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Metabolic control theory: its role in microbiology and biotechnology

(Flux control; optimisation; elasticity; concentration control; productivity; metabolism; genetic engineering)

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1. SUMMARY

Many areas of microbiology and biotechnology are directly concerned with the isolation, study or engineering of cells capable of (over)producing metabolites of commercial significance. Yet the study, production or improvement of such strains has often been at best semi-empirical. The metabolic control theory developed by Kacser, Burns, Heinrich and Rapoport can provide a rational and quantitative basis for the description and improvement of such processes.

2. INTRODUCTION

'It is now becoming generally accepted that understanding the control of biochemical processes within living organisms is no longer served by qualitative arguments but must involve quantitative methods of a very special kind' [1].

That the foregoing is something of a truism does little to hide our comparative ignorance of the exact way in which living cells, or complex ensembles of enzymes derived therefrom, control the rate of flux of substrates through metabolic pathways of interest to the microbiologist or biotechnologist. This is in contrast to the rather well-developed insights into how rates of reactions catalysed by single enzymes are affected by changes in the concentration of their substrates, products or allosteric modifiers. Yet, apart from questions of product recovery and the like, the aim in almost every area of biotechnology is to maximise the rate of conversion of substrate(s) S into product(s) P catalysed by a 'black box' system, the cell, or to maximise the intracellular concentration of a product. Thus, a proper understanding of how best in principle to circumvent normal metabolic controls so as to lead ultimately to the overproduction of a particular metabolite or protein is prerequisite to the rational design of many microbiological or biotechnological research programmes.

Now, as foreshadowed in the opening quotation, powerful, general and quantitative methods do exist with which one may properly seek to characterise the state, control structure and pro-

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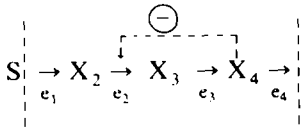
ductivity of (i.e., flux through) a metabolic pathway. However, we are not aware of a single application of these methods, to date, to microbiological or biotechnological processes. Thus, our primary aim in the present article is to give a very elementary introduction to, and an entrée to the literature concerning, these 'methods of a very special kind', with a view to fostering their more widespread adoption by the microbiological community. We believe not only that these methods will be of great benefit to the microbiologist, but also that the many interesting and 'unnatural' systems exploited by the microbial technologist will provide an important body of data of use in the further development of these methods.

The approach which we shall describe is, not only from a mathematical standpoint [2], of a very general kind, and can be applied to any system in which catalytic or energy-transducing centres (enzymes) change diffusible substances from one form into another, at rates which depend upon the concentrations of all other diffusible intermediates in the system. Such systems thus include the production of interferons from corn-steep liquor by genetically modified *Escherichia coli*, and the creation of Gross National Product by socioeconomic systems on a macroscopic scale. However, we shall confine our review to 'simple' metabolic systems in which a 'cheap' substrate is converted into a value-added 'product'.

The 'metabolic control theory' to which we allude in our title actually refers to a formalism, a conceptual approach involving a number of ideas, definitions and theorems, by which one can obtain a quantitative, rigorous and easily interpretable understanding of what controls what in a metabolic pathway. Following contributions by Higgins [3] and Savageau [4,5], the formalism originates with the work of Kacser and Burns [6] in Edinburgh, and of Heinrich and Rapoport [7,8] in Berlin: we shall hencefort refer to its salient ideas as the *metabolic control theory*. More recent reviews of the theory are by Groen et al. [9], by Westerhoff et al. [10], by Westerhoff and van Dam [11] and by Porteous [12]. It is best illustrated with reference to a generalised, unbranched metabolic pathway of the type given in Scheme 1:



Scheme 1

In this scheme, an extracellular substrate S at a certain, constant concentration is converted via a series of intermediates (X_i) to an extracellular product P at essentially constant concentration. In the steady state ($d[P]/dt$ = constant = J, the pathway flux; and $d[X_i]/dt = 0$), every enzymic step is turning over at the same rate, and the levels of intermediates have adjusted themselves so as to achieve this. One might then, as a microbiologist or fermentation technologist, ask oneself the traditional question of what step is rate-determining to the pathway flux, with the implicit assumption that the next stage in a mutation and selection (or cloning) programme aimed at maximising J should be directed at that step. (We shall see, however, that the idea of a *single* rate-determining step is inappropriate.) Yet, from observations of a single steady state alone one cannot even in principle state the extent to which any enzymic step is flux-controlling, since of course all are proceeding at the same rate.

3. FLUX-CONTROL COEFFICIENTS

Now we know, of course, that if we were by some means to effect a complete removal of any one of the enzymes in the pathway, the pathway flux would drop to zero. This is, of course, one criterion by which we can assess which enzymes are actually in the pathway, but cannot tell us the extent to which they are controlling the flux in a given steady state. To determine this we must make use of what Kacser and Burns [6] call the *method of modulation*. If we were to change the amount of an enzyme in the system by a small amount, ΔE, the metabolite concentrations would change and the system would relax to a new steady state, with a flux J′ different from J by an amount ΔJ, so that a measure of the control of enzyme E on J would be the ratio $\Delta J/\Delta E$ [3]. However, since the absolute changes ΔE and ΔJ depend on the units used to measure them, it is preferable to describe this in proportional (and hence dimen-

sionless) terms, so that, by using such fractional terms, the effect is now represented as $(\Delta J/J)/(\Delta E/E)$, where J is the initial flux and E is the initial enzyme concentration [6,7]. Further, since such relationships may be highly non-linear (Fig. 1), in the limit of a small (strictly infinitesimal) change in E we obtain a ratio $(dJ/J)/(dE/E)$ that is independent of the step size:

$$C^J_{e_i} \overset{\text{def}}{=} \left(\frac{dJ}{d\,e_i} \cdot \frac{e_i}{J} \right)_{ss} = (d \ln |J|/d \ln e_i)_{ss} \qquad (1)$$

where the subscript ss refers to the steady state and $C^J_{e_i}$ or C^J_i is known as the *flux-control coefficient of enzyme* e_i. (Because of the independent derivations of this approach, the terms and symbols equivalent to $C^J_{e_i}$ have had several different manifestations in the literature; those given here accord with the terminology agreed on by the originators of the control analysis (see [13]). In Eqn. 1, ln refers to the natural logarithm, but the two natural logarithms may simultaneously be replaced by base-10 logarithms. Thus, the flux-control coefficient is the slope of the dependence of flux upon enzyme concentration in a doubly-logarithmic plot. |J| refers to the absolute magnitude of flux J; from here on we shall omit the absolute magnitude symbol and will consider it implied in every logarithm. Importantly, the definition is such that the change in J is considered when the concentration (or rather, activity [14]) of enzyme e_i *alone* is changed; the concentrations of all other enzymes, as well as the values of all other parameters (see below) are to be kept constant.

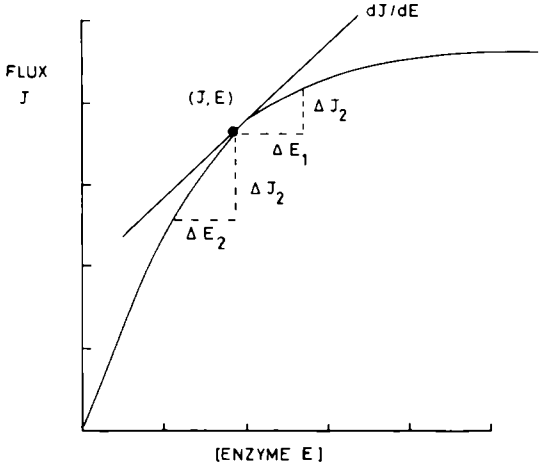


Fig. 1. The flux-control coefficient of an enzyme E. If the amount of an enzyme E in a metabolic pathway is changed by an amount ΔE then the pathway flux J will change by an amount ΔJ. Because of the non-linear relation between J and E, $\Delta J/\Delta E$ depends not only on the value of (J, E) but on the magnitude and sign of ΔE. We therefore consider limiting (small) changes in E inducing small changes in J, so that the flux-control coefficient of enzyme E is defined by $C^J_E = (dJ/J)/(dE/E)$. Since this = $(d \ln |J|/d \ln E)$ we may also obtain C^J_E as the slope of a log-log plot of J vs. E at the value of (log J, log E) prevailing.

