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The derivation and interpretation of control coefficients

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1. Equations for control coefficients are derived by using a method that generates all the control coefficients for a system in a single procedure. This requires solving fewer simultaneous equations than an equivalent method based on 'control theorems'. 2. The interpretation of control coefficients is discussed: in particular, it is shown that these functions are unsatisfactory as measures of 'control' and are perhaps best used as a means of testing control theories (models).

1. INTRODUCTION

The strength of a metabolic control system can be measured as the relative change in the response to a given relative change in stimulus. When the changes are 'near-infinitesimal', the resulting functions are differential coefficients, which we prefer to call 'sensitivities' [1]. A group of workers has recently proposed a very different system of nomenclature (see [2,3]), which, since it is based on a general 'systems' approach [4,5], is not suitable for our 'flux-orientated' approach [1]. However, the 'systems' approach has identified an important class of sensitivities, which have been termed 'control coefficients' [2,3]. These sensitivities describe the response of a flux and its associated metabolic intermediates to an infinitesimal change of an enzyme activity. For example, the control coefficients for the system in Fig. 1 are (i) the sensitivities of the flux, J, to changes in the activities of the enzymes E_1 and E_2 ($s^J_{E_1}$, $s^J_{E_2}$) and (ii) the sensitivities of the internal effector, S, to changes in the activities of E_1 and E_2 ($s^S_{E_1}$, $s^S_{E_2}$). Although we believe that the term 'control coefficient' is inappropriate, because these sensitivities do not measure physiological 'control' (see section 4), it is now in such widespread use that confusion would only result from proposing a more logical name. However, we prefer to use the symbol 's', instead of the recommended symbol, 'C', to make it clear that control coefficients are just a special type of sensitivity.

Control coefficients are useful because they can be measured experimentally without requiring any knowledge of the nature of the internal communications that control the flux; these measured values can therefore be used to test the validity of proposed theories (models) describing these internal communications. For this it is necessary to be able to derive control coefficients from the component sensitivities of the assumed model(s), and this can be done by using several procedures. One procedure is based on 'control theorems', which are relationships between the control coefficients themselves (e.g. 'summation' theorems) or between the control coefficients and other component sensitivities ('connectivity' theorems) [3,4,6,7]. Another procedure involves a simultaneous solution of the set of rate equations, in either differential (linear) form [8] or as power equations [1,9]. We prefer to use rate equations, because it is a more direct procedure that can be used to calculate the overall sensitivity of any communication sequence (and not just control coefficients). Moreover, with complex systems involving extensive branching and different types of flux, the use of control theorems becomes very cumbersome (e.g. [10]) and requires a much larger number of equations to obtain the entire set of control coefficients (section 3).

The present paper outlines a systematic method, based on elementary matrix algebra, in which the component rate equations (in differential linear form) are used directly to derive algebraic expressions for all the control coefficients of a system in a single procedure.

2. GENERATION OF CONTROL COEFFICIENTS DIRECTLY FROM RATE EQUATIONS AND BRANCHING EQUATIONS

We have previously used power equations [9] to calculate the overall sensitivity of a complex response [1]. Although this method is straightforward (and has the advantage of using equations that resemble those of chemical kinetics), it is preferable to express the component equations in linear rather than power form when solving for a large number of sensitivities. This is because a set of linear equations can be solved simultaneously by standard procedures (e.g. by using determinants or substitutions; see ref. [11]). However, a manual solution becomes increasingly tedious as the number of equations increases; consequently, a solution by computer is essential for most metabolic systems, and this is aided by writing the equations in matrix form (see [5], [6], [7] and [9]). Since matrix representations also provide a very concise way of writing the set of equations and, in particular, of indexing their solutions, they provide a very useful basis for the general method outlined in the present paper, even with systems that are not sufficiently complex to require solving by computer.

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(i) Simple linear flux (Fig. 1)

The simple system outlined in Fig. 1 has been analysed by several workers (e.g. [1], [4], [5] and [8] and the rate equations for its component reactions, E_1 and E_2, can be written in the following linear form [1,8,9]:

$$\overset{r}{v}_1 = \sigma \cdot \overset{r}{S} + \gamma \cdot \overset{r}{X} = \overset{r}{J}$$

$$\overset{r}{v}_2 = \mu \cdot \overset{r}{S} + \delta \cdot \overset{r}{Y} = \overset{r}{J}$$

where the notation $\overset{r}{X}$ denotes an infinitesimal relative change [$\overset{r}{X} = dX/X$ or $d(\ln X)$] and σ, μ, γ and δ represent the respective intrinsic sensitivities (Fig. 1).

