$ is square and, in principle, invertible.

CALCULATING ELASTICITIES FROM CHANGES IN VARIABLES

In this section we show how to calculate the elasticities from small relative changes in the steady-state variables, the essence of the double-modulation method. Initially we consider metabolic systems in which all the metabolite concentrations are independent, but in the Conserved concentrations section we shall examine the more general case where some concentrations may be constrained by conservation equations.

The matrices $\mathbf{C}^{\mathbf{j}}_{\mathbf{v}}$ $[r \times r]$, $\mathbf{C}^{\mathbf{s}}_{\mathbf{v}}$ $[m \times r]$ and $\boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}}$ $[r \times m]$ are related by the following equation [25,26]:

$$\mathbf{C}^{\mathbf{j}}_{\mathbf{v}} = \mathbf{I}_{\mathbf{r}} + \boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}} \tag{1}$$

in which $\mathbf{I_r}$ is the $[r \times r]$ identity matrix. This equation can be partitioned into four blocks:

$$\begin{bmatrix} \mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}_I} & \mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}_D} \\ \mathbf{C}^{\mathbf{j}_D}_{\mathbf{v}_I} & \mathbf{C}^{\mathbf{j}_D}_{\mathbf{v}_D} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{\mathbf{r}_I} & \mathbf{O} \\ \mathbf{O} & \mathbf{I}_{\mathbf{r}_D} \end{bmatrix} + \begin{bmatrix} \boldsymbol{\varepsilon}^{\mathbf{v}_I}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_I} & \boldsymbol{\varepsilon}^{\mathbf{v}_I}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D} \\ \boldsymbol{\varepsilon}^{\mathbf{v}_D}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_I} & \boldsymbol{\varepsilon}^{\mathbf{v}_D}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D} \end{bmatrix} \tag{2}$$

where each $\mathbf{O}$ represents a null matrix of the appropriate dimensions. Note that the independent and dependent rates $\mathbf{v_I}$ and $\mathbf{v_D}$ correspond to the independent and dependent fluxes $\mathbf{j_I}$ and $\mathbf{j_D}$ respectively. In what follows we shall use the top-right block, i.e.:

$$\mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}_D} = \boldsymbol{\varepsilon}^{\mathbf{v}_I}_{\mathbf{s}}\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D} \tag{3}$$

As we assume for the moment that the metabolite concentrations are not subject to conservation relationships, the total number of metabolites is equal to the number of dependent rates. Eqn. (3) can be solved for the matrix of elasticities:

$$\boldsymbol{\varepsilon}^{\mathbf{v}_I}_{\mathbf{s}} = \mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}_D}(\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D})^{-1} \tag{4}$$

The result indicates that the elasticity matrix corresponding to the independent rates can be obtained by modulating the dependent rates and measuring the changes in metabolite concentrations and independent fluxes.

However, the dependent rates are not modulated directly, but by changes in parameters. The question is now to decide which parameters have to be modulated. To answer this we return to the definitions of the two control coefficient matrices that appear in eqn. (4) [21,25–27]:

$$\mathbf{C}^{\mathbf{j}_I}_{\mathbf{v}_D} = \mathbf{R}^{\mathbf{j}_I}_{\mathbf{p}}(\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}})^{-1} \tag{5a}$$

$$\mathbf{C}^{\mathbf{s}}_{\mathbf{v}_D} = \mathbf{R}^{\mathbf{s}}_{\mathbf{p}}(\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}})^{-1} \tag{5b}$$

in which $\mathbf{R}^{\mathbf{j}_I}_{\mathbf{p}}$ and $\mathbf{R}^{\mathbf{s}}_{\mathbf{p}}$ are the matrices of response coefficients of the independent fluxes and concentrations with respect to a vector of parameters $\mathbf{p}$, and $\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}}$ is a matrix of elasticities of the dependent rates with respect to the parameters.

The calculations implied by eqns. (5a–5b) can only be done if $\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}}$ is invertible; we must therefore find a vector of parameters of dimension r_D, the number of dependent rates, and each rate law corresponding to a dependent rate must be affected by at least one of these parameters. Although a different parameter is associated with each rate, a change in one parameter may effect more than one dependent rate. Algebraically the simplest solution, of course, is to choose parameters such that each is specific for one dependent rate, and in this case $\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}}$ is diagonal and invertible. However, the algebraically simplest solution may not be the simplest experimentally; indeed, it may not always be possible, but one can modulate parameters that affect more than one rate as long as $\boldsymbol{\pi}^{\mathbf{v}_D}_{\mathbf{p}}$ is invertible. (For example, we can use two inhibitors such that each acts only on the same two enzymes, provided that their effects on the two enzymes are not proportional to one another.) In addition, modulation of the chosen parameters must not affect the independent rates $\mathbf{v_I}$.

We now show how to express the control coefficients on the right-hand side of eqn. (4) in terms of ratios of increments. The elements of a response coefficient matrix $\mathbf{R}^{\mathbf{y}}_{\mathbf{p}}$ (where $\mathbf{y}$ is any vector of variables) are normalized partial derivatives of the form

$\frac{p}{y}\frac{\partial y}{\partial p}$ or, equivalently, $\frac{\partial \ln y}{\partial \ln p}$. (More strictly we should write the latter as $\frac{\partial \ln |y|}{\partial \ln p}$, as some metabolic variables, such as fluxes, can be negative; we shall take this to be understood.) For small changes these elements can be approximated by the corresponding ratios of increments, i.e. $\frac{p}{y}\frac{\partial y}{\partial p} = \frac{\partial \ln y}{\partial \ln p} \approx \frac{p}{y}\frac{\delta y}{\delta p} \approx \frac{\delta \ln y}{\delta \ln p}$, though it is important to realize that the two approximate expressions given here are different from one another, becoming identical only in the limit. We shall symbolize the matrix of increment ratios corresponding to the response coefficient matrix $\mathbf{R}^{\mathbf{y}}_{\mathbf{p}}$ as $\bar{\mathbf{R}}^{\mathbf{y}}_{\mathbf{p}}$. It is important to note that the elements of any one column of an $\bar{\mathbf{R}}$-matrix share the same relative changes in p as denominator. It follows that the $\bar{\mathbf{R}}$-matrices can be decomposed into two factors:

$$\mathbf{R}^{\mathbf{y}}_{\mathbf{p}} \approx \bar{\mathbf{R}}^{\mathbf{y}}_{\mathbf{p}} = {}^{\mathbf{p}}\left[\frac{\boldsymbol{\delta}\mathbf{y}}{\mathbf{y}}\right]\left[\mathrm{Diag}\left(\frac{\boldsymbol{\delta}\mathbf{p}}{\mathbf{p}}\right)\right]^{-1} \approx {}^{\mathbf{p}}(\boldsymbol{\delta}\ln\mathbf{y})\,[\mathrm{Diag}\,(\boldsymbol{\delta}\ln\mathbf{p})]^{-1} \qquad (6)$$

The second factor on the right-hand side of this equation is the inverse of a diagonal matrix whose elements are the relative changes in **p**. The first factor is a matrix for which the element ij is the relative change in the variable y_i caused by the change in the parameter p_j. In eqn. (6) and elsewhere in the present paper the pre-superscript (**p** in this case) indicates the modulated parameter; this contrasts with the common use of subscripts in partial derivatives to indicate the independent variables that are held constant during the differentiation.

The matrix $\boldsymbol{\pi}^{\mathbf{v}}_{\mathbf{p}}$ can also be approximated by an expression similar to eqn. (6), i.e.:

$$\boldsymbol{\pi}^{\mathbf{v}}_{\mathbf{p}} \approx \bar{\boldsymbol{\pi}}^{\mathbf{v}}_{\mathbf{p}} = {}^{\mathbf{p}}\left[\frac{\boldsymbol{\delta}\mathbf{v}}{\mathbf{v}}\right]\left[\mathrm{Diag}\left(\frac{\boldsymbol{\delta}\mathbf{p}}{\mathbf{p}}\right)\right]^{-1} \approx {}^{\mathbf{p}}(\boldsymbol{\delta}\ln\mathbf{v})\,[\mathrm{Diag}\,(\boldsymbol{\delta}\ln\mathbf{p})]^{-1} \qquad (7)$$

In the conditions where $\boldsymbol{\pi}^{\mathbf{v}}_{\mathbf{p}}$ is invertible (see above), its inverse can be written as:

$$(\boldsymbol{\pi}^{\mathbf{v}}_{\mathbf{p}})^{-1} \approx \mathrm{Diag}\left(\frac{\boldsymbol{\delta}\mathbf{p}}{\mathbf{p}}\right)\left[{}^{\mathbf{p}}\left(\frac{\boldsymbol{\delta}\mathbf{v}}{\mathbf{v}}\right)\right]^{-1} \approx \mathrm{Diag}\,(\boldsymbol{\delta}\ln\mathbf{p})\,[{}^{\mathbf{p}}(\boldsymbol{\delta}\ln\mathbf{v})]^{-1} \qquad (8)$$

Combining eqns. (6) and (8) with eqns. (5a) and (5b) allows the control coefficients to be expressed approximately in terms of increment ratios, i.e.

$$\mathbf{C}^{\mathbf{j_I}}_{\mathbf{v_D}} \approx {}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta}\mathbf{j_I}}{\mathbf{j_I}}\right)\left[{}^{\mathbf{p}}\left(\frac{\boldsymbol{\delta}\mathbf{v_D}}{\mathbf{v_D}}\right)\right]^{-1} \approx {}^{\mathbf{v_D}}(\boldsymbol{\delta}\ln\mathbf{j_I})\,[{}^{\mathbf{p}}(\boldsymbol{\delta}\ln\mathbf{v_D})]^{-1} \qquad (9a)$$

$$\mathbf{C}^{\mathbf{s}}_{\mathbf{v_D}} \approx {}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta}\mathbf{s}}{\mathbf{s}}\right)\left[{}^{\mathbf{p}}\left(\frac{\boldsymbol{\delta}\mathbf{v_D}}{\mathbf{v_D}}\right)\right]^{-1} \approx {}^{\mathbf{v_D}}(\boldsymbol{\delta}\ln\mathbf{s})\,[{}^{\mathbf{p}}(\boldsymbol{\delta}\ln\mathbf{v_D})]^{-1} \qquad (9b)$$

In these equations the superscript **p** corresponding to the relative changes in the variables has been replaced with $\mathbf{v_D}$, as a reminder that the parameters modulated are those that affect the dependent rates, as discussed above.

Finally, substitution of eqns. (9a) and (9b) into eqn. (4) gives the central result of this paper:

$$\boldsymbol{\varepsilon}^{\mathbf{v_I}}_{\mathbf{s}} \approx {}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta}\mathbf{j_I}}{\mathbf{j_I}}\right)\left[{}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta}\mathbf{s}}{\mathbf{s}}\right)\right]^{-1} \approx {}^{\mathbf{v_D}}(\boldsymbol{\delta}\ln\mathbf{j_I})\,[{}^{\mathbf{v_D}}(\boldsymbol{\delta}\ln\mathbf{s})]^{-1} \qquad (10)$$

This tells us how to calculate the elasticities related to a set of independent rates from relative changes in the variables. For this calculation we need to measure the relative changes in a set of independent fluxes and metabolite concentrations produced by small relative changes in parameters appearing in the rate laws for dependent rates. The element kl of the first matrix on the right-hand side of eqn. (10) is the relative change in the kth independent flux produced by a small change in the parameter associated with the lth dependent rate. Similarly, the element hl of the second matrix on the right-hand side of eqn. (10) is the relative change in the hth metabolite concentration produced by a small change in the same parameter. The invertibility of this second matrix is ensured by an adequate choice of parameters.