Most often, flux-control coefficients have values lying between 0 (no flux control) and 1 ('completely rate-limiting enzyme'), although negative values (e.g. [15]) and values greater than one are possible. The important point, however, is that the magnitude of the flux-control coefficient gives an exact and proper measure of the extent to which a given enzyme (i.e., enzymic step) in a pathway controls the flux through that pathway.

3.1. The flux-control summation theorem

Repeating the above exercise for all the enzymes $e_1 \ldots e_4$ in the pathway of Scheme 1, we can define for each enzyme the extent to which it is rate-controlling, regardless, at this stage, of the mechanism by which it is exerting its rate control. Since we may imagine, correctly, that each enzyme in (or affecting) the pathway can potentially contribute to the flux control, one might imagine that the sum of the flux-control coefficients of the enzymes in (or affecting) the pathway would, when normalised, be equal to 1. Actually, the definition of the flux-control coefficients (Eqn. 1) achieves this normalisation, so that the sum of the flux-control coefficients of the enzymes in (or affecting) the pathway = 1, a theorem which is known as the *flux-control summation theorem*, and a proof of which is given elsewhere [6,14]:

$$C^J_{e_1} + C^J_{e_2} \cdots + C^J_{e_4} = \sum_{i=1}^{n} C^J_{e_i} = 1 \qquad (2)$$

It may be mentioned that the flux-control summation theorem will only in general be true under conditions in which the substrates and effectors behave as a freely diffusible pool (for most applications this is likely to be approximately true). This question is discussed in more detail elsewhere [16,17].

3.2. Distinguishing the controllers from the controlled in steady-state systems

In discussing metabolic control it is important to distinguish clearly between the properties that are set by the system itself and those that are set by the outside world. In a system with unchanging gene expression, for instance, the concentrations and kinetic properties of the enzymes are predetermined. In contrast, the concentrations of most (i.e., the freely variable) metabolites, and hence the reaction rates, are not preset. They only attain a constant value as the system reaches its steady state. To distinguish between the preset properties and the properties only having a constant value when the system is in a steady state, we call the former *parameters* and the latter *variables*. Thus, the steady-state magnitude of any variable (the 'controlled') is determined by the value of all the parameters (the 'controllers'). Appreciation of this distinction between variables and parameters greatly reduces the confusion which may arise in discussions of metabolic control. In fact, the question of how a given variable controls a flux should simply not be asked. The following example illustrates this.

If we were to increase the concentration $[X_3]$ in the pathway of Scheme 1 (above) by adding an aliquot of X_3 to the system, we would observe that the system would initially be perturbed from its steady state (V_3 will initially exceed V_2 and V_4), but would then relax back to the same steady-state condition which it was in before the extra X_3 was added. This is because the steady state of the system is completely determined by the parameters, in this case the concentrations and kinetic properties of enzymes 1 to 4 and the concentrations of S and P. Consequently the control of $[X_3]$ (or of any other variable) on the steady-state flux through the pathway of scheme 1 (or on the *steady-state* value of any *variable*) is zero.

It should be noted that not all metabolite concentrations in pathways are variables. Often the concentration of the first substrate and that of the final product is effectively constant over 'long' periods: these concentrations are then parameters. The concentrations of allosteric enzyme effectors may also be either parameters or variables; if they are metabolites intermediary in the pathway, we call them variables. We then call them internal effectors to contrast them with external effectors whose concentrations are set by the outside world. Added inhibitors are an example of the latter. We shall designate variable metabolites by X, externally clamped pathway substrates and products by S and P and external effectors by I.

In the preceding two sections we have discussed the steady-state control of one type of *variable* (the fluxes or reaction rates) by one type of *parameter* (the enzyme concentration). We shall now discuss two other cases of the steady-state control of variables by parameters.

3.3. Flux control by pathway substrates and external effectors

Changes in the concentration of the pathway substrate S or of an external effector will lead, potentially, to changes in the pathway flux, just as can changes in the concentration of an enzyme. Thus, the metabolic control theory defines flux-control coefficients for the control of pathway flux by pathway substrate S or by external effector I_n in the same way as it defines the flux-control coefficient of enzyme e_i, dropping square (concentration) brackets and absolute magnitude signs for clarity:

$$C_S^J \overset{\text{def}}{=} \left(\frac{dJ}{dS} \cdot \frac{S}{J} \right)_{SS} = (d \ln J / d \ln S)_{SS} \qquad (3)$$

$$C_{I_n}^J \overset{\text{def}}{=} \left(\frac{dJ}{dI_n} \cdot \frac{I_n}{J} \right)_{SS} = (d \ln J / d \ln I_n)_{SS} \qquad (4)$$

It may be noted that in the absence of an external effector ($I_n = 0$) the flux-control coefficient for the control of pathway flux by that effector equals zero.

3.4. Concentration-control coefficients and their summation

Complementarily, the level or activity of enzymes can effect ('control') the concentrations of metabolites and, by interaction with enzymes, any external effector can control (affect) the concentration of any metabolite. Thus, the metabolic control theory defines a family of *concentration-control coefficients*. The concentration-control coefficient for the control exerted by enzyme e_i on metabolite X_j is given by:

$$C^{X_j}_{e_i} \overset{\text{def}}{=} \left(\frac{dX_j}{d\,e_i} \cdot \frac{e_i}{X_j} \right)_{ss} = (d \ln X_j / d \ln e_i)_{ss} \qquad (5)$$

and the concentration-control coefficient of pathway substrate S on metabolite X_j by:

$$C^{X_j}_{S} \overset{\text{def}}{=} \left(\frac{dX_j}{dS} \cdot \frac{S}{X_j} \right)_{ss} = (d \ln X_j / dS)_{ss} \qquad (6)$$

The concentration-control coefficients, like the flux-control coefficients, also obey a summation theorem. In this case, however, their sum equals zero [8]. For the control on the concentration of any metabolite X:

$$C^{X}_{e_1} + C^{X}_{e_2} + \ldots C^{X}_{e_n} = 0 \qquad (7)$$

It may be noted that this implies that at least one of the enzymes must exert a negative control on X, in the sense that an increase in the activity of that enzyme will lead to a decreased steady-state concentration of X. Intuitive considerations of the control structure of metabolic pathways are in line with this theorem.

4. ELASTICITY COEFFICIENTS

We have noted that steady-state reaction rates (fluxes) and metabolite concentrations are functions of the parameters of the system (such as concentrations and kinetic properties of the enzymes) but not of the variables such as concentrations of (most) intermediary metabolites. Yet we know that when we change the concentration of a metabolite, the 'instantaneous' reaction rate of at least the enzyme for which it is a substrate changes. Although this change will disappear as the system returns to its original steady state, its magnitude is nevertheless important for describing the control structure of the metabolic system. In metabolic control theory, this initial change in rate relative to the causative change in metabolite concentration (both taken as fractional changes) is called an *elasticity coefficient.* In contrast to *control coefficients,* where changes between steady states are considered, an elasticity coefficient relates a change in a reaction rate to a change in one of the enzyme's effectors, at constant magnitude of all other factors which may affect the reaction rate. The product concentration, for instance, is considered fixed if one considers the elasticity coefficient of an enzyme e_i for its substrate X_i. In this sense as elasticity coefficient shares features with an 'apparent K_m' or '$S_{1/2}$'. Thus we have:

$$\epsilon^{V_i}_{X_i} \overset{\text{def}}{=} \left(\lim_{\delta X_i \to 0} \left(\frac{\delta V_i}{V_i} \right) \Big/ \left(\frac{\delta [X_i]}{[X_i]} \right) \right)_{[S],[P],[X_j],[e_k]} \qquad (8)$$

Through the definition of partial differentials, this may be rewritten in two different ways:

$$\epsilon^{V_i}_{X_i} = \frac{[X_i]}{V_i} \cdot \left(\frac{\partial V_i}{\partial [X_i]} \right)_{[S],[P],[X_j],[e_k]} = \left(\frac{\partial \ln |V_i|}{\partial \ln [X_i]} \right)_{[S],[P],[X_j],[e_k]} \qquad (9)$$

In these equations, the subscript variables and parameters are to be held constant in the differentiation. Indeed, elasticity coefficients differ from control coefficients in that they are *partial* derivatives (∂), measuring a change with all other potential variables kept constant. The control coefficients are *total* derivatives (d) because they take account of the total change in the variable concerned, allowing for effects through changes in all other variables. A second important difference between elasticity coefficients and control coefficients results: at given metabolite concentrations, the former are properties of single enzymes (they are 'local' properties) whilst the latter are properties of the system as a whole.

