

The Control of Flux

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Foreword

The Control of Flux by Kacser & Burns [1], originally published in 1973, together with the work independently developed by Heinrich & Rapoport [2, 3], started the development of the field now called Metabolic Control Analysis. It is still considered today the most readable account of the subject. The article is reprinted here (with the permission of the Society for Experimental Biology) with two types of alteration. The first is that certain of the terms and symbols originally used have been replaced by those adopted by international agreement in 1985 [4] and now in common use in the literature of Metabolic Control Analysis. The intention behind this is to make this important paper more accessible to those who have been drawn into the field through more recent articles. The changes are indicated where they first occur and a Glossary of them all is given at the end. The second type of alteration is the insertion of comments to indicate where subsequent work has led to support and development of the original ideas. These additions can be recognised by the different style in which they are laid out, as in this section. The references to the additional work are given in a second reference list following the original.

D. A. Fell & H. Kacser.

Introduction

The literature on Control is distinguished by a vast quantity of detailed information on particular systems and very little discussion on general problems. There also appears to be confusion, or at least, disagreement — disagreement on definitions, on concepts, on criteria and on methodology. There are probably several reasons for this. Firstly, we are concerned with the properties of very complex systems, complex in the sense that there are many different types of component which interact in a variety of ways and hence can display many modes of behaviour. Secondly, there is no established general idea of control of biochemical systems, There are bits of theory but no comprehensive theory.

One bit of theory is usually represented as metabolic maps. These maps give information on the structure of the system : they tell us about transformations, syntheses and degradations and they represent the molecular anatomy. They tell us 'what goes' but not 'how much'. Another bit of theory is called enzymology. We have many data on individual enzymes, their kinetics, inhibition characteristics, molecular structure and some theory of catalysis. All these give us elementary functional information.

It is an obvious step to combine the two parts. And here we immediately come to a stop since, as anybody who has tried this realises, we end up with a large set of simultaneous non-linear equations for which there is no explicit solution (Appendix A). We can, of course, use computers to solve particular cases. In the hands of an expert such simulation can lead to insight (e.g, see Garfinkel, 1971). More often it will simply confirm that our assumptions about the system are correct. In either case, however, it cannot yield a general theory of control.

Discussions on 'control' or 'regulation' usually centre on the question of which enzymes are controlling the flux in a pathway and suggest experimental means of identification. In the absence of a general theory of control, the basis of such experimental identification will remain obscure, being at best intuitively plausible. (See, however, the excellent review by Rolleston, 1972.) The measurements which are made for this identification include the levels of enzymes and of pools, inhibition characteristics of enzymes and feedbacks, the levels of inhibitors or activators, the measurement of fluxes and their changes under a variety of conditions. The discussions suggest that 'controlling enzymes' can be identified as those which satisfy a number of different criteria (e.g., see Chance, Holmes, Higgins and Connelly, 1958; Newsholme and Gevers, 1967, Scrutton and Utter, 1968) which are rather directly related to the measurements. It is, however, not clear in what way diverse criteria are related or how consistent they are with one another. But principally, what appears to be lacking are agreed definitions of the various aspects of control to which these criteria could be applied. The establishment of the necessary definitions and their

inter-relations requires a rigorous theoretical approach.

Higgins (1963) has presented a kinetic treatment of sequential reactions and has analysed system responses in terms of 'reflection coefficients'. Our treatment is based on essentially the same approach. We shall outline a general theory of steady-state enzyme networks and apply it to the problem of the control of flux. It involves concepts to which we find it useful to attach new names and a methodology which is basic to our approach. Four control measures will be defined, given operational meaning and set into the framework of a quantitative systems theory.

Parameters, Variables and Responses

We must begin by being clear about the distinction between parameters and variables in a biochemical system. On the one hand parameters represent the constant constraints of any organism. We can identify these with the genetically determined constants of enzymes such as turnover numbers, Michaelis constants, inhibition constants, etc., and with the externally set concentrations of the nutrients, effectors and other substances. Under a wide variety of circumstances enzyme concentrations are found to be uninfluenced by changes in metabolism, in which case they too can be treated as parameters. There are, in addition, general thermodynamic constraints represented by the equilibrium constants. The system parameters concerned with enzyme quantity and quality and with the environmental conditions, are constant for most interpretable experiments, but are, within limits, under *our* control. We can set, say, glucose or arginine outside the system to any desired value and we can similarly, though not quite as easily, alter the enzymic values by e.g., either changing the dose or the alleles of certain genes.

The variables, on the other hand, represent the levels of metabolites and the diverse molecular forms arising from them. It is their characteristic that they move and settle to certain values. In, e.g., the steady state, which is both algebraically and experimentally a convenient state to consider, the concentrations of the pools are time-invariant because each has reached a balance of formation and removal which in turn depends on other rates, extends right through the system and involves, in principle, all the enzymes¹.