The procedure as we have described supplies the elasticities associated with the independent rates. We now show how to calculate the remaining elasticities, namely those associated with the dependent rates. To achieve this goal the general strategy consists of redefining which rates we designate dependent or independent, recalling that there is some arbitrariness (see the Structural properties section) in how this is done at the outset. In particular, it is completely arbitrary whether we designate the first rate to be considered in a metabolic scheme as dependent or independent. It is always therefore possible to select different sets of independent rates such that every rate in the scheme is in at least one such set, and when eqn. (10) is applied in turn to each set, all of the elasticities can be calculated from measurements of steady-state changes in the variables.

For example, in an unbranched chain of r steps there is only one independent rate, but any one of the rates can be designated as independent, and eqn. (10) can be applied r times to give all of the elasticities of the system. In the case of a simple branch there are three rates (say v_1, v_2 and v_3): as any two of these can be independent, we can first, for example, designate v_1 and v_2 as independent and then v_1 and v_3; eqn. (10) has to be solved only twice (i.e. as many times as the number of rates at the branch minus one). Notice in particular that as v_1 was not considered a dependent rate in either of the sets used, it is not necessary to modulate it, i.e. we do not need to have a parameter that affects v_1. More generally, we need to answer the question of how many rates (and which rates) need to be modulated to calculate all of the elasticities in an arbitrary system.

As we have described, obtaining the matrix of elasticities of the independent rate requires modulation of a number of rates equal to the number of dependent rates, and calculation of the remaining elasticities of the dependent rates requires redefinition of which rates are independent. For the moment, let us assume that among r_{I} independent rates there are $r_{\mathrm{I}}-1$ that are not proportional to some other rate. It follows that the remaining independent rate can be replaced by any of the dependent rates to obtain a new set of independent rates. In such a case there are $r_{\mathrm{I}}-1$ rates that are never designated as dependent and, therefore, do not need to be modulated. It follows that the number of rates (or parameters) to be modulated must be at least $r_{\mathrm{D}}+1$. The minimum of $r_{\mathrm{D}}+1$ is possible only if there are at least $r_{\mathrm{I}}-1$ independent rates that are not proportional to some other rate, but more than this otherwise, with one additional modulation for each of the $r_{\mathrm{I}}-1$ rates that are proportional to other rates. It follows that the number r_{mod} of rates to be modulated in order to determine all the elasticities is:

$$r_{\mathrm{mod}} = r - \mathrm{Min}\,(r_{\mathrm{I}}-1, r_{\mathrm{np}}) \qquad (11)$$

where r is the total number of rates, r_{np} is the number of rates that are not proportional to other rates, and $\mathrm{Min}\,(r_{\mathrm{I}}-1, r_{\mathrm{np}})$ is the smaller of $r_{\mathrm{I}}-1$ and r_{np}.

How do we choose which r_{mod} rates to modulate? First, we determine which rates are the r_{np} that are not proportional to other rates, and a systematic way of achieving this is described in the Appendix. If $r_{\mathrm{np}} \geqslant r_{\mathrm{I}}-1$ we retain $r_{\mathrm{I}}-1$ of the rates that are not proportional to other rates as invariable members of the sets of independent rates, leaving the other $r_{\mathrm{D}}+1$ as rates to be modulated. If $r_{\mathrm{np}} < r_{\mathrm{I}}-1$ all the r_{np} non-proportional rates are

retained in this way, and the remaining $r-r_{np}$ are the rates to be modulated.

It is easy to show that the calculation of all the elasticities requires measurement of the changes in the independent fluxes and metabolite concentrations. Let us first consider the fluxes. As described above, every flux in the system must play the role of independent flux at least once. We therefore need to know the relative changes in all the fluxes, but as the dependent fluxes can be calculated from the independent fluxes, only the latter need to be measured. According to our initial assumption, all metabolite concentrations are independent, and so according to eqn. (10) it is necessary to measure the changes in all of them, but this point will be discussed further in the Conserved concentrations section in the context of systems with conserved moieties.

Calculation of the elasticities involves relative (not absolute) changes in the variables. This fact is of practical importance, because it is well known that the determination of cellular concentrations and fluxes may be subject to non-negligible systematic errors, a major source of such errors being the evaluation of cellular volume. The values of relative changes is very much less affected by volume errors than those of absolute changes because, under identical experimental conditions, the volumes cancel. Similar considerations apply to other types of systematic error, and in general, therefore, these are less important than they may at first appear.

EXAMPLES

In this section we shall illustrate the application of the double-modulation method with two simple metabolic schemes that do not contain conserved cycles.

Unbranched chain

Scheme 1 shows an unbranched pathway with three rates ($r = 3$), of which two are dependent ($r_D = 2$) and one is independent ($r_I = 1$). From eqn. (11) and eqn. (A4) of the Appendix the number r_{mod} of rates to be modulated is three, as $r_{np} = 0$ and $r_I - 1 = 0$, i.e. all of the rates must be modulated. From eqn. (10), the elasticities are given by the following expressions:

$$[\epsilon^{v_1}_{S_1} \quad \epsilon^{v_1}_{S_2}] \approx \left[{}^{v_2}\left(\frac{\delta J_1}{J_1}\right) \; {}^{v_3}\left(\frac{\delta J_1}{J_1}\right) \right] \begin{bmatrix} {}^{v_2}\left(\frac{\delta S_1}{S_1}\right) & {}^{v_3}\left(\frac{\delta S_1}{S_1}\right) \\ {}^{v_2}\left(\frac{\delta S_2}{S_2}\right) & {}^{v_3}\left(\frac{\delta S_2}{S_2}\right) \end{bmatrix}^{-1}$$

$$\approx [{}^{v_2}(\delta \ln J_1) \quad {}^{v_3}(\delta \ln J_1)] \begin{bmatrix} {}^{v_2}(\delta \ln S_1) & {}^{v_3}(\delta \ln S_1) \\ {}^{v_2}(\delta \ln S_2) & {}^{v_3}(\delta \ln S_2) \end{bmatrix}^{-1} \quad (12a)$$