A convenient device by which one may more clearly discern the meaning of the shorthand embodied in the symbols for these control and elasticity coefficients is to note that their subscripts relate to the *causes* of a potential change, and their superscripts to an *effect.* Thus the symbol $C^{J}_{e_i}$ is the flux-control coefficient of enzyme e_i on the flux J, and $\epsilon^{v_i}_{X_i}$ or $\epsilon^{i}_{X_i}$ is the elasticity coefficient of enzyme e_i with respect to metabolite X_i.

The turnover number of many enzymes may of course be modulated not only by the concentration of their substrate(s) (and product(s)) but by other (usually allosteric) metabolites or 'internal effectors', substances which, in formal terms, may be some distance from the primary pathway of interest. Thus, an enzyme will have a family of

elasticity coefficients towards metabolites X_j, defined exactly as in Eqn. 9:

$$\epsilon^{V_i}_{X_j} = \left(\frac{[X_j]}{V_i} \cdot \frac{\partial V_i}{\partial [X_j]} \right)_{[S],[P],[X_k],[E],K_m,T}$$

$$= \left(\frac{\partial \ln |V_i|}{\partial \ln [X_j]} \right)_{[S],[P],[X_k],[E],K_m,T} \quad (10)$$

Up to this point, we have only considered the direct effects on reaction rates of changes in variables, i.e., concentrations of internal metabolites. Changes in parameters may also produce direct effects upon reaction rates, effects which are also quantified by means of elasticity coefficients. Thus, one has an elasticity coefficient of enzyme e_i with respect to the external inhibitor I_n, defined by:

$$\epsilon^{V_i}_{I_n} = \left(\lim_{\delta I_n \to 0} \left(\frac{[I_n]}{\delta [I_n]} \cdot \frac{\delta V_i}{V_i} \right) \right)_{[S],[P],[X_k],e,K_m,T} \quad (11)$$

If the external inhibitor is already present in the system ($I_n \neq 0$), then this definition may be rewritten as:

$$\epsilon^{V_i}_{I_n} = \left(\frac{\partial \ln |V_i|}{\partial \ln [I_n]} \right)_{S,P,X_k,e,K_m,T} \quad (12)$$

Regarding elasticities, changes in two types of parameter warrant extra attention. One is a change in total enzyme concentration. Here we will not pursue enzyme-enzyme interactions and monomer-dimer equilibria. The elasticity coefficient of an enzyme with respect to itself then becomes 1 and its elasticity coefficient with respect to any other enzyme becomes 0:

$$\epsilon^{V_i}_{e_i} = 1 \quad (13)$$

$$\epsilon^{V_i}_{e_j} = 0 \quad \text{if } i \neq j \quad (14)$$

The other change is one in a Michaelis constant for a substrate or a product. In almost all rate equations, the concentration of every metabolite (substrate, product or internal effector) occurs as its ratio with respect to its Michaelis or binding constant. As a consequence, the elasticity coefficient of an enzyme with respect to the concentration of a metabolite equals *minus* its elasticity coefficient with respect to the corresponding Michaelis or binding constant, here designated as K_j:

$$\epsilon^{V_i}_{X_j} = -\epsilon^{V_i}_{K_j} \quad (15)$$

Like control coefficients, elasticities are dimensionless quantities. For simple reactions not close to equilibrium, the elasticity coefficient for the substrate tends to lie between 0 and 1 and that for the product between 0 and -1. For reactions that are close to equilibrium, these elasticity coefficients can become much greater than 1, and much lower than -1, respectively. If the kinetic rate equation of an enzyme-catalysed reaction is known, its elasticity coefficients may be calculated directly by partial derivatisation (differentiation) in line with Eqns. 9–11. For instance, for the simple irreversible, product-inhibited reaction rate:

$$V_j = \frac{V_j \cdot (X_j / K_j)}{\frac{X_j}{K_j} + \frac{X_{j+1}}{K^r_{j+1}}} \quad (16)$$

one finds:

$$\epsilon^{V_j}_{X_j} = \left(\frac{X_{j+1}}{K^r_{j+1}} \right) \Big/ \left(\frac{X_j}{K_j} + \frac{X_{j+1}}{K^r_{j+1}} \right) \quad (17)$$

It is seen from this that if the concentration of the product of reaction j is far above its product inhibition constant K_{j+1}, the elasticity coefficient for the substrate X_j tends to 1. If the enzyme is saturated with respect to its substrate, however, its elasticity coefficient for the substrate X_j tends to zero. Importantly, the elasticity coefficient of an enzyme (towards a given effector) is not a *constant* property of that enzyme; it is a property that depends upon the conditions (including the metabolite concentrations) under which the enzyme is working. Indeed, this is the reason why the extent to which an enzyme controls the flux through a pathway may vary with conditions (see e.g. [18]). Of course, since in any steady-state situation the concentrations of metabolites are determined by the values of the parameters, the elasticity coefficients are also ultimately functions of the parameter values.

Now, we are well aware that these terminologies, by their unfamiliarity, and the above equations, with their many subscripts, superscripts and

Greek letters, may (as they did to us) appear daunting on the first reading. However, we hope that the beauty, power and rigour of the metabolic control theory of Kacser, Burns, Heinrich and Rapoport, and the improved understanding of metabolic control (and how to circumvent such control) which it brings, will more than compensate the short time necessary to become acquainted with these notions. Armed with the definitions above, we are now in a position to see how we make use of the theory in real life. However, we begin with one other crucial set of theorems, the connectivity theorems.

5. THE CONNECTIVITY THEOREM(S)

Enzymes which have high elasticities tend to have low flux-control coefficients, a statement which may be most simply visualised as follows. Imagine that we inhibit a given enzyme e_i by a small amount, as we do when assessing its flux-control coefficient. The initial effect will thus be an increase in the concentration of its substrate X_i, which in turn will either serve significantly to increase the flux through the step catalysed by such enzymes ('if $X_i <$ or $\simeq K_m$') or will fail to do so ('$X_i \gg K_m$'). The former circumstance is associated with a high elasticity coefficient (significant change in rate for small change in X_i), whilst the latter implies a low elasticity with respect to the substrate (small change in rate for a given change in X_i). By extending such qualitative notions to all enzymes in the pathway we have the *flux-control connectivity theorem*:

$$C_1^J \cdot \epsilon_X^1 + C_2^J \cdot \epsilon_X^2 \cdots + C_n^J \cdot \epsilon_X^n = 0 \tag{18}$$

or

$$\sum_{i=1}^{n} C_i^J \cdot \epsilon_X^i = 0 \tag{19}$$

where $e_1 \ldots e_n$ are any enzymes which are related or connected to each other by sharing any intermediate pool X, which may be any metabolite or internal effector [6].

In particular, for two adjacent pathway enzymes lacking feedback inhibition, such as enzymes 1 and 2 in Scheme 1, Eqns. 18 and 19 reduce to:

$$C_{e_1}^J \cdot \epsilon_{X_2}^{e_1} + C_{e_2}^J \cdot \epsilon_{X_2}^{e_2} = 0 \tag{20}$$

or

$$C_{e_1}^J / C_{e_2}^J = -\epsilon_{X_2}^{e_2} / \epsilon_{X_2}^{e_1} \tag{21}$$

In other words, the flux-control coefficients of two adjacent enzymes in a metabolic pathway tend to be inversely related to their elasticity coefficients to their common metabolite.