These two equations may be solved simultaneously, by simple substitution, to obtain the changes in variables J and S, in response to given changes in X and Y. For example, when $\overset{r}{Y} = 0$, solving the two equations gives:

$$\overset{r}{J} = \frac{\gamma \cdot \mu \cdot \overset{r}{X}}{(\mu + \bar{\sigma})}$$

where $\bar{\sigma} = -\sigma$. Consequently, the intrinsic sensitivity of J to X, i.e. the infinitesimal response when all other regulators are held constant [1], is given by the equation:

$$s^J_X = \overset{r}{J}/\overset{r}{X} = \frac{\gamma \cdot \mu}{\mu + \bar{\sigma}}$$

However, to calculate control coefficients (as opposed to discussing their physiological significance; section 4), there is no need to specify the nature of the regulator, and the above rate equations may be written more generally as:

$$\overset{r}{J} = \sigma \cdot \overset{r}{S} + \overset{r}{E}_1$$

$$\overset{r}{J} = \mu \cdot \overset{r}{S} + \overset{r}{E}_2$$

where the term 'E' represents the effect of unspecified totally external regulators [8]; the control coefficients are then obtained as the ratios $\overset{r}{J}/\overset{r}{E}_1$, $\overset{r}{J}/\overset{r}{E}_2$, $\overset{r}{S}/\overset{r}{E}_1$ and $\overset{r}{S}/\overset{r}{E}_2$. For example, solving the equations by simple substitution:

$$s^J_{E_1} = \overset{r}{J}/\overset{r}{E}_1 \quad (\text{when } E_2 = 0) = \frac{\mu}{\mu + \bar{\sigma}}$$

where $\bar{\sigma} = -\sigma$.

However, these equations and their solutions can be expressed more concisely in matrix form. To do this, the original linear equations are written with all the variables on the left-hand side and all the external interactions on the right-hand side, i.e.:

$$\overset{r}{J} - \sigma \cdot \overset{r}{S} = \overset{r}{E}_1$$

$$\overset{r}{J} - \mu \cdot \overset{r}{S} = \overset{r}{E}_2$$

The matrix representation is then:

$$\begin{vmatrix} 1 & -\sigma \\ 1 & -\mu \end{vmatrix} \begin{vmatrix} \overset{r}{J} \\ \overset{r}{S} \end{vmatrix} = \begin{vmatrix} \overset{r}{E}_1 \\ \overset{r}{E}_2 \end{vmatrix} \qquad (1)$$

The format of this matrix equation is quite straightforward. Each row across the entire representation represents one of the original linear equations. On the right-hand side no changes occur, but on the left-hand side the variables ($\overset{r}{J}$, $\overset{r}{S}$) are placed in a separate matrix from their coefficients (μ and σ for $\overset{r}{S}$; 1 for $\overset{r}{J}$). The order in which the variables appear in their 'single-column' matrix (referred to as a 'vector') is determined by the order in which they appear in the original equations: since $\overset{r}{J}$ precedes $\overset{r}{S}$ in the original equations (reading from left to right), $\overset{r}{J}$ is placed above $\overset{r}{S}$ in the vector of variables (for further details see refs. [11] and [13].

The matrix equation is solved by applying 'transformations', which involve the addition and subtraction of appropriate multiples of the rows of each matrix to each other, until the square matrix on the left-hand side becomes the 'identity' matrix. (These transformations are only applied to the square matrix and the vector on the right-hand side: the vector of variables is not transformed). The solution is then obtained from the rows of the resulting solution vector on the right-hand side.

A possible sequence of transformations for eqn. (1) is as follows: (i) subtract row 2 from row 1; (ii) add $[\sigma/(\sigma-\mu)] \times$ row 2 to row 1; (iii) divide row 2 by $(\sigma-\mu)$. These result in the equation:

$$\begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix} \begin{vmatrix} \overset{r}{J} \\ \overset{r}{S} \end{vmatrix} = \begin{vmatrix} \frac{\sigma}{\sigma-\mu}(\overset{r}{E}_2 - \overset{r}{E}_1) + \overset{r}{E}_1 \\ \frac{1}{\sigma-\mu}(\overset{r}{E}_2 - \overset{r}{E}_1) \end{vmatrix} \qquad (1a)$$

where the square matrix on the left-hand side is the (2×2) identity matrix. No terms in $\overset{r}{S}$ now appear in the

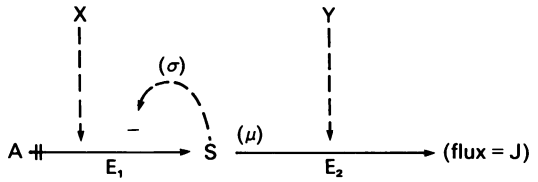


Fig. 1. Simple linear flux

S is an internal effector of flux J, whereas X and Y are totally external effectors: σ ($= s^{E_1}_{fS}$) and μ ($= s^{E_2}_{fS}$) are component, and in this case intrinsic, sensitivities (see [1]). In more complex systems (e.g. Fig. 3) the component sensitivities may be complex functions of the intrinsic sensitivities. ⊣⟶ denotes saturation with substrate A [1], which means that the interactions of X and S with E_1 do not include any competitive components with respect to A.

top row of the representation, which may be written out (expanded) as follows:

$$\dot{J} = \frac{\sigma}{\sigma-\mu}(\dot{E}_2-\dot{E}_1)+\dot{E}_1 \qquad (2)$$