$$[\epsilon^{v_2}_{S_1} \quad \epsilon^{v_2}_{S_2}] \approx \left[{}^{v_1}\left(\frac{\delta J_2}{J_2}\right) \quad {}^{v_3}\left(\frac{\delta J_2}{J_2}\right) \right] \begin{bmatrix} {}^{v_1}\left(\frac{\delta S_1}{S_1}\right) & {}^{v_3}\left(\frac{\delta S_1}{S_1}\right) \\ {}^{v_1}\left(\frac{\delta S_2}{S_2}\right) & {}^{v_3}\left(\frac{\delta S_2}{S_2}\right) \end{bmatrix}^{-1}$$

$$\approx [{}^{v_1}(\delta \ln J_2) \quad {}^{v_3}(\delta \ln J_2)] \begin{bmatrix} {}^{v_1}(\delta \ln S_1) & {}^{v_3}(\delta \ln S_1) \\ {}^{v_1}(\delta \ln S_2) & {}^{v_3}(\delta \ln S_2) \end{bmatrix}^{-1} \quad (12b)$$

$$[\epsilon^{v_3}_{S_1} \quad \epsilon^{v_3}_{S_2}] \approx \left[{}^{v_1}\left(\frac{\delta J_3}{J_3}\right) \quad {}^{v_2}\left(\frac{\delta J_3}{J_3}\right) \right] \begin{bmatrix} {}^{v_1}\left(\frac{\delta S_1}{S_1}\right) & {}^{v_2}\left(\frac{\delta S_1}{S_1}\right) \\ {}^{v_1}\left(\frac{\delta S_2}{S_2}\right) & {}^{v_2}\left(\frac{\delta S_2}{S_2}\right) \end{bmatrix}^{-1}$$

$$\approx [{}^{v_1}(\delta \ln J_3) \quad {}^{v_2}(\delta \ln J_3)] \begin{bmatrix} {}^{v_1}(\delta \ln S_1) & {}^{v_2}(\delta \ln S_1) \\ {}^{v_1}(\delta \ln S_2) & {}^{v_2}(\delta \ln S_2) \end{bmatrix}^{-1} \quad (12c)$$

$$X_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} S_2 \xrightarrow{v_3} X_3$$

Scheme 1 An unbranched pathway consisting of three steps

The symbol X is used for metabolites with concentrations that are fixed independently of the enzymes in the system; S is used for metabolites with variable concentrations that are determined by the system.

Because of flux conservation the three relative (or logarithmic) changes in flux are equal:

$${}^{v_i}\left(\frac{\delta J_1}{J_1}\right) = {}^{v_i}\left(\frac{\delta J_2}{J_2}\right) = {}^{v_i}\left(\frac{\delta J_3}{J_3}\right) \equiv {}^{v_i}\left(\frac{\delta J}{J}\right) \quad (13a)$$

$${}^{v_i}(\delta \ln J_1) = {}^{v_i}(\delta \ln J_2) = {}^{v_i}(\delta \ln J_3) \equiv {}^{v_i}(\delta \ln J), \quad (13b)$$

for $i = 1, 2, 3$. Introducing this into eqns. (12a), (12b) and (12c) and operating, we obtain

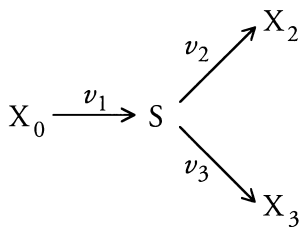
$$\epsilon^{v_1}_{S_1} \approx \frac{{}^{v_2}\left(\frac{\delta S_2}{S_2}\right) \cdot {}^{v_3}\left(\frac{\delta J}{J}\right) - {}^{v_3}\left(\frac{\delta S_2}{S_2}\right) \cdot {}^{v_2}\left(\frac{\delta J}{J}\right)}{{}^{v_3}\left(\frac{\delta S_1}{S_1}\right) \cdot {}^{v_2}\left(\frac{\delta S_2}{S_2}\right) - {}^{v_2}\left(\frac{\delta S_1}{S_1}\right) \cdot {}^{v_3}\left(\frac{\delta S_2}{S_2}\right)} \approx \frac{\frac{{}^{v_3}(\delta \ln J)}{{}^{v_3}(\delta \ln S_2)} - \frac{{}^{v_2}(\delta \ln J)}{{}^{v_2}(\delta \ln S_2)}}{\frac{{}^{v_3}(\delta \ln S_1)}{{}^{v_3}(\delta \ln S_2)} - \frac{{}^{v_2}(\delta \ln S_1)}{{}^{v_2}(\delta \ln S_2)}}$$

$$\approx \frac{{}^{v_3}O^{J}_{S_2} - {}^{v_2}O^{J}_{S_2}}{{}^{v_3}O^{S_1}_{S_2} - {}^{v_2}O^{S_1}_{S_2}} \quad (14)$$

as well as similar expressions for the other five elasticities. Each of the four fractions within the logarithmic form of this equation is a ratio of relative changes in two variables in response to the same rate change; in the terminology of co-response analysis [12–14], therefore, each approximates to a co-response coefficient and can be written as shown at the right, where ${}^{p}O^{x}_{y} \equiv {}^{p}(\mathrm{d} \ln x)/{}^{p}(\mathrm{d} \ln y)$. (Note that, as x and y are not independent of one another, this is a fraction, not a derivative.)

Calculation of all the elasticities from eqn. (14) and the other five similar equations requires measurement of three independent variables S_1, S_2 and J, and perturbation of three parameters of which each affects only one rate. (As elsewhere in this paper, by independent variables we mean variables that are linearly independent in the kinetic model; all three of these variables are, however, dependent variables in the sense used in statistical analysis, and hence in terms of measurement.) However, if we wanted to determine the elasticity corresponding to one particular rate only two rates would need to be modulated, though we should still need to measure all three variables.