Similarly, there are two connectivity theorems [10,19] which relate metabolite concentration-control coefficients to elasticity coefficients:

$$\sum_{i=1}^{n} C_{e_i}^X \cdot \epsilon_X^{e_i} = -1 \tag{22}$$

and

$$\sum_{i=1}^{n} C_{e_i}^X \cdot \epsilon_Y^{e_i} = 0 \tag{23}$$

where in the latter case X and Y are different metabolites. Finally, for a specific *external* effector I acting on any enzyme e_i, it may be shown [6] that:

$$C_I^J = C_{e_i}^J \cdot \epsilon_I^{e_i} \tag{24}$$

It may be noted that in the absence of any external effector (I = 0) this equation is trivially satisfied. Groen et al. [9] have shown that in that case the following non-trivial equation holds:

$$C_{e_i}^J = \left(\frac{\mathrm{d} \ln J}{\mathrm{d} \ln I}\right)_{ss} \Big/ \left(\frac{\partial \ln V_i}{\partial \ln I}\right) = \left(\frac{\mathrm{d}J}{\mathrm{d}I}\right)_{ss} \Big/ \left(\frac{\partial V_i}{\partial I}\right) \tag{25}$$

5.1. Expressing the flux-control coefficients in terms of the elasticity coefficients

For an unbranched pathway, the connectivity and summation theorems provide sufficient equations to solve for the control coefficients. For the pathway given in scheme 1, with the possibility of feedback inhibition of X_4 on e_2 one finds [20]:

$$C_1^J = \rho_2 \, \rho_3 \, \rho_4 / \sum \tag{26}$$

$$C_2^J = \rho_3 \, \rho_4 / \sum \tag{27}$$

$$C_3^J = \rho_4 / \sum \tag{28}$$

$$C_4^J = \left(1 + \rho_4 \frac{\epsilon_4^2}{\epsilon_4^3}\right) / \sum \tag{29}$$

Table 1

A calculated example of flux-control coefficients for the case of scheme 1 in the absence and presence of feedback inhibition by X_4 on e_2

Assumed magnitudes for the elasticity coefficients were zero except for $\epsilon^1_{X_2} = -0.9$; $\epsilon^2_{X_2} = 0.5$; $\epsilon^2_{X_3} = -0.2$; $\epsilon^3_{X_3} = 0.7$; $\epsilon^2_{X_4} = 0$ (no feedback inhibition) or -1 (feedback inhibition); $\epsilon^3_{X_4} = -0.1$; and $\epsilon^4_{X_4} = 0.9$.

	No feedback inhibition	Feedback inhibition
C^J_1	0.30	0.19
C^J_2	0.53	0.34
C^J_3	0.15	0.10
C^J_4	0.02	0.38

with

$$\sum \overset{\text{def}}{=} \rho_2 \rho_3 \rho_4 + \rho_3 \rho_4 + 1 + \rho_4 \frac{\epsilon^2_4}{\epsilon^3_4} \tag{30}$$

and

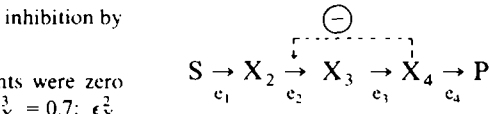
$$\rho_i \overset{\text{def}}{=} -\epsilon^i_{X_i} / \epsilon^{i-1}_{X_i} \tag{31}$$

ρ_i is thus the ratio of the two elasticity coefficients of the adjacent enzymes with respect to metabolite X_i, i.e., that to which X, is a substrate and that to which X_i is a product, respectively. Most often values of ρ exceed 1. A negative ϵ^2_4 implies that X_4 inhibits e_2. By way of a numerical example, we have calculated the flux-control coefficients for a case without and with feedback inhibition of X_4 on e_2. The results, which are perhaps counter-intuitive, are given in Table 1.

6. A BIOTECHNOLOGICAL QUESTION

Taken together, the summation and connectivity theorems provide the microbiologist and biotechnologist with a tool suitable for finding quantitative and mechanistic answers to questions such as: 'which enzymes are most rate determining to the flux through a metabolic pathway (and why)?' and 'what manipulations should I do to increase the flux through a certain pathway?' (e.g., a pathway synthesising and excreting a desirable substance) or 'what should I do to increase the intracellular concentration of a certain metabolite?' (e.g., if the substance is to be obtained by harvesting and lysing the cells). We shall here address the former types of question.

Let us suppose that we have a pathway like that of Scheme 1:



From unspecified evidence, we may (i) suspect X_4 to exert a significant feedback inhibition on e_2. There may also be other reasons why, from traditional considerations, we may expect that reaction 2 in the pathway may be providing a bottleneck, such as (ii) the concentration of X_2 may be much lower than the $S_{1/2}$ of enzyme 2 for X_2 (i.e., enzyme 2 is operating far below its maximum capacity) or (iii) the 'concentration' of enzyme e_2 (in terms of V_{max} or k_{cat}) is much lower than that of the other enzymes in the pathway. Since enzyme 2 is perceived as the bottleneck, a general strategy directed at increasing the flux through the pathway would seem to be to increase the concentration of enzyme 2, for instance by placing its synthesis under the control of a stronger promoter. More specifically, for case (i) above one might seek to modify enzyme 2 so that it is less subject to feedback inhibition, whilst for case (ii) one might opt to modify enzyme 2 so that it has a lower K_m (or higher k_{cat}/K_m) for its substrate.

Given the availability of modern methods of genetic and protein engineering, the question arises as to which of these is the best strategy, or is there perhaps a set of strategies we have overlooked? Metabolic control theory allows us to obtain a clear and quantitative answer.

Suppose we might in one strategy be able to decrease the binding constant of enzyme 2 for X_4 (which we shall call K^2_4) by a%. If a is small, equation 17 gives the effect of the decrease in K^2_4 on the flux:

$$\mathrm{d} \ln J = -C^J_{e_2} \cdot \epsilon^2_{K^2_4} \cdot \mathrm{a}\% \tag{32}$$

With the help of Eqn. 15 we may also write this as:

$$\mathrm{d} \ln J = C^J_{e_2} \cdot \epsilon^2_{X_4} \cdot \mathrm{a}\% \tag{33}$$

Similarly, the strategy which would decrease the K_m of enzyme 2 for its substrate by b% (whilst keeping the V_{max} constant) would lead to an increase in the steady-state flux of:

$$\mathrm{d} \ln J = C^J_{e_2} \cdot \epsilon^2_{X_2} \cdot \mathrm{b}\% \tag{34}$$

If the third strategy were to increase the concentration of enzyme 2 by c%, it would increase the steady-state flux by:

$$d \ln J = C^J_{e_2} \cdot c\% \tag{35}$$

Consequently, a rational choice between these strategies would be to compare the magnitudes $a\epsilon^2_{X_4}$ to $b\epsilon^2_{X_2}$ to c, and pick the largest.

If intuition had not already done so, the metabolic control theory analysis would tell us that if indeed enzyme e_2 is subject to strong feedback inhibition by metabolite X_4, the strategy of increasing the concentration of enzyme e_2 is probably not the best. As suggested by Eqn. 29 (at high $\epsilon^2_4/\epsilon^3_4$) and demonstrated by our numerical example in Table 1, such a feedback inhibition will actually shift the flux control to enzyme 4. Consequently, in a case with such feedback inhibition it may be wiser to increase the concentration of enzyme 4 or even (depending upon the magnitude of $\epsilon^4_{X_4}$) decrease the K_m of that enzyme for X_4 than to increase the concentration of enzyme 2. If d% and e% are the percentage changes we are able to impose on the K_m of enzyme 4 for X_4 and the concentration of enzyme 4 respectively, the quantitative evaluation should consider:

$$d \ln J = -C^J_{c_4} \cdot \epsilon^4_{X_4} \cdot d\% \tag{36}$$

$$d \ln J = -C^J_{e_4} \cdot e\% \tag{37}$$

Thus far we have only considered changing parameters which a combination of intuition and control theory most obviously suggest. We may still have overlooked changes which may be even more effective in increasing the flux. Elsewhere [20] we develop a completely systematic strategy, which also incorporates matrix forms of the metabolite concentration-control and connectivity theorems. We illustrate this approach [20] here for the case of a linear 4-enzyme pathway such as that of Scheme 1. The largest element of the following matrix turns out to correspond to the best strategy:

$$\begin{pmatrix} -C^J_1 \cdot \epsilon^1_{X_2} \cdot \delta \ln(K^1_2) & -C^J_1 \cdot \epsilon^1_{X_3} \cdot \delta \ln(K^1_3) & -C^J_1 \cdot \epsilon^1_{X_4} \cdot \delta \ln(K^1_4) & C^J_1 \cdot d \ln e_1 \\ -C^J_2 \cdot \epsilon^2_{X_2} \cdot \delta \ln(K^2_2) & -C^J_2 \cdot \epsilon^2_{X_3} \cdot \delta \ln(K^2_3) & -C^J_3 \cdot \epsilon^2_{X_4} \cdot \delta \ln(K^2_4) & C^J_2 \cdot d \ln e_2 \\ -C^J_3 \cdot \epsilon^3_{X_2} \cdot \delta \ln(K^3_2) & -C^J_3 \cdot \epsilon^3_{X_3} \cdot \delta \ln(K^3_3) & -C^J_3 \cdot \epsilon^3_{X_4} \cdot \delta \ln(K^3_4) & C^J_3 \cdot d \ln e_3 \\ -C^J_4 \cdot \epsilon^4_{X_2} \cdot \delta \ln(K^4_2) & -C^J_4 \cdot \epsilon^4_{X_3} \cdot \delta \ln(K^4_3) & -C^J_4 \cdot \epsilon^4_{X_4} \cdot \delta \ln(K^4_4) & C^J_4 \cdot d \ln e_4 \end{pmatrix} \tag{38}$$