Similarly, no terms in J appear in the bottom row, which may be expanded as:

$$\dot{S} = \frac{1}{\sigma-\mu}(\dot{E}_2-\dot{E}_1) \qquad (3)$$

From eqn. (2) the control coefficient, $s^J_{E_1}$, is given by the equation:

$$s^J_{E_1} = \dot{J}/\dot{E}_1 \quad (\text{when } \dot{E}_2 = 0)$$

$$= 1-\frac{\sigma}{\sigma-\mu}$$

$$= \frac{\mu}{\mu+\bar{\sigma}}$$

Since $s^J_{E_1} < 1$, the net sensitivity of J to any regulator of E_1 is less than the intrinsic sensitivity of E_1 to that regulator: this is because the action of regulators of E_1 (e.g. X) is opposed by changes of S (see [1]).

Also from eqn. (2):

$$s^J_E = \dot{J}/\dot{E}_2 \quad (\text{when } E_1 = 0)$$

$$= \frac{\bar{\sigma}}{\mu+\bar{\sigma}}$$

Similarly, from eqn. (3), the control coefficient, $s^S_{E_1}$, is given by the equation:

$$s^S_{E_1} = \dot{S}/\dot{E}_1 \quad (\text{when } E_2 = 0)$$

$$= \frac{1}{\mu+\bar{\sigma}}$$

Since $s^S_{E_1} > 0$, activation of E_1 tends to increase S, as would be expected from the model.

Also from eqn. (3):

$$s^S_{E_2} = \dot{S}/\dot{E}_2 \quad (\text{when } \dot{E}_1 = 0)$$

$$= -\frac{1}{\mu+\bar{\sigma}}$$

Since $s^S_{E_2} < 0$, activation of E_2 tends to decrease S, which is also as expected from the model.

Values for the control coefficients can therefore be calculated from the values of the component sensitivities, μ and σ, which in turn are obtained from the kinetic parameters of the reactions *in situ* (see [1] and [3]).

The above analysis shows that all four control coefficients for the system occur as coefficients of the respective 'E' terms in the solution vector of eqn. (1a): those for J occur in the first ($\dot{J}$) row and those for S occur in the second ($\dot{S}$) row. In general, the control coefficient, $s^{\text{variable}}_{E_j}$, is the coefficient of E_j in the row of the solution vector corresponding to that variable. The use of matrix methods therefore provides a very straightforward and concise way of deriving all the control coefficients and of indexing their positions in the final solution vector. As mentioned above, matrix representations are also appropriate for a solution by computer, which is essential for metabolic systems. Moreover, recent developments in computer technology [14] should soon be able to provide algebraic solutions of the set of rate equations, thereby giving the actual equations for the control coefficients directly.

The effects of specific external regulators, e.g. X and Y (Fig. 1), may be re-incorporated as the product of the control coefficient and the relevant intrinsic sensitivity [1,4], and this should always be done before assessing the physiological importance of a control site [15,16]. Thus:

$$s^J_X = s^E_{iX} \cdot s^J_{E_1}$$
$$= \gamma \cdot s^J_{E_1}$$
$$= \frac{\gamma \cdot \mu}{\mu+\bar{\sigma}}$$

If enzyme-bound intermediates transmit the flux between reactions (so that the enzyme plays more than just a catalytic role; ref. [12]), these must be included in the rate equation. For example, in the sequence:

$$S + e \underset{v_r}{\overset{v_t}{\rightleftharpoons}} eP \rightarrow Q$$

(E) (F)

where enzyme-bound P (eP) is used directly by reaction F, the rate equation for E is:

$$\dot{v} = R\cdot\dot{S} + R\cdot\dot{e} - (R-1)\cdot\dot{eP} + \dot{E}$$

where $R = v_t/v$ (see ref. [1]). The extra variable, $\dot{e}$, can be removed by using the 'enzyme conservation', $e + eP = E_t$ (which, in differential form, becomes $\dot{e} + \dot{eP} = 0$), to give the equation:

$$\dot{v} = R\cdot\dot{S} - [R\cdot(E_t/e) - 1]\cdot\dot{eP} + \dot{E}$$

(ii) Simple branched flux (Fig. 2)

This system (which is also analysed in refs. [1] and [8]) consists of three fluxes J, J_a and J_b which are all of the same type, i.e. carbon or C fluxes [1], and the rate equations (in linear form) are as follows:

For E_1:

$$\dot{J} = \alpha\cdot\dot{S} + \dot{E}_1$$

For E_2:

$$\dot{J}_a = \beta\cdot\dot{S} + \dot{E}_2$$

For E_3:

$$\dot{J}_b = \gamma\cdot\dot{S} + \dot{E}_3$$

Since there are now four variables (J, J_a, J_b and S) a complete solution requires a fourth equation, which is provided by the branch-point relationship:

$$J = J_a + J_b$$

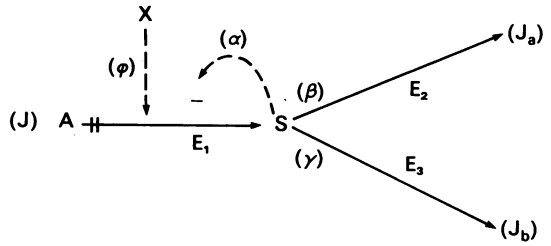


Fig. 2. Simple branched flux

As in Fig. 1, the Greek letters denote component sensitivities and ⫲⟶ denotes saturation with A.