It is important to notice that $\epsilon^{v_1}_{S_2}$ is a feedback elasticity and $\epsilon^{v_3}_{S_1}$ is a feedforward elasticity: the general matrix equations that we have used allow, in principle, all the possible elasticities to be non-zero. In the experimental system some interactions may be absent, and if so the values measured for the corresponding elasticities will be zero. If additional information about the regulatory structure of the pathway is available, we may be able to use it to set to zero some of the general expressions for elasticities as functions of changes in concentrations and fluxes. As a consequence some of the previously independent changes in variables will be dependent. This possibility is advantageous as it requires less experimental effort. On the other hand, one should be very cautious about assuming an *in situ* value for an elasticity if the information was derived from an *in vitro* experiment; in general it is safer to measure the quantities of interest rather than assume them.



Scheme 2 A simple branch point

Simple branch

Scheme 2 shows a simple branch: there are three rates ($r = 3$), as in the first example, but only one variable metabolite, so $r_D = 1$, $r_I = 2$; $r_{np} = 3$ and $r_I - 1 = 1$, so from eqn. (11) and eqn. (A3) of the Appendix the number of rates to be modulated is $r_{mod} = 2$. The choice of these can be determined in an experimental system according to the practical ease of modulating one parameter rather than another; here we shall arbitrarily select v_2 and v_3. From eqn. (10), the elasticities are given by the following expressions:

$$\begin{bmatrix} \epsilon_S^{v_1} \\ \epsilon_S^{v_2} \end{bmatrix} \approx \begin{bmatrix} {}^{v_3}\left(\frac{\delta J_1}{J_1}\right) \\ {}^{v_3}\left(\frac{\delta J_2}{J_2}\right) \end{bmatrix} \left[{}^{v_3}\left(\frac{\delta S}{S}\right)\right]^{-1} \approx \begin{bmatrix} {}^{v_3}(\delta \ln J_1) \\ {}^{v_3}(\delta \ln J_2) \end{bmatrix} [{}^{v_3}(\delta \ln S)]^{-1} \quad (15a)$$

$$\begin{bmatrix} \epsilon_S^{v_1} \\ \epsilon_S^{v_3} \end{bmatrix} \approx \begin{bmatrix} {}^{v_2}\left(\frac{\delta J_1}{J_1}\right) \\ {}^{v_2}\left(\frac{\delta J_3}{J_3}\right) \end{bmatrix} \left[{}^{v_2}\left(\frac{\delta S}{S_S}\right)\right]^{-1} \approx \begin{bmatrix} {}^{v_2}(\delta \ln J_1) \\ {}^{v_2}(\delta \ln J_3) \end{bmatrix} [{}^{v_2}(\delta \ln S)]^{-1} \quad (15b)$$

Flux conservation introduces the following constraint:

$${}^{v_i}\left(\frac{\delta J_1}{J_1}\right) = \frac{J_2}{J_1} \cdot {}^{v_i}\left(\frac{\delta J_2}{J_2}\right) + \left(1 - \frac{J_2}{J_1}\right) \cdot {}^{v_i}\left(\frac{\delta J_3}{J_3}\right) \quad (16a)$$

$$\text{or} \quad (\delta \ln J_1) = \alpha \cdot {}^{v_i}(\delta \ln J_2) + (1-\alpha) \cdot {}^{v_i}(d \ln J_3) \quad (16b)$$

for $i = 1, 2, 3$, where $\alpha = J_2/J_1$. Let us assume for illustration that, for practical reasons, J_2 and J_3 are easier to measure than J_1. Introducing eqn. (16) into eqns. (15) and operating we obtain the following expressions for the three elasticities, in which the right-hand form are again written in terms of co-response coefficients:

$$\begin{aligned} \epsilon_S^{v_1} &\approx \left[\alpha \cdot {}^{v_3}\left(\frac{\delta J_2}{J_2}\right) + (1-\alpha) \cdot {}^{v_3}\left(\frac{\delta J_3}{J_3}\right)\right] \Big/ {}^{v_3}\left(\frac{\delta S}{S}\right) \\ &\approx \frac{\alpha \cdot {}^{v_3}(\delta \ln J_2) + (1-\alpha) \cdot {}^{v_3}(\delta \ln J_3)}{{}^{v_3}(\delta \ln S)} \approx \alpha \cdot {}^{v_3}O_S^{J_2} + (1-\alpha) \cdot {}^{v_3}O_S^{J_3} \\ &\approx \left[\alpha \cdot {}^{v_2}\left(\frac{\delta J_2}{J_2}\right) + (1-\alpha) \cdot {}^{v_2}\left(\frac{\delta J_3}{J_3}\right)\right] \Big/ {}^{v_2}\left(\frac{\delta S}{S}\right) \\ &\approx \frac{\alpha \cdot {}^{v_2}(\delta \ln J_2) + (1-\alpha) \cdot {}^{v_2}(\delta \ln J_3)}{{}^{v_2}(\delta \ln S)} \approx \alpha \cdot {}^{v_2}O_S^{J_2} + (1-\alpha) \cdot {}^{v_2}O_S^{J_3} \end{aligned} \quad (17a)$$

$$\epsilon_S^{v_2} \approx {}^{v_3}\left(\frac{\delta J_2}{J_2}\right) \Big/ {}^{v_3}\left(\frac{\delta S}{S}\right) \approx {}^{v_3}(\delta \ln J_2)/{}^{v_3}(\delta \ln S) \approx {}^{v_3}O_S^{J_2} \quad (17b)$$

$$\epsilon_S^{v_3} \approx {}^{v_2}\left(\frac{\delta J_3}{J_3}\right) \Big/ {}^{v_2}\left(\frac{\delta S}{S}\right) \approx {}^{v_2}(\delta \ln J_3)/{}^{v_2}(\delta \ln S) \approx {}^{v_2}O_S^{J_3} \quad (17c)$$

Thus calculation of all the elasticities requires measurement of three independent variables (S and two fluxes) and the modulation of two parameters (affecting two rates). To measure one particular elasticity it is sufficient to modulate one parameter and to measure S and one flux. As seen in eqn. (17a), there are different ways of calculating $\epsilon_S^{v_1}$ according to whether we choose to modulate v_2 (last two lines) or v_3 (first two lines). In general, when any two rates in Scheme 2 are modulated, there are two ways of calculating the elasticity corresponding to the third rate. Redundancy of this kind can be used to test the reproducibility of the results, and, of course, redundancy can be increased by modulating all three rates even though modulation of just two is in principle sufficient.