Here K^j_k is the K_m, K_d or K_i of enzyme j for metabolite k, $\delta \ln K^j_k$ the change in K^j_k one can achieve by genetic manipulation and $\delta \ln e_j$ the change in enzyme concentration one can achieve by genetic manipulation. In our numerical example with feedback inhibition and assuming equal 1% changes in all the parameters, the matrix **A** becomes [20]:

$$100\,\mathbf{A} = \begin{pmatrix} -0.17 & 0 & 0 & 0.19 \\ 0.17 & -0.07 & -0.34 & 0.34 \\ 0 & 0.07 & -0.01 & 0.10 \\ 0 & 0 & 0.34 & 0.38 \end{pmatrix} \tag{39}$$

Clearly this would plead for increasing e_4 or e_2, decreasing K^4_4 or increasing K^2_4. In this particular example these four strategies would be approximately equally effective.

7. MEASUREMENT OF FLUX-CONTROL COEFFICIENTS

From the definition of the flux-control coefficient of an enzyme (Eqn. 1), i.e. $C^J_{e_i} = (dJ/J)/(de_i/e_i)$, it is obvious that a more or less direct measurement of $C^J_{e_i}$ comes from a study of the effect of (small) changes in enzyme concentration on the pathway flux. One way to change the concentration of an enzyme is of course by genetic means, and Kacser and his colleagues (e.g., [21,22]; see also [23]) have used this approach with *Neurospora crassa* by creating heterokaryons expressing different amounts of the enzyme of interest. The principle is illustrated in Fig. 3. Possible pitfalls in the simplest type of interpretation of experiments of this type include the presence of pleiotropic effects (see below). Nonetheless, this experimental program has given a very clear explanation for the fact that most mutations in diploid organisms are recessive: since the flux-control coefficient of most

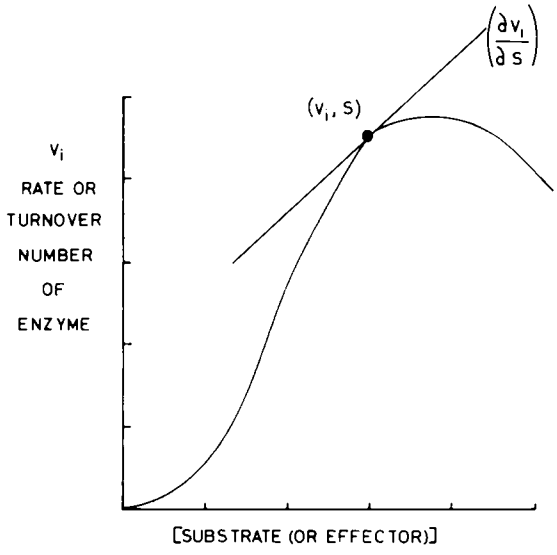


Fig. 2. The elasticity coefficient of an enzyme with respect to its substrate. The curve shows a hypothetical enzyme (with highly non-linear kinetics with respect to its substrate). The elasticity coefficient is defined as the fractional change in enzyme turnover number divided by the fractional change in substrate concentration (activity) as these tend to infinitesimally small values under conditions in which all other effectors are present and maintained at their in vivo levels. Each enzyme thus has a family of elasticity coefficients, one for every substrate and effector. The elasticity coefficients may loosely be thought of as relating to whether the system is operating below or above the K_m or K_i of its substrate or effector.

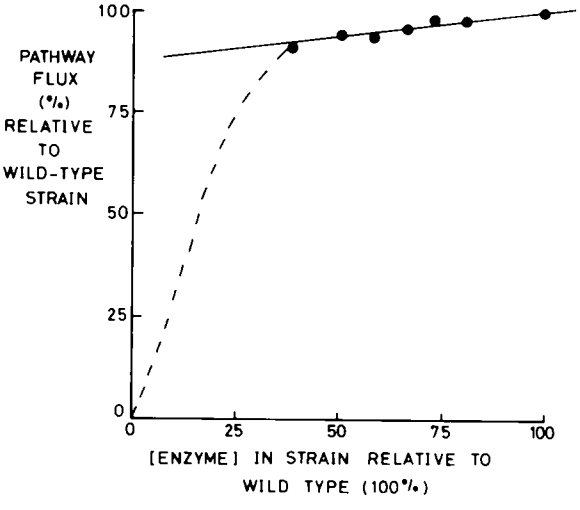


Fig. 3. Estimation of the flux-control coefficient of an enzyme by varying its level of expression in a heterokaryotic organism. As in Fig. 1, $C_E^J = (dJ/J)/(dE/E)_{SS}$.

enzymes is low, decreasing their concentration by even 50% has a rather modest effect on the flux through the pathway(s) in which they are involved [24]. This fact (that the flux-control coefficient of most enzymes is relatively small, especially in long pathways) might be anticipated from the fact that $\Sigma\, C_i^J = 1$, and also serves to explain why *so many* rounds of mutation and selection are generally required to produce a 'productive' strain (e.g. [25,26]); even the penicillin fermentation, which through countless rounds of more or less empirical mutation and selection has improved its productivity from 2 U/ml to 50000 U/ml in the last 40 years or so, still only converts some 6% of the added carbon source to the desired product [27,28].

With the modern ability to place the gene specifying as individual enzyme under the control of promoters of different strengths, one now has the ability to do very clean and elegant experiments of this type so as to measure the flux-control coefficient, although we know of only one such approach to date [29]. A related approach cloning different enzymes of lysine biosynthesis into pBR322 was also described [30].

A simpler method of obtaining the flux-control coefficient is by the use of specific inhibitors (Fig. 4) if these are available. In the case of a tight-binding ('irreversible') inhibitor, the flux-control coefficient may be read directly from a plot of the flux against the inhibitor concentration [9,10,17], although for weaker or competitive inhibitors more complex expressions must be used [9]. Again, in the modern era, one might seek to apply monoclonal antibodies against individual enzymes in permeabilised cells [31], so that this method should become generally suitable even in the absence of a previously known inhibitor. (Pitfalls here may include the presence of inhibitor-binding sites different from those of active target enzymes; their presence will lead to an underestimation of the flux-control coefficients.)

In the case of mixed cultures or consortia, one may similarly describe flux-control coefficients for *organisms*. Since the principles involved are identical to those for assessing the flux-control coefficients of *enzymes*, we do not here consider this topic further.