which may be written as:

$$\mathbf{J}\cdot\vec{\mathbf{J}} = \mathbf{J}_a\cdot\vec{\mathbf{J}}_a + \mathbf{J}_b\cdot\vec{\mathbf{J}}_b$$

(see [1]). Writing the four equations in matrix form as explained in section 2(i):

$$\begin{vmatrix} 1 & 0 & 0 & -\alpha \\ 0 & 1 & 0 & -\beta \\ 0 & 0 & 1 & -\gamma \\ \mathbf{J} & -\mathbf{J}_a & -\mathbf{J}_b & 0 \end{vmatrix} \begin{vmatrix} \vec{\mathbf{J}} \\ \vec{\mathbf{J}}_a \\ \vec{\mathbf{J}}_b \\ \vec{\mathbf{S}} \end{vmatrix} = \begin{vmatrix} \vec{\mathbf{E}}_1 \\ \vec{\mathbf{E}}_2 \\ \vec{\mathbf{E}}_3 \\ 0 \end{vmatrix}$$

As in the previous example, each row across the entire matrix represents one of the linear equations, and the order of the variables in their vector is that in which they appear in the original equations. By the use of transformations similar to those outlined previously, this set of equations eventually becomes:

$$\begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{vmatrix} \begin{vmatrix} \vec{\mathbf{J}} \\ \vec{\mathbf{J}}_a \\ \vec{\mathbf{J}}_b \\ \vec{\mathbf{S}} \end{vmatrix} = \begin{vmatrix} \vec{\mathbf{E}}_1 + \frac{\alpha}{D}\left(-\vec{\mathbf{E}}_1 + \frac{\mathbf{J}_a}{\mathbf{J}}\vec{\mathbf{E}}_2 + \frac{\mathbf{J}_b}{\mathbf{J}}\vec{\mathbf{E}}_3\right) \\ \vec{\mathbf{E}}_2 + \frac{\beta}{D}\left(-\vec{\mathbf{E}}_1 + \frac{\mathbf{J}_a}{\mathbf{J}}\vec{\mathbf{E}}_2 + \frac{\mathbf{J}_b}{\mathbf{J}}\vec{\mathbf{E}}_3\right) \\ \vec{\mathbf{E}}_3 + \frac{\gamma}{D}\left(-\vec{\mathbf{E}}_1 + \frac{\mathbf{J}_a}{\mathbf{J}}\vec{\mathbf{E}}_2 + \frac{\mathbf{J}_b}{\mathbf{J}}\vec{\mathbf{E}}_3\right) \\ -\frac{1}{D}\left(-\vec{\mathbf{E}}_1 + \frac{\mathbf{J}_a}{\mathbf{J}}\vec{\mathbf{E}}_2 + \frac{\mathbf{J}_b}{\mathbf{J}}\vec{\mathbf{E}}_3\right) \end{vmatrix}$$

where $D = (\alpha - \beta\frac{\mathbf{J}_a}{\mathbf{J}} - \gamma\frac{\mathbf{J}_b}{\mathbf{J}})$ and the square matrix on the left-hand side is the (now 4 × 4) identity matrix.

As before, the solution vector on the right-hand side contains all the control coefficients of the system as the coefficients of the relevant $\vec{\mathbf{E}}$ terms. Thus $s^{\mathbf{J}}_{\mathbf{E}_1}$ is the coefficient of $\vec{\mathbf{E}}_1$ in the $\vec{\mathbf{J}}$ (first) row, so that:

$$s^{\mathbf{J}}_{\mathbf{E}_1} = 1 - \frac{\alpha}{D}$$

$$= \frac{\beta\mathbf{J}_a + \gamma\mathbf{J}_b}{\bar{\alpha} + \beta\mathbf{J}_a + \gamma\mathbf{J}_b}$$

where $\bar{\alpha} = -\alpha$ (since $\alpha < 0$).