CONSERVED CONCENTRATIONS

In this section we shall extend the double-modulation method to schemes in which some metabolite concentrations are constrained by conservation relationships. We shall follow a similar itinerary to that given in the 'Calculating elasticities from changes in variables' section, but will present it more briefly. The first step is to define a 'reduced' system of equations, by replacing the dependent concentrations $\mathbf{s_D}$ in the rates $\mathbf{v}$ by appropriate expressions of the independent concentrations $\mathbf{s_I}$, to obtain the reduced rates $\mathbf{v^*}$. (Details of this approach may be found in Section 9 of [24]). The advantage of this strategy is that one can apply to the reduced scheme all of the results obtained in the section on calculating elasticities for schemes without conserved concentrations.

For the reduced system eqn. (3) takes the following form:

$$\mathbf{C}^{\mathbf{j_I}}_{\mathbf{v_D}} = \boldsymbol{\varepsilon}^{\mathbf{v_I^*}}_{\mathbf{s_I}} \mathbf{C}^{\mathbf{s_I}}_{\mathbf{v_D}} \quad (18)$$

the number of independent concentrations m_I being equal to the number of dependent rates, r_D. Solving for this elasticity matrix, we obtain:

$$\boldsymbol{\varepsilon}^{\mathbf{v_I^*}}_{\mathbf{s_I}} = \mathbf{C}^{\mathbf{j_I}}_{\mathbf{v_D}} (\mathbf{C}^{\mathbf{s_I}}_{\mathbf{v_D}})^{-1} \quad (19)$$

Following the same steps as in the 'Calculating elasticities from changes in variables' section, this can be transformed into an approximate solution in terms of the relative changes in the variables (cf. eqn. 10):

$$\boldsymbol{\varepsilon}^{\mathbf{v_I^*}}_{\mathbf{s_I}} \approx {}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta} \mathbf{j_I}}{\mathbf{j_I}}\right) \left[{}^{\mathbf{v_D}}\left(\frac{\boldsymbol{\delta} \mathbf{s_I}}{\mathbf{s_I}}\right)\right]^{-1} \approx {}^{\mathbf{v_D}}(\boldsymbol{\delta} \ln \mathbf{j_I}) [{}^{\mathbf{v_D}}(\boldsymbol{\delta} \ln \mathbf{s_I})]^{-1} \quad (20)$$

The first matrix on the right-hand side is the same as the corresponding matrix in eqn. (10); the second has elements such that element hl is the relative change in the hth independent metabolite concentration produced by a small change in the parameter associated with the lth dependent rate.

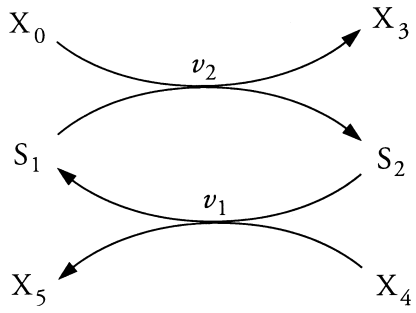
The remaining elasticities $\boldsymbol{\varepsilon}^{\mathbf{v_D^*}}_{\mathbf{s_I}}$ of the reduced system may be obtained by redefining which rates are dependent and independent, as described in the section on calculating elasticities. All of this allows us, therefore, to calculate all the elasticities $\boldsymbol{\varepsilon}^{\mathbf{v^*}}_{\mathbf{s_I}}$ of the reduced system in terms of the relative changes in the variables.

It remains to transform these into the elasticities $\boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}}$ of the original system. We start from the following relationship, which was eqn. (64) of ref. 24:

$$\boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}} = [\boldsymbol{\varepsilon}^{\mathbf{v^*}}_{\mathbf{s_I}} \quad \mathbf{R}^{\mathbf{j}}_{\mathbf{t}}] [\mathbf{L}_n \mathbf{R}^{\mathbf{s}}_{\mathbf{t}}]^{-1} \quad (21)$$

in which $\mathbf{R}^{\mathbf{j}}_{\mathbf{t}}$ and $\mathbf{R}^{\mathbf{s}}_{\mathbf{t}}$ are the response coefficient matrices for fluxes and concentrations respectively generated by modulation of the moiety-conserved vector $\mathbf{t}$, and $\mathbf{L}_n$ is given by:

$$\mathbf{L}_n = \begin{bmatrix} & \mathbf{I} & \\ \mathbf{S_D^{-1}} & \mathbf{L} & \mathbf{S_I} \end{bmatrix} \quad (22)$$



Scheme 3 A simple conserved cycle

Although the individual concentrations of S_1 and S_2 can change, their sum is fixed by the stoichiometry of the system.

where $\mathbf{S_I}$ and $\mathbf{S_D}$ are diagonal matrices whose elements are the independent and dependent concentrations respectively. $\mathbf{L}$ depends on the stoichiometry only: premultiplying the matrix of the linearly independent rows of the stoichiometry matrix by $\mathbf{L}$ produces the matrix of the linearly dependent rows of the stoichiometry matrix.