As we have seen, an assessment of each of the

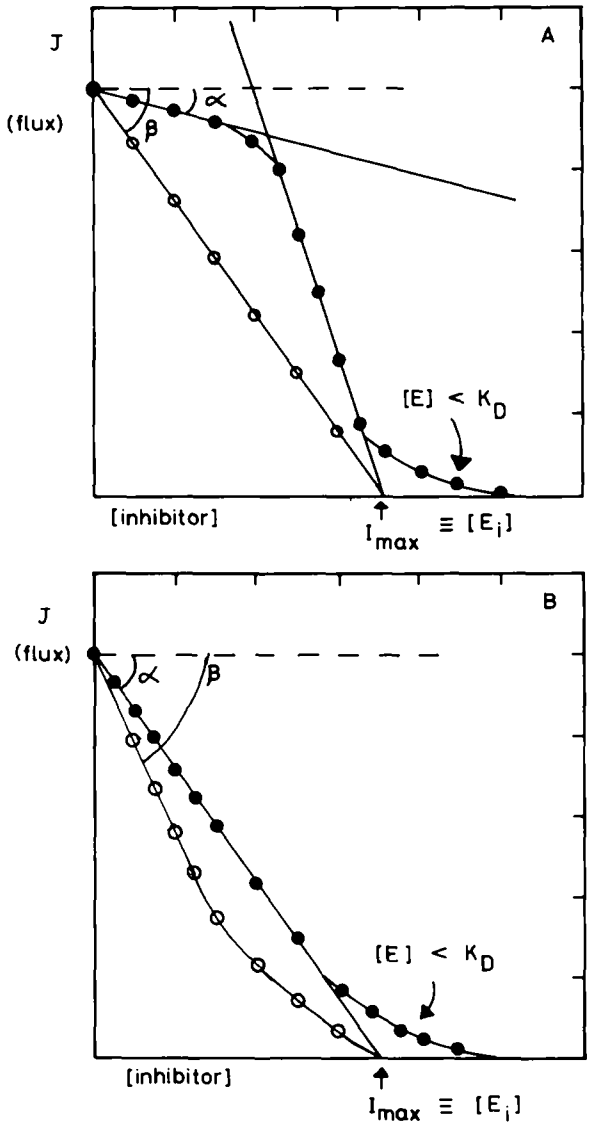


Fig. 4. Estimation of the flux-control coefficient of an enzyme by titration of the pathway flux with a specific inhibitor. Since one is usually interested in the flux-control coefficient of an enzyme in the absence of inhibitor, equation 25 is the preferred method of analysis. For a tight-binding, all-or-none type of inhibitor (A) the titration of the rate V_i at constant metabolite concentrations would give the line of open circles; $\partial V_i/\partial I$ would amount to $\tan(\beta)$. The point I_{max} may be obtained reasonably accurately by simply extrapolating the lower region of the titration of the steady-state flux. dJ/dI amounts to $\tan(\alpha)$. For a non-tight-binding inhibitor (B), the titration of the enzymic rate at constant metabolite concentrations is not a straight line. Consequently, $\tan(\alpha)$ can only be obtained empirically or calculated from the rate equation of the inhibited enzyme. In both A and B, $C_E^J = \tan(\alpha)/\tan(\beta)$.

flux-control coefficients will tell us which steps are the most rate-limiting and to what extent. To find out *why* we must measure the elasticity coefficients.

8. MEASUREMENT OF ELASTICITY COEFFICIENTS

From the definition of elasticities given in equation 8 ($\epsilon_{X_j}^{V_i} = (X_j/V_i)/(\partial V_i/\partial X_j)$, at constant concentrations of all other effectors), we find that the elasticity of an enzyme towards metabolite or effector X_j = the relative change in turnover number caused by a small relative change in the concentration of metabolite X_j. For some enzymes the kinetic rate equations have been determined and the elasticity coefficient can be calculated by differentiating this rate equation with respect to the effector considered. The most common expressions, developed by Heinrich et al. [14] and Groen et al. [9], may also be used to make rough estimates of elasticity coefficients even if the properties of the enzyme are only approximately known (e.g. if $S \triangleq K_m$ and $P \triangleq 0.1\ K_P$ and the enzyme catalyses an 'irreversible' reaction).

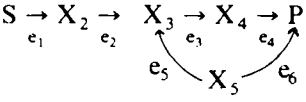
Preferably, one would determine the elasticity coefficients experimentally. This might be done by isolating the enzyme, either physically or functionally, incubating it under what one thinks to be the in vivo conditions (of pH, metabolite concentrations, temperature, etc) and measuring its rate before and after adding a small concentration (i.e., δX_j) of X_j (see Fig. 2). This approach has some difficulties, but perhaps less than might be anticipated. There is the obvious problem that during isolation e_i has been damaged and its kinetic properties (and hence its elasticity coefficient) changed. Also, allosteric effectors influencing the activity in vivo might be absent in vitro. On the other hand, the fact that the activity coefficient (γ) of the metabolite for which the elasticity coefficient is measured probably differs from that in vivo is not generally in fact a very severe problem: because *relative* changes are considered, γ tends to drop out. In the definition of elasticity coefficients, the effect of a change in say X_j, is sought in the absence of changes in the concentration of the

other intermediates. In an in vitro system, this is readily achieved.

One may also measure elasticity coefficients in systems closer to the in vivo situation (e.g., non-growing, washed cell suspensions). The problem then becomes to perturb the concentration of a specific metabolite at constant concentrations of the other metabolites and measure the effect on the reaction rate of interest. In an intact system, however, it is often impossible to measure a change in reaction rate before the concentrations of certain metabolites have changed. There are two ways round this problem. The first [6,32] uses two different steady perturbations. For the reaction rate of enzyme 3 in scheme 1 the following equation holds:

$$\delta \ln V_3 = \epsilon_3^3 \cdot \delta \ln[X_3] + \epsilon_4^3 \cdot \delta \ln[X_4] \qquad (40)$$

This equation remains valid for the transition to a new steady state. Two different (i.e., one from the left and one from the right) stationary perturbations give two equations from which the two unknown elasticity coefficients may be calculated. The second method [33] can be used when a branch is present in (or can be introduced into) the pathway. If we consider:



then one may vary both e_4 and e_5 such that X_4 is varied, V_3 changes but X_3 is constant. ϵ_4^3 then equals $\delta \ln V_3/\delta \ln X_4$.

Yet another way to determine the elasticity coefficient is by measuring the control coefficients and then calculate 'backwards' to the elasticity coefficients. This may seem to introduce a circularity if later these elasticity coefficients are to be used in calculations of flux-control coefficients. This is not always so, however. If one keeps the initial part of a pathway the same but varies its terminal stages then elasticity coefficients of the initial enzymes do not vary but their control coefficients do. An extensive example of the estimation of elasticity coefficients by combinations of the above methods for gluconeogenesis may be found in [34].

9. MORE COMPLEX PATHWAYS

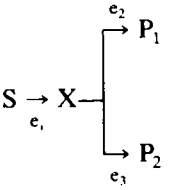
From a consideration of simple linear pathways, albeit incorporating feedback loops and allosteric effectors, we have seen (i) how the flux-control coefficients give us a direct measure of the extent to which a given enzymic step is rate-controlling; (ii) how the elasticity coefficients give us quantitative *mechanistic* information about why a step exerts strong rate control; and (iii) how flux-control, elasticity and concentration-control coefficients are related to each other by connectivity theorems. These are the fundamental parts of the metabolic control theory. We have also seen how the theory provides a simple, natural and rational explanation for why one has to go through so many rounds of mutation and selection to obtain highly overproducing strains, since flux control is shared by *all* enzymes in a pathway [16].

The theory may be extended to treat metabolic pathways of arbitrary complexity, and in this section we draw attention to some situations of more particular interest to the biotechnologist. We have thus far only discussed steady states. However, transients between (asymptotically stable) steady states may also be treated [14,35]; experimental difficulties connected with the rapid measurement of fluxes and metabolite concentrations in transient conditions suggest that the transient analyses will not find as much use as steady-state analyses, although it should be suitable for the study of the enhanced productivities of continuous cultures of certain bacteria when operated under non-steady-state conditions, an area of intense current interest [36,37].

In Scheme 1 we allowed S and P to diffuse across the cell membrane as if by magic; in practice, of course, carrier-mediated fluxes can often exert a considerable rate-limitation, and a good example is given by the improvement in fermentor productivity caused by the selective permeabilisation of the *Corynebacterium glutamicum* plasma membrane to glutamate (induction of carrier slip [38]) by biotin limitation or with certain amphiphiles [39,40]. Any reaction step is simply incorporated into the metabolic control theory as an enzymic step, whether it is associated with a chemical transformation or transport.

Cell growth, even in an exponential culture, is not a strictly (stationary) steady state but an expanding steady state [1]. Only minor modifications to the simple theory given above are needed to accommodate this, and over short-term periods a growing culture may adequately be treated as a (stationary) steady state.