Similarly, $s^{\mathbf{S}}_{\mathbf{E}_3}$ is the coefficient of $\mathbf{E}_3$ in the S (fourth) row, so that:

$$s^{\mathbf{S}}_{\mathbf{E}_3} = \frac{\mathbf{J}_b}{D\cdot\mathbf{J}}$$

$$= \frac{-\mathbf{J}_b}{\alpha\mathbf{J} + \beta\mathbf{J}_a + \gamma\mathbf{J}_b}$$

(This control coefficient is negative because an increased activity of $\mathbf{E}_3$ tends to decrease S.) As before, all the control coefficients are contained in the final solution vector, and can then be combined with the interaction of specific regulators: for example, the sensitivity of flux J to the external regulator X (Fig. 2) is obtained from the equation:

$$s^{\mathbf{J}}_{\mathbf{X}} = \phi\cdot s^{\mathbf{J}}_{\mathbf{E}_1}$$

$$= \frac{\phi(\beta\mathbf{J}_a + \gamma\mathbf{J}_b)}{(\alpha\mathbf{J} + \beta\mathbf{J}_a + \gamma\mathbf{J}_b)}$$

(iii) Branched system with different types of flux (Fig. 3)

In this system, which is designed to illustrate some of the principles involved in the regulation of glycolytic ATP production [15], there are both carbon (C) and 'energy' (E) fluxes (i.e. fluxes of ATP to ADP). If n is not equal to m, this system provides a complex feedback in which a change in $\mathbf{J}_a$ (≡ lactate production) or $\mathbf{J}_b$ (≡ pyruvate oxidation) changes the net yield of ATP by glycolysis and hence changes the total rate of glycolysis, J, as a result of the effect of ATP on J. This effect of $\mathbf{J}_a$ or $\mathbf{J}_b$ on J occurs in the absence of any direct feedback from either branched flux to J and would be overlooked if attention were concentrated only on these three carbon (C) fluxes. It should also be noted that, in this system, γ is not an intrinsic sensitivity, because it includes the interactions of ATP with other important auxiliary

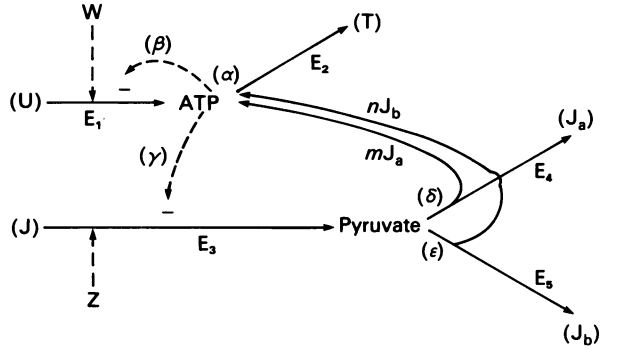


Fig. 3. Complex branched system with carbon and energy (ATP) fluxes

This system illustrates some basic principles involved in the control of glycolysis by ATP and related metabolites; however, it is not a full control model of this pathway. The system contains carbon (C) fluxes (J, $\mathbf{J}_a$ and $\mathbf{J}_b$) and 'energy' (E) fluxes (U ≡ other ATP-producing pathways; T ≡ ATP-using pathways, $m\mathbf{J}_a$ and $n\mathbf{J}_b$). For simplicity each component flux is shown as a single reaction catalysed by 'single enzyme activities' $\mathbf{E}_1$–$\mathbf{E}_5$. In practice $\mathbf{E}_1$–$\mathbf{E}_5$ represent the net effects of all component regulatory reactions; for example, $\mathbf{E}_1$ represents the overall effect of all the enzymes in flux U that communicate with U. For further details see the text.

regulators of glycolysis, for example ADP, AMP and P_i (see [1]): an intrinsic sensitivity to ATP describes the response when the concentrations of these other regulators remain constant.

As previously, the five rate equations for the system are written in their linear forms:

$$(1)\ \vec{U} = \beta \cdot \vec{ATP} + \vec{E}_1$$

$$(2)\ \vec{T} = \alpha \cdot \vec{ATP} + \vec{E}_2$$

$$(3)\ \vec{J} = \gamma \cdot \vec{ATP} + \vec{E}_3$$

$$(4)\ \vec{J}_a = \delta \cdot \vec{Pyr} + \vec{E}_4$$

$$(5)\ \vec{J}_b = \epsilon \cdot \vec{Pyr} + \vec{E}_5$$

In addition, there are now two branch-point conservation equations, one for the C flux:

$$J \cdot \vec{J} = J_a \cdot \vec{J}_a + J_b \cdot \vec{J}_b$$

and one for the E flux:

$$T \cdot \vec{T} = U \cdot \vec{U} + mJ_a \cdot \vec{J}_a + nJ_b \cdot \vec{J}_b$$

The above seven equations form the following matrix equation:

$$\begin{vmatrix} 1 & 0 & 0 & 0 & 0 & 0 & -\beta \\ 0 & 1 & 0 & 0 & 0 & 0 & -\alpha \\ 0 & 0 & 1 & 0 & 0 & 0 & -\gamma \\ 0 & 0 & 0 & 1 & 0 & -\delta & 0 \\ 0 & 0 & 0 & 0 & 1 & -\epsilon & 0 \\ 0 & 0 & J & -J_a & -J_b & 0 & 0 \\ -U & T & 0 & -mJ_a & -nJ_b & 0 & 0 \end{vmatrix} \cdot \begin{vmatrix} \vec{U} \\ \vec{T} \\ \vec{J} \\ \vec{J}_a \\ \vec{J}_b \\ \vec{Pyr} \\ \vec{ATP} \end{vmatrix} = \begin{vmatrix} \vec{E}_1 \\ \vec{E}_2 \\ \vec{E}_3 \\ \vec{E}_4 \\ \vec{E}_5 \\ 0 \\ 0 \end{vmatrix}$$

which, although more complex than those of the previous examples, can be transformed in exactly the same way to give the equation:

$$\mathbf{I} \times \begin{vmatrix} \vec{U} \\ \vec{T} \\ \vec{J} \\ \vec{J}_a \\ \vec{J}_b \\ \vec{Pyr} \\ \vec{ATP} \end{vmatrix} = \mathbf{V}$$

where **I** is the (7 × 7) identity matrix and the components of the right-hand side solution vector, **V**, are given in Table 1.