$\mathbf{R}^{\mathbf{j}}_{\mathbf{t}}$ and $\mathbf{R}^{\mathbf{s}}_{\mathbf{t}}$ can be approximated by the matrices of increment ratios, using the same procedure that led to eqn. (6):

$$\mathbf{R}^{\mathbf{j}}_{\mathbf{t}} \approx {}^{\mathbf{t}}\left[\frac{\boldsymbol{\delta}\mathbf{j}}{\mathbf{j}}\right]\cdot\left[\mathrm{Diag}\left(\frac{\boldsymbol{\delta}\mathbf{t}}{\mathbf{t}}\right)\right]^{-1} \approx {}^{\mathbf{t}}[\boldsymbol{\delta}\ln\mathbf{j}]\cdot[\mathrm{Diag}\,(\boldsymbol{\delta}\ln\mathbf{t})]^{-1} \tag{23}$$

$$\mathbf{R}^{\mathbf{s}}_{\mathbf{t}} \approx {}^{\mathbf{t}}\left[\frac{\boldsymbol{\delta}\mathbf{s}}{\mathbf{s}}\right]\cdot\left[\mathrm{Diag}\left(\frac{\boldsymbol{\delta}\mathbf{t}}{\mathbf{t}}\right)\right]^{-1} \approx {}^{\mathbf{t}}[\boldsymbol{\delta}\ln\mathbf{s}]\cdot[\mathrm{Diag}\,(\boldsymbol{\delta}\ln\mathbf{t})]^{-1} \tag{24}$$

Substitution of eqns. (20) and (22)–(24) into eqn. (21) gives expressions for the elasticities that depend on the steady-state values of the variables and the relative changes in the total concentrations of the conserved moieties $\boldsymbol{\delta}\mathbf{t}/\mathbf{t}$ or $\boldsymbol{\delta}\ln\mathbf{t}$, which are parameters. This is a difference from the double-modulation method applied to systems without conserved cycles, for which the elasticities depend only on the values of the variables, as discussed in the Elasticities from changes in variables section.

Finally, we shall illustrate the application of the double-modulation method to the simple conserved cycle of Scheme 3, in which the variable concentrations S_1 and S_2 are subject to the following conservation constraint:

$$S_1 + S_2 = T \tag{25}$$

T being the constant total concentration of the conserved moiety. We shall choose S_1 as the independent concentration and S_2 as the dependent concentration. There are two rates, v_1 and v_2, which must both be modulated, as $r_{\mathrm{mod}} = 2$ (cf. eqn. 11). We shall initially consider v_1 as the independent rate and v_2 as the dependent rate, and in this case eqn. (20) takes the following form:

$$\epsilon^{v_1^*}_{S_1} \approx {}^{v_2}\left(\frac{\delta J_1}{J_1}\right)\Big/{}^{v_2}\left(\frac{\delta S_1}{S_1}\right) \approx {}^{v_2}(\delta\ln J_1)/{}^{v_2}(\delta\ln S_1) \approx {}^{v_2}O^{J_1}_{S_1} \tag{26a}$$

Redefining the independent rate as dependent and vice versa, the new form of eqn. (20) is the following:

$$\epsilon^{v_2^*}_{S_1} \approx {}^{v_1}\left(\frac{\delta J_2}{J_2}\right)\Big/{}^{v_1}\left(\frac{\delta S_1}{S_1}\right) \approx {}^{v_1}(\delta\ln J_2)/{}^{v_1}(\delta\ln S_1) \approx {}^{v_1}O^{J_2}_{S_1} \tag{26b}$$

Because of flux conservation,

$${}^{v_i}\left(\frac{\delta J_1}{J_1}\right) = {}^{v_i}\left(\frac{\delta J_2}{J_2}\right) \equiv {}^{v_i}\left(\frac{\delta J}{J}\right) \tag{27a}$$

$${}^{v_i}(\delta\ln J_1) = {}^{v_i}(\delta\ln J_2) \equiv {}^{v_i}(\delta\ln J) \tag{27b}$$

for $i = 1, 2$, only one of the fluxes needs to be measured.

Eqns. (26a) and (26b) provide the elasticities of the reduced system. Now we must calculate those of the original system by means of eqn. (21), which for the system under consideration takes the following form:

$$\boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}} = \begin{bmatrix} \epsilon^{v_1^*}_{S_1} & R^{J_1}_T \\ \epsilon^{v_2^*}_{S_1} & R^{J_2}_T \end{bmatrix}\begin{bmatrix} 1 & R^{S_1}_T \\ -S_1/S_2 & R^{S_2}_T \end{bmatrix}^{-1} \tag{28a}$$

where:

$$R^{J_2}_T = R^{J_1}_T \equiv R^J_T \tag{28b}$$

$$R^{S_2}_T = \frac{T - R^{S_1}_T S_1}{S_2} \tag{28c}$$

and S_2 is given by eqn. (25).

The last step is to replace R^J_T and $R^{S_1}_T$ by the approximate expressions obtained from eqns. (23) and (24) respectively, i.e.:

$$R^J_T \approx \left(\frac{\delta J}{J}\right)_T\Big/\frac{\delta T}{T} \approx \frac{\delta\ln J}{\delta\ln T} \tag{29}$$

$$R^{S_1}_T \approx \left(\frac{\delta S_1}{S_1}\right)_T\Big/\frac{\delta T}{T} \approx \frac{\delta\ln S_1}{\delta\ln T} \tag{30}$$

The final equations for the elasticities in terms of the variables and T are then

$$\epsilon^{v_1}_{s_1} \approx \frac{[\delta T - {}^{T}(\delta S_1)]\cdot{}^{v_2}\left(\frac{\delta J}{J}\right) + {}^{v_2}(\delta S_1)\cdot{}^{T}\left(\frac{\delta J}{J}\right)}{{}^{v_2}\left(\frac{\delta S_1}{S_1}\right)\delta T}$$

$$\approx \frac{[\delta T - {}^{T}(\delta S_1)]\cdot{}^{v_2}(\delta\ln J) + {}^{v_2}(\delta S_1)\cdot{}^{T}(\delta\ln J)}{{}^{v_2}(\delta\ln S_1)\,\delta T} \tag{31a}$$

$$\epsilon^{v_1}_{s_2} \approx \frac{\left[{}^{T}\left(\frac{\delta S_1}{S_1}\right)\cdot{}^{v_2}\left(\frac{\delta J}{J}\right) - {}^{v_2}\left(\frac{\delta S_1}{S_1}\right)\cdot{}^{T}\left(\frac{\delta J}{J}\right)\right](S_1 - T)}{{}^{v_2}\left(\frac{\delta S_1}{S_1}\right)\delta T}$$

$$\approx \frac{[{}^{T}(\delta\ln S_1)\cdot{}^{v_2}(\delta\ln J) - {}^{v_2}(\delta\ln S_1)\cdot{}^{T}(\delta\ln J)](S_1 - T)}{{}^{v_2}(\delta\ln S_1)\cdot\delta T} \tag{31b}$$

with a similar pair of expressions for the other two elasticities $\epsilon^{v_2}_{S_1}$ and $\epsilon^{v_2}_{S_2}$.