Up to this point our examples have been limited to unbranched pathways. This is because for such pathways the summation and connectivity theorems provide sufficient equations to express the control coefficients in terms of the elasticity coefficients. With branched pathways, however, whilst the summation and connectivity theorems remain valid, additional theorems are needed to obtain expressions for the control coefficients in terms of elasticity coefficients and flux ratios. The general form of these equations [14,19,41,42] is somewhat complex; we will here illustrate them for the pathway of Scheme 2:



Scheme 2

The following equations follow from the fact that the steady-state production rate of X must always equal zero [14]:

$$\delta_r^1 V_r - V_1 \epsilon_X^1 \cdot C_r^X - V_2 \cdot \epsilon_X^2 \cdot C_r^X - V_3 \epsilon_X^3 \cdot C_r^X = 0 \tag{41}$$

δ_r^1 (the Kronecker delta) equals 1 if $r = 1$, and 0 if $r \neq 1$. For this simple example the equations readily allow one to solve for the concentration-control coefficients. For instance, taking $r = 1$ we find:

$$1/C_1^x = \epsilon_X^1 + \epsilon_X^2 \cdot \frac{V_2}{V_1} + \epsilon_X^3 \left(1 - \frac{V_2}{V_1}\right) \tag{42}$$

Chen and Westerhoff [42] elaborated a numerical example of a more complicated metabolic pathway and showed how, using solely equations of the type of Eqn. 41, the concentration-control coefficients can be expressed in terms of elasticity coefficients and flux ratios at the branches [14]. Additional equations relate flux-control coefficients to concentration-control coefficients. For Scheme 2:

$$C_{e_r}^{v_1} = \epsilon_X^1 \cdot C_r^X \tag{43}$$

which gives for $r = 1$:

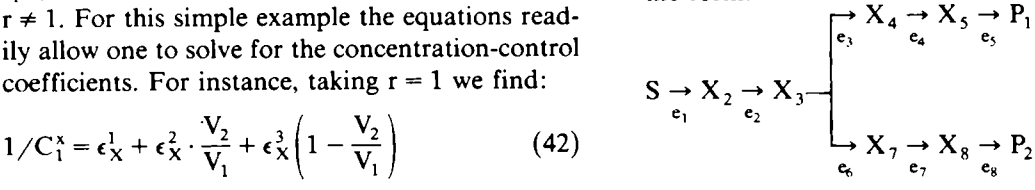
$$1/C_1^{v_1} = 1 + \frac{\epsilon_X^2}{\epsilon_X^1} \cdot \frac{V_2}{V_1} + \frac{\epsilon_X^3}{\epsilon_X^1} \cdot \left(1 - \frac{V_2}{V_1}\right) \tag{44}$$

Because one may change e_2 and e_3 such that the total flux through the two branches does not change, the following theorem also holds for scheme 2 [41]:

$$V_2 \cdot C_3^{v_1} = V_3 \cdot C_2^{v_1} \tag{45}$$

Fell and Sauro [41] devised a simple algorithm which also uses this equation plus the summation and connectivity theorems to express the control coefficients in terms of the elasticity coefficients. Both the procedure of Heinrich et al. [14] (cf. [42]), and that of Fell and Sauro [41] involve matrix inversion. However, with the present ubiquity of microcomputers this is no longer a problem. The take-home message is that, for pathways of arbitrary complexity, the control coefficients can be expressed in terms of enzymic properties (i.e., the elasticity coefficients) and the flux ratios at the branches.

Although the validity of metabolic control theory is general, there are a number of caveats, in the sense that its definitions should be carefully adhered to. For instance, for branched pathways care must be taken in assessing the sign of the flux-control coefficients. It is easy to appreciate this [1,15] by considering a branched pathway of the form:



Scheme 3

In this case, e_3, e_4 and e_5 will (usually) have negative flux-control coefficients with respect to the flux towards P_2 (and positive flux-control coefficients with respect to the flux to P_1), so that an experimental program designed to maximise the flux to P_2 will, of course, have to devote some attention to minimising that to P_1. Thus, in Scheme 3, the flux-control summation theorem would ap-

parently be violated if we only (and incorrectly) considered the pathway leading to P_2 (i.e., $C_1^J + C_2^J + C_6^J + C_7^J + C_8^J = X > 1$); however, $C_3^J + C_4^J + C_5^J = -X$ leaves the flux-control summation theorem unmodified. Another caveat is that elasticity coefficients of enzymes with respect to their products will tend to be negative (an increase in product concentration usually leads to a decrease in reaction rate).

Control coefficients are defined with respect to a change in enzyme concentration. If enzyme concentrations are of the same order of magnitude as metabolite concentrations, metabolic control theory remains valid provided that it is interpreted in terms of total enzyme concentration (i.e., [E] + [ES] + [EP]) [14]. In the case of monomer-dimer equilibria of enzymes where only the dimer is active, the definitions should be taken as referring to the dimer concentration. Special care must also be exercised when enzymes are present that couple two reactions with a variable stoichiometry (slipping enzymes), rate (isozymes) or participate in 'substrate cycles'.

The control coefficients have been defined as the change in flux (or metabolite concentration) relative to a change in enzyme activity at constant activities of other enzymes in the system. In real systems, amplification of the expression of one gene may well lead to an altered expression of other genes. In that case, prediction of the result is obtained by multiplying the control coefficient of each gene (enzyme) affected by the relative extent to which its activity has been changed and summing the results.

Perhaps the most serious caveat pertains to those metabolic systems where metabolites are not freely diffusible but are instead 'channelled' directly from one enzyme to another [16,17,43]. Here again it may well be impossible to increase the activity of one enzyme in a metabolic sequence without affecting the activity of others (the enzymes may form a supercomplex, or may even be constituted by a single polypeptide chain). In these cases, the flux-control coefficient determined by increasing the enzyme concentration by a small amount will differ from that determined by decreasing the enzyme concentration by the same small amount. Sections of metabolic pathways that are organised in a channelled fashion are most appropriately treated as a unit within the framework of metabolic control theory.

10. RELATIONSHIP OF METABOLIC CONTROL THEORY TO THE OPTIMISATION OF MICROBIAL ACTIVITIES

The optimal control strategy for a metabolic [10] or free energy-transducing [44,45] systems depends upon the 'purpose' of that pathway or system, and whilst such a discussion is only freed with difficulty from teleological arguments, it is reasonable in the present context to enquire briefly, and in the most general terms, as to what type of control structure might be one's goal in the 'design' of a system aimed at maximising the flux through a particular pathway.

Alone, the control analysis does not tell us how to maximise a metabolic flux so that it is at its theoretical, diffusion-controlled limit. What it does tell us, however, is (i) how to seek to approach this ideal and (ii) that different conditions may show (or require) that particular types of enzyme have relatively high or relatively low elasticities with respect to particular effectors [10]. We here consider the particular case in which, regardless of the energetic efficiency, it is desirable to maximise the flux through a particular pathway.

It is usual to regard the perfectness or 'efficiency' of an individual enzyme in terms of the ratio k_{cat}/K_m [46,47] or k_{cat}/K_S [48]. When considering a whole pathway, however, the description of the 'efficiency' of a particular enzyme is better phrased in terms which take account of the *amount* of that enzyme, to give the so-called 'kinetic power' [49]. Thus, in the terminology of the metabolic control theory: if the pathway is to be under diffusion control, the elasticities of enzymes towards their substrates should be high, whereas their elasticities towards feedback modifiers should be low.

11. CONCLUDING REMARKS

We hope that this introduction to the metabolic control theory will have served to indicate its

potentially great utility in microbiology and biotechnology. However, we would urge readers who are stimulated to adopt the formalism to read the original reference given, where a much fuller treatment may be obtained (but bearing in mind the changes in terminology for flux-control and elasticity coefficients). Armed with the formalism, much useful progress in the rational design of microbiological processes may be anticipated; conversely, deeper considerations, based upon appropriate experimental programs, of the structure of metabolic systems of interest to microbiologists and biotechnologists will provide a stimulus to the academic biochemical community further to develop the formalism.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Kacser, H. (1983) The control of enzyme systems in vivo: elasticity analysis of the steady state. Biochem. Soc. Trans. 11, 35–40.

[2] Cruz, J.B. (1973) System Sensitivity Analysis. Dowden, Hutchinson and Ross, Stroudsburg, PA.

[3] Higgins, J.J. (1965) in Control of Energy Metabolism (Chance, B., Estabrook, R. and Williamson, J.R., Eds.) pp. 13–46. Academic Press, New York.

[4] Savageau, M.A. (1972) The Behaviour of Intact Biochemical Control Systems. Curr. Top. Cell. Reg. 6, 63–130.

[5] Savageau, M.A. (1976) Biochemical Systems Analysis; a Study of Function and Design in Molecular Biology. Addison-Wesley, Reading, MA.