As before, the solution vector **V** contains all the control coefficients as coefficients of the respective $\vec{E}$ terms. For example, the control coefficient of 'glycolysis' (J) with respect to E_1 is the coefficient of $\vec{E}_1$ in the $\vec{J}$ (third) row of **V** (Table 1); so that:

$$s^J_{E_1} = \frac{\gamma \cdot q}{x} \cdot \gamma \cdot J \cdot U$$

$$= \frac{U\gamma(\delta J_a + \epsilon J_b)}{(\alpha T - U\beta) \cdot (\delta J_a + \epsilon J_b) - J\gamma(\delta m J_a + \epsilon n J_b)}$$

and this function can then be used to calculate the sensitivity of J to the external regulator W, by using the equation:

$$s^J_W = s^{E_1}_{tW} \cdot s^J_{E_1}$$

With this system it is interesting to consider the sensitivity of flux J to regulator Z. The required control coefficient, i.e. the control coefficient of J with respect to reaction E_3, is the coefficient of $\vec{E}_3$ in the $\vec{J}$ (third) row of vector **V** (Table 1):

$$s^J_{E_3} = \frac{qJ\gamma(\alpha T - U\beta)}{x}$$

This function is zero if α and U are both zero, i.e. if E_2 is saturated with ATP and all the ATP derives from J (a situation which may occur in the flight muscles of some insects, notably flies and bees, during flight; see refs. [17] and [18]). Consequently, under these conditions, a 'titration' of flux J with regulator Z (Fig. 3) would give a flux control coefficient of zero. If control coefficients are interpreted as measuring the 'control' of J exerted by E_3 (e.g. [19] and [20]), it would then be concluded that E_3 is not regulatory for J. However, as can be appreciated from Fig. 3, the control of J is actually exerted, via ATP, at E_3, which communicates with, and therefore is regulatory for, J [1]. This apparent paradox can be resolved by noting that control coefficients, measured experimentally, relate to the interaction of totally external regulators (i.e. 'open' regulatory sequences); they do not relate to, and hence do not detect, 'closed sequences' initiated by 'partially external' regulators, such as the interaction of ATP with E_3 and hence J in this system [15] (these terms, which, in our approach, are always defined relative to a given flux, are explained in more detail in section 5). In the extreme situation when U = 0 and α = 0, the control of J is exerted totally by the closed sequence; no 'open' regulatory sequences for J can then be initiated at E_3, and hence the control coefficient, $s^J_{E_3}$, is zero. Since even a qualitative analysis of the control of any given flux must include both 'open' and 'closed' regulatory sequences, a function relating to only one of them is not satisfactory even as an 'index' of control, unless the discussions are concerned specifically with totally external interactions. In other words, physiological control cannot always be simulated by 'adding enzymes' to systems.

3. CALCULATION OF CONTROL COEFFICIENTS BY USING CONTROL THEOREMS

Control coefficients may also be derived by using relationships referred to as 'summation' and 'connec-

Table 1. Components of vector, V, for the system in Fig. 3

Row	Vector V
1 ($\dot{U}$)	$\dot{E}_1+\frac{\beta}{J\gamma}\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)+\frac{\beta q}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
2 ($\dot{T}$)	$\dot{E}_2+\frac{\alpha}{J\gamma}\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)+\frac{\alpha q}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
3 ($\dot{J}$)	$\dot{E}_3+\frac{1}{J}\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)+\frac{\gamma q}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
4 ($\dot{J}_a$)	$\dot{E}_4+\frac{\delta}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
5 ($\dot{J}_b$)	$\dot{E}_5+\frac{\epsilon}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_1\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_b\dot{E}_4-J\dot{E}_3\right)\right]$
6 ($\dot{Pyr}$)	$\frac{1}{J\gamma}\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)+\frac{q}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
7 ($\dot{ATP}$)	$\frac{1}{x}\left[J\gamma\left(nJ_b\dot{E}_5+mJ_a\dot{E}_4+U\dot{E}_1-T\dot{E}_2\right)-\left(\alpha T-\beta U\right)\left(J_b\dot{E}_5+J_a\dot{E}_4-J\dot{E}_3\right)\right]$
where	$q=\frac{\delta J_a+\epsilon J_b}{J\gamma}$ and $x=(\alpha T-\beta U)(\delta J_a+\epsilon J_b)-J\gamma(\delta m J_a+\epsilon n J_b)$