To calculate the four elasticities we have to modulate v_1, v_2 and T and to measure J, S_1 and S_2 (this last concentration being needed for the calculation of T and δT). However, if our objective is just to calculate the elasticities associated with one step (say v_1), it is sufficient to modulate a parameter in the other step (i.e. v_2) and T.

DISCUSSION

The generalized double-modulation method developed in the present paper indicates which parameters to modulate and which variables to measure in order to calculate the elasticities of a metabolic system of any structure and size. Determination of all possible elasticities implies, of course, a substantial experimental effort, so it is important to note that the procedure makes it possible to determine only the elasticities associated with a particular rate of interest, thereby requiring less experimental effort (see, for example, eqn. 14).

Application of the generalized double-modulation method requires, strictly speaking, measurement of infinitesimal changes in the steady-state values of the variables, but these may, of course, be approximated by small finite changes. An important practical point is then to decide what is the best way to estimate the changes in the variables. To decide this it will be useful to begin by reviewing three simple ways of approximating a derivative with finite increments. Let us assume that we need the derivative at a point (y_0, p_0) and that we have measurements of two other points (y_{-1}, p_{-1}) and (y_{+1}, p_{+1}), with $p_{-1} < p_0 < p_{+1}$. Three simple approximations to the derivative are $(y_{+1}-y_0)/(p_{+1}-p_0)$, $(y_{-1}-y_0)/(p_{-1}-p_0)$, and $(y_{+1}-y_{-1})/2h$, where $h = (p_{+1}-p_0) = (p_0-p_{-1})$; these are called the right, left and central approximations respectively. The central approximation has the interesting property that, if the second derivative of the function y does not change sign in the interval (p_{-1}, p_{+1}), then there is some compensation for the systematic errors introduced by approximating infinitestimal with finite changes. For our purposes, however, the problem is that this approximation assumes that $(p_{+1}-p_0) = (p_0-p_{-1})$, i.e. that there are equal parameter changes. However, as one of the main advantages of the generalized double-modulation method is that knowledge of the magnitudes of the parameter changes is not required (see eqns. 10 and 20–24), we cannot assume this and should not assume that the central approximation is a good choice for using with the method. It is better to use the right or left approximations, ideally using both to calculate two sets of values of the elasticities: if these sets are in reasonable agreement the results can be considered acceptable.

There are other ways to check the reliability of the values obtained, as exemplified in the second case considered in the Examples section, where we showed that there might be multiple ways of calculating the elasticities. This type of test may sometimes require the modulation of more parameters and measurements of more variables than are strictly necessary, but as there are many sources of error in metabolic experiments appropriate verification to validate the results can hardly be avoided.

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APPENDIX

Proportionality between fluxes

Here we describe a procedure for working out the proportionality relationships between fluxes in a metabolic model. We shall be particularly interested in identifying the fluxes that are not proportional to any other fluxes.

Let us consider a metabolic scheme in which all the variable metabolite concentrations are independent. The possible values that this flux vector **j** can take that are compatible with the stoichiometry matrix **N** are as follows (cf. [24]):

$$\mathbf{j} = (\mathbf{I_r} - \mathbf{N^T}(\mathbf{N}\mathbf{N^T})^{-1}\mathbf{N})\,\mathbf{x} \tag{A1}$$

where $\mathbf{I_r}$ is the identity matrix, $\mathbf{N^T}$ is the transpose of **N** and **x** is an arbitrary vector. Particular values of **j** are produced by assigning values to the elements of **x**.

We symbolize by **[1/j]** the vector whose elements are the reciprocals of the corresponding elements of **j**, and by **Q** the matrix whose elements are the ratios of fluxes. This can be calculated as follows:

$$\mathbf{Q} = [\mathbf{1/j}]\,\mathbf{j^T} \tag{A2}$$

where $\mathbf{j^T}$ is the transpose of **j**. The diagonal elements of **Q** are always one. Any other element may be a constant number if the corresponding fluxes are proportional or a function of the elements of the vector **x** if they are not. For example if the element (3, 7) is a number then the third flux is proportional to the seventh.

The number r_{np} of fluxes that are not proportional to any other flux is equal to the number of rows (or columns) of the matrix **Q** whose elements are all dependent on **x** (apart from the diagonal element, which is always one). The fluxes that are not proportional to any other flux are, of course, those corresponding to these rows (or columns).

As examples we give **Q** for two metabolic schemes. For the simple branch in Scheme 2 we have:

$$\mathbf{Q} = \begin{bmatrix} 1 & \dfrac{x_1+2x_2-x_3}{2x_1+x_2+x_3} & \dfrac{x_1-x_2+2x_3}{2x_1+x_2+x_3} \\ \dfrac{2x_1+x_2+x_3}{x_1+2x_2-x_3} & 1 & \dfrac{x_1-x_2+2x_3}{x_1+2x_2-x_3} \\ \dfrac{2x_1+x_2+x_3}{x_1-x_2+2x_3} & \dfrac{x_1+2x_2-x_3}{x_1-x_2+2x_3} & 1 \end{bmatrix} \quad \text{(A3)}$$

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In this case all the non-diagonal elements of all the three rows depend on **x**, and so $r_{np} = 3$. By contrast, for Scheme 1 the **Q** matrix is:

$$\mathbf{Q} = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \quad \text{(A4)}$$

and in consequence for a linear chain of steps any single flux is proportional to any other single flux and, therefore, $r_{np} = 0$.