[6] Kacser, H. and Burns, J.A. (1973) The control of flux, in Rate Control of Biological Processes (D.D. Davies, Ed.), Symp. Soc. Exp. Biol., Vol. 27, pp. 65–104. Cambridge University Press, Cambridge.

[7] Heinrich, R. and Rapoport, T.A. (1973) Acta Biol. Med. Germ. 31, 479–494.

[8] Heinrich, R. and Rapoport, T.A. (1974) A linear steady-state treatment of enzymatic chains: general properties, control and effector-strength. Eur. J. Biochem. 42, 89–95.

[9] Groen, A.K., van der Meer, R., Westerhoff, H.V., Wanders, R.J.A., Akerboom, T.P.M. and Tager, J.M. (1982) Control of metabolic fluxes, in Metabolic Compartmentation (H. Sies, Ed.), pp. 9–37. Academic Press, New York.

[10] Westerhoff, H.V., Groen, A.K. and Wanders, R.J.A. (1984) Modern theories of metabolic control and their applications. Biosci. Rep. 4, 1–22.

[11] Westerhoff, H.V. and van Dam, K. (1986) Mosaic Non-Equilibrium Thermodynamics and the Control of Biological Free Energy Transduction. Elsevier, Amsterdam.

[12] Porteous, J.W. (1986) Control and Constraint in Metabolism. Cambridge University Press, Cambridge.

[13] Burns, J.A., Cornish-Bowden, A., Groen, A.K., Heinrich, R., Kacser, H., Porteous, J.W., Rapoport, S.M., Rapoport, T.A., Stucki, J.W., Tager, J.M., Wanders, R.J.A. and Westerhoff, H.V. (1985) Control Analysis of Metabolic Systems. Trends Biochem. Sci. 10, 16.

[14] Heinrich, R., Rapoport, S.M. and Rapoport, T. (1977) Metabolic Regulation and Mathematical Models. Prog. Biophys. Mol. Biol. 32, 1–83.

[15] Westerhoff, H.V. and Arents, J.C. (1984) Two (completely) rate-limiting steps in one metabolic pathway? The resolution of a paradox using bacteriorhodopsin liposomes and the control theory. Biosci. Rep. 4, 23–31.

[16] Kell, D.B. and Westerhoff, H.V. (1985) Catalytic facilitation and membrane bioenergetics, in Organized Multienzyme Systems; Catalytic Properties (G.R. Welch, Ed.) pp. 63–139. Academic Press, New York.

[17] Westerhoff, H.V. and Kell, D.B. (1986) A control theoretical analysis of inhibitor titrations of metabolic channelling. Comments Mol. Cell. Biophys., in press.

[18] Groen, A.K., Wanders, R.J.A., Westerhoff, H.V., van der Meer, R. and Tager, J.M. (1982) Quantification of the contribution of various steps to the control of mitochondrial respiration. J. Biol. Chem. 257, 2754–2757.

[19] Westerhoff, H.V. and Chen, Y. (1984) How do enzyme activities control metabolite concentration? An additional theorem in the theory of metabolic control. Eur. J. Biochem. 142, 425–430.

[20] Westerhoff, H.V. and Kell, D.B. (1986) A matrix method for determining the steps most rate-limiting to metabolic fluxes in biotechnological processes. Submitted for publication.

[21] Flint, H.J., Porteous, D.J. and Kacser, H. (1980) Control of the flux in the arginine pathway of *Neurospora crassa*. The flux from citrulline to arginine. Biochem. J. 190, 1–15.

[22] Flint, H.J., Tateson, R.W., Barthelmess, I.B., Porteous, D.J., Donachie, W.D. and Kacser, H. (1981) Control of the flux in the arginine pathway of *Neurospora crassa*. Modulations of enzyme activity and concentration. Biochem. J. 200, 231–246.

[23] Middleton, R.J. and Kacser, H. (1983) Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in *Drosophila melanogaster*. Genetics 105, 633–650.

[24] Kacser, H. and Burns, J.A., Eds. (1981) The molecular basis of dominance. Genetics 97, 639–666.

[25] Herrmann, K.M. and Somerville, R.L. (1983) Amino Acids: Biosynthesis and Genetic Regulation. Addison-Wesley, Reading, MA.

[26] Tosaka, O., Enei, H. and Hirose, Y. (1983) The Production of L-Lysine by Fermentation. Trends Biotechnol. 1, 70–73.
[27] Cooney, C.L. (1979) Conversion yields in penicillin production: theory vs. practice. Process Biochem. 14 (5), 31–33.
[28] Kell, D.B. (1980) The role of ion-selective electrodes in improving fermentation yields. Process Biochem. 15 (1), 18–29.
[29] Walsh, K. and Koshland Jr., D.E. (1985) Characterization of rate-controlling steps in vivo by use of an adjustable expression vector. Proc. Natl. Acad. Sci. USA 82, 3577–3581.
[30] Dauce-Le Reverend, B., Boitel, M., Deschamps, A.M., Lebeault, J.M., Sano, K., Takinami, K. and Patte, J.C. (1982) Improvement of *Escherichia coli* overproducing lysine using recombinant DNA techniques. Eur. J. Appl. Microbiol. Biotechnol. 15, 227–231.
[31] Felix, H. (1982) Permeabilized cells. Anal. Biochem. 120, 211–234.
[32] Kacser, H. and Burns, J.A. (1979) Molecular democracy: who shares the controls? Biochem. Soc. Trans. 7, 1149–1160.
[33] Wanders, R.J.A., Groen, A.K., van Roermund, C.W.T. and Tager, J.M. (1984) Factors determining the relative contribution of the adenine nucleotide translocator and the ADP-regenerating system to the control of oxidative phosphorylation in isolated rat-liver mitochondria. Eur. J. Biochem. 142, 417–424.
[34] Groen, A.K. (1984) Quantification of control in studies of intermediary metabolism. Thesis, University of Amsterdam.
[35] Heinrich, R. and Rapoport, T.A. (1975) Mathematical analysis of multienzyme systems, II. Steady state and transient control. Biosystems 7, 130–136.
[36] Bazin, M.J., Ed. (1982) Microbial Population Dynamics. CRC Press, Boca Raton, FL.
[37] Cunningham, A. and Nisbet, R.M. (1983) Transients and oscillations in continuous culture, in Mathematics in Microbiology (Bazin, M.J., Ed.) pp. 77–103. Academic Press, London.
[38] Eddy, A.A. (1980) Slip and leak models of gradient-coupled solute transport. Biochem. Soc. Trans. 8, 271–273.
[39] Demain, A.L. and Birnbaum, M.J. (1968) Alteration of permeability for the release of metabolites from the microbial cell. Curr. Topics Microbiol. 46, 1–25.
[40] Clement, Y., Escoffier, B., Trombe, M.-C. and Lanéelle, G. (1984) Is glutamate excreted by its uptake system in *Corynebacterium glutamicum*? A working hypothesis. J. Gen. Microbiol. 130, 2589–2594.
[41] Fell, D.A. and Sauro, H.M. (1985) Metabolic control and its analysis. Additional relationships between elasticities and control coefficients. Eur. J. Biochem. 148, 555–561.
[42] Chen, Y. and Westerhoff, H.V. (1986) How do inhibitors and modifiers of individual enzymes affect steady-state fluxes and concentrations in metabolic systems? Adv. Math. Comp. Med., in press.
[43] Welch, G.R. and Clegg, J.S., Eds. (1986) Organization of Cell Metabolism. Plenum Press, New York.
[44] Stucki, J.W. (1980) The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. Eur. J. Biochem. 109, 269–283.
[45] Westerhoff, H.V., Hellingwerf, K.J. and van Dam, K. (1983) Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. Proc. Natl. Acad. Sci. USA 80, 305–309.
[46] Albery, W.J. and Knowles, J.R. (1976) Efficiency and evolution of enzyme catalysis. Angew. Chem. Int. Ed. Engl. 16, 285–293.
[47] Fersht, A.R. (1985) Enzyme Structure and Mechanism, 2nd ed. W.H. Freeman, San Francisco.
[48] Brocklehurst, K. and Cornish-Bowden, A. (1976) The pre-eminence of k_{cat} in the manifestation of optimal enzyme activity delineated by using the Briggs-Haldane two-step irreversible kinetic model. Biochem. J. 159, 165–166.
[49] Keleti, T. and Welch, G.R. (1984) The evolution of enzyme kinetic power. Biochem. J. 223, 299–303.