tivity' theorems [6,7,21]. With simple systems (e.g. those in Figs. 1 and 2) this procedure is quite satisfatory, although less direct than solving a set of rate and branch-point equations. However, with more complex systems such as that in Fig. 3, the theorems become extremely complicated (e.g. [10]). Moreover, as the number of reactions increases, the approach based on theorems becomes less efficient for generating equations for all the control coefficients of the system. By using the method outlined in the present paper, all the control coefficients are obtained by solving $(r+b)$ simultaneous equations, where r is the number of reactions and b is the number of branch points. However, when control theorems are used, the control coefficients themselves become the variables, instead of the $(r+b)$ system variables such as J, J_a, J_b etc. Since each system variable generates r control coefficients of the type $s_{E_j}^{\text{variable}}$, the total number of such coefficients is $r\cdot(b+r)$. Consequently, to derive equations for all the control coefficients of a system by using control theorems, it is necessary to solve $r\cdot(b+r)$ simultaneous equations, which is $r\times$ the number involved when using the set of rate and branch-point approximations. For example, the system in Fig. 1, for which $r=2$ and $b=0$, has four control coefficients ($s^J_{E_1}$, $s^J_{E_2}$, $s^S_{E_1}$ and $s^S_{E_2}$) and therefore four (i.e. r^2) equations (two summation and two connectivity theorems) are required for a solution based on control theorems. However, only two (i.e. r) equations are needed to derive all four control coefficients when using the set of rate equations directly [section 2(i)].

It is therefore more efficient to derive equations for control coefficients directly from the set of rate and branch-point approximations, especially when the number of reactions (r) is large.

4. HOW SHOULD CONTROL COEFFICIENTS BE INTERPRETED?

It is important to realize that 'control coefficients' measure only the response of a system variable (e.g. a flux) to an infinitesimal change of an enzyme activity. They do not therefore measure the 'control exerted' in any physiological sense of that term. The reasons for this have been discussed in detail elsewhere [1,15,16], and so only a brief summary is given here.

Firstly, since all metabolic sensitivities vary continuously during a physiological (i.e. 'large') response [1,4,20,22], a single value referring only to an infinitesimal response does not reflect the overall 'strength' of the corresponding physiological response. This limitation also applies to the use of control coefficients for pharmacology or genetic engineering. For example, if two sites have measured control coefficients of 0.3 and 0.7, these values do not necessarily indicate that the latter is a better site for large external interactions (i.e. 'engineering'). In other words, the values of control coefficients can only serve to identify possible sites for external interaction and should not be used to rank them in order of importance. This also means that, for this type of analysis, it is unnecessary to determine control coefficients with high accuracy: a semi-quantitative approach similar to that developed by Rognstad [23] may be sufficient.

Secondly, because control coefficients relate to 'enzyme activities', they do not include the interactions of the totally external regulators that produce these changes in enzyme activity *in situ* [partially external interactions are included, as discussed in sections 2(iii) and 5]. However, these totally external interactions may be major factors

determining the importance of an enzyme as a control site *in situ*. For example, if an enzyme having a low flux control coefficient interacts with a totally external regulator (e.g. a hormone) having a large intrinsic sensitivity, the net sensitivity of the flux to that regulator (i.e. the product of the control coefficient and the intrinsic sensitivity [1]) may be sufficiently large to make the enzyme an important control site. This situation could arise when the interaction of the regulator involves a system of enzymically interconvertible forms, which, in theory, can provide a very high intrinsic sensitivity [1,24]. At the other extreme, an enzyme may have a measurable flux control coefficient (i.e. be a potential regulatory site) and yet have no physiological regulators [15]. Such 'inactive' or 'silent' regulatory sequences clearly play no part in controlling the flux under physiological conditions and therefore the values of their flux control coefficients do not reflect (even qualitatively) the physiological 'control' exerted by these reactions.

Thirdly, as shown in section 2(iii), the operation of a 'closed' regulatory sequence resulting from the interaction of a 'partially external' regulator can provide physiological control that is not detected by control coefficients; in an extreme situation this can result in a flux control coefficient of zero at a reaction that is nevertheless an important physiological control site.

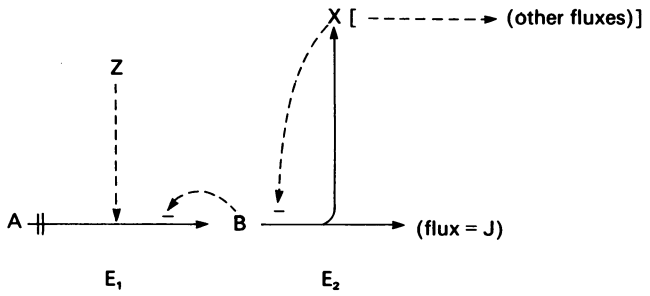
Consequently, we believe that control coefficients do not reflect (even qualitatively), and therefore should not be used to measure, the 'control' exerted by a site or an enzyme under physiological conditions. However, we accept that some workers find these functions preferable to a purely qualitative description and, provided that the above limitations are fully recognized, control coefficients can serve as approximate 'indices' of totally external control.

In our opinion control coefficients are important because they can be measured experimentally [3,7,22,25] without making any assumptions about the nature of the internal interactions that provide the control (i.e. the communications that comprise the rate equations). A comparison of experimental values with those calculated theoretically (as described in the present paper) therefore provides an additional method for testing the validity of the rate equations (i.e. the control model). Indeed, it must be stressed that the values of any control coefficients, calculated theoretically, that have not been subjected to experimental testing (e.g. those for gluconeogenesis in ref. [21]) apply only to the assumed control model and not necessarily to the relevant pathway or flux *in situ*. For example, if the branched system consisting of J, J_a and J_b in Fig. 3 had been modelled by ignoring its interactions with fluxes U and T, the calculated value of $s^J_{E_3}$ would be unity. However, because of the (overlooked) inhibitory effect of ATP (γ), this value would be greater than that measured experimentally, e.g. by 'titration' with Z; indeed, as shown above, if α and U are both zero, $s^J_{E_3}$ will be zero. Without such a comparison of experimental and theoretical values the erroneous modelling would almost certainly go undetected. However, we do not suggest that a simple test of experimental versus theoretically calculated control coefficients will always validate (or otherwise) a model. Errors in both the measured coefficients and in the kinetic data needed to calculate them will often make the result of such a test less clear-cut. Nevertheless, it can provide a valuable additional test to complement the more familiar ones [26,27]. In fact an example already exists. In a study of the control of aromatic amino acid metabolism [25], control coefficients measured by techniques such as inhibitor titration and hormonal manipulations agreed well with those calculated theoretically by using what the authors described as 'simple minded assumptions' (i.e. a very simple control model), thereby validating this simple model.

These cautionary notes apply especially to procedures for calculating theoretical values of control coefficients for pharmacological or microbiological 'engineering' [6,7]. Since these calculations require a full knowledge of the internal control structure of the pathways and since this information is very unlikely to be available, any control coefficients derived in this way will be conjectural and potentially misleading. Moreover, if the control structure is known, it would be preferable to use it directly to simulate the actual physiological or pharmacological responses, instead of basing conclusions on control coefficients that apply only to infinitesimal changes.

5. CONTROL COEFFICIENTS AND 'CLOSED' REGULATORY SEQUENCES

Metabolic systems frequently involve the sharing of intermediates (especially cofactors such as adenine and nicotinamide nucleotides) between fluxes. One example has already been discussed in relation to glycolytic ATP production [section 3(iii)], and its essential characteristics are outlined below:



Metabolite X is a regulator of flux J, but is shared between J and other, unspecified, fluxes. Since the sharing of X between the fluxes means that its concentration is partially determined by J, X is termed a 'partially external' regulator of J and its interaction with J forms a 'closed' regulatory sequence for J [15]. In contrast, the concentration of Z is not determined (even partially) by J; Z is therefore a totally external regulator of J and its interaction forms an 'open' regulatory sequence for J [15].

Since a change in concentration of the partially external regulator, X, does not affect that of a totally external regulator such as Z, the response of J to X may be calculated by treating J as if it were completely 'open', i.e. by assuming that X is a totally external regulator. A 'pseudo flux-control coefficient', $s^J_{E_{2(X)}}$, is then derived and s^J_X is calculated as the product of this and the intrinsic sensitivity, $s^{E_2}_{iX}$. (The nature of the subscript of the pseudo flux-control coefficient indicates that it is specific for the interaction of X, and does not apply to other regulators such as Z.)

However, since [X] is partially determined by J and hence by [Z], changes of [Z] will result in changes of [X],

which must therefore be included in any quantitative analysis of the response of J to Z. Consequently, in this case the relevant control coefficient cannot be calculated by treating J as a completely 'open' structure, i.e. by ignoring X. This raises a problem because, in terms of the matrix equations, the changes of [X] add an extra variable, $\dot{X}_r$, which is not matched by a corresponding equation; consequently, there are more variables than equations and the system of equations cannot be solved uniquely. The most satisfactory way of resolving this problem would be to incorporate the unspecified fluxes to form a completely open system; for example, the control coefficients for flux J in Fig. 3 are calculated from a wider system involving fluxes U and T, which together form a completely open structure. Unfortunately, this is usually not possible in practice, because the ultimate 'open' structure may include the entire metabolism of the cell or even the organism!

An alternative solution would be to measure changes of [X] experimentally and eliminate X by using an empirical function relating [X] to changes of J. However, this may be difficult technically because of the problems associated with measuring intracellular metabolite concentrations (see [1]).

Since erroneous values for control coefficients, and hence sensitivities to regulators, will often result from treating a complex metabolic pathway as a completely 'open' regulatory structure (e.g. [21]), problems caused by the operation of partially external regulators (a class to which most common regulators belong) will have to be seriously considered in future quantitative analyses of metabolic control systems.

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