¹It has often been said that pools 'must be maintained at their proper levels' or that there is a 'normal' level, 'excess' of which would either be uneconomic or would upset 'the delicate equilibrium' so necessary to 'integrate the different metabolic functions'. Natural selection has been invoked as being responsible for this amazing feat of juggling. Those who are aware of the forces responsible for coming to a steady state realise, of course, that this is a fanciful delusion. Almost any set of enzymes will generate a steady state with all fluxes in operation. The existence of the vast array of genetic variation shows that there are very many different 'delicate equilibria' which are just right. As Mark Twain observed, while marvelling at our amazing adaptation: 'Our legs are just long enough to reach the

The pools are in fact the 'links' in the system's interactions. Thus pool concentrations and fluxes (which are flows into and out of pools) are determined by the constellation of the system's parameters. Flux is a systemic property and questions of its control cannot be answered by looking at one step in isolation — or even each step in isolation. An analysis must consequently be in terms of the quantitative relations between the parts as much as in terms of the gross structure or the molecular architecture of its catalysts. Any particular pool or flux is thereby not determined by a single parameter, but in general, by all of them acting and interacting as a system. The situation outlined above can be represented mathematically in a fairly general way (Appendix A).

This raises the first question: Are all parameters equally important in influencing the value which a particular variable, say a flux, settles to? Or an alternative form of the same question: What is the quantitative influence of one parameter on, say, one particular flux and how do we ascertain it?

Clearly if we want quantitative answers we must ask quantitative questions. Let us take a flux through a pathway and focus our attention on one of the enzymes. If we could, in a thought experiment or in a real experiment, make a change in the quantity of this enzyme, and this enzyme alone, and observed any change in flux which may be caused, this would be a relevant observation. We would get an idea how sensitive the flux is to changes in this enzyme's concentration.

Secondly, if we extracted the enzyme and investigated how its rate of reaction varied with, say, an inhibitor concentration, this would be a relevant observation. This might tell us how controllable this enzyme is, particularly if we knew what the inhibitor concentration *in vivo* was. Thirdly, we could investigate how changes in the pool concentrations, from the values they have, would affect the rate of the reaction. This might tell us how 'elastic' that step is in response to pool changes.

If it is agreed that these three vague questions are not unlike what many people have in mind when talking about control, we can proceed to make them more precise. We will in fact show that they allow us to formulate a quantitative systems theory of control. But first we must translate these questions into a form which is suitable for both experimental procedures and theoretical evaluation.

The following example will suggest how this can be approached. Take an external parameter, say an effector acting as a specific enzyme inhibitor, I , which we allow to act on an enzyme in the system.

Let us measure by some suitable method, the flux, J , carried by that enzyme step in the presence of a certain fixed external concentration I' .

Flux was symbolised by F in the original.

ground.'

For this part of the argument it is assumed that the internal level, I , depends only on the external level I' . In the case of compartmentation the effective internal concentration may be difficult to ascertain.

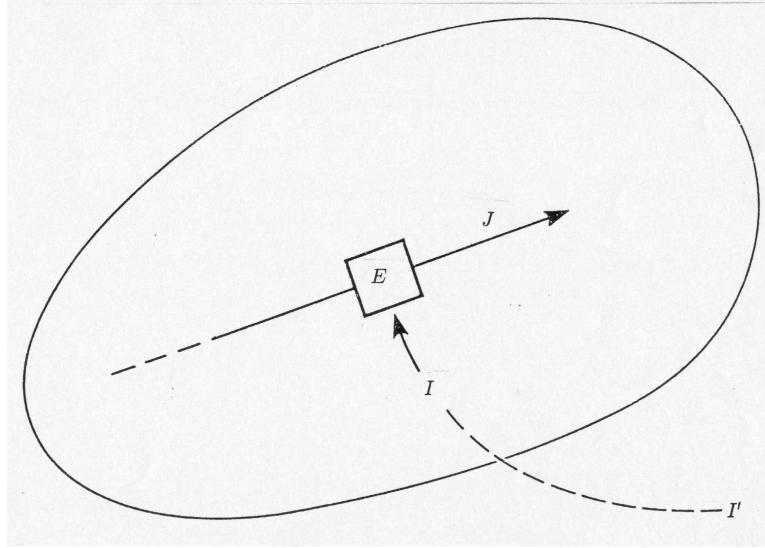


Figure 1:

How does variation in I affect J ? Let us impose such a variation, ΔI , and observe how J changes. Since the absolute changes, ΔI and ΔJ , depend on the units chosen to measure these, it is best to use fractional changes to describe this, $\Delta J/J$ and $\Delta I/I$, independent of units.

A measure of the effect of I on J would be the ratio

$$\frac{\Delta J}{J} \bigg/ \frac{\Delta I}{I}$$

But since, in general, the relationship is non-linear, this ratio will depend on the size of the fractional change made. If, however, we reduce the size of ΔI , then, in the limit, we obtain a ratio of differentials independent of step size

$$\frac{\Delta J}{J} \bigg/ \frac{\Delta I}{I} \rightarrow \frac{dJ}{J} \bigg/ \frac{dI}{I} = R_I^J.$$

This coefficient, R_I^J [Note: R in the original], will be recognised as the scaled slope at the value of I

$$\frac{dJ}{dI} \cdot \frac{I}{J} = R_I^J = \text{slope} \cdot \frac{I}{J}.$$

In practice, an infinitesimal change cannot be achieved, but very small intervals, δ , can approximate this:

$$\frac{\delta J}{J} \bigg/ \frac{\delta I}{I} \approx R_I^J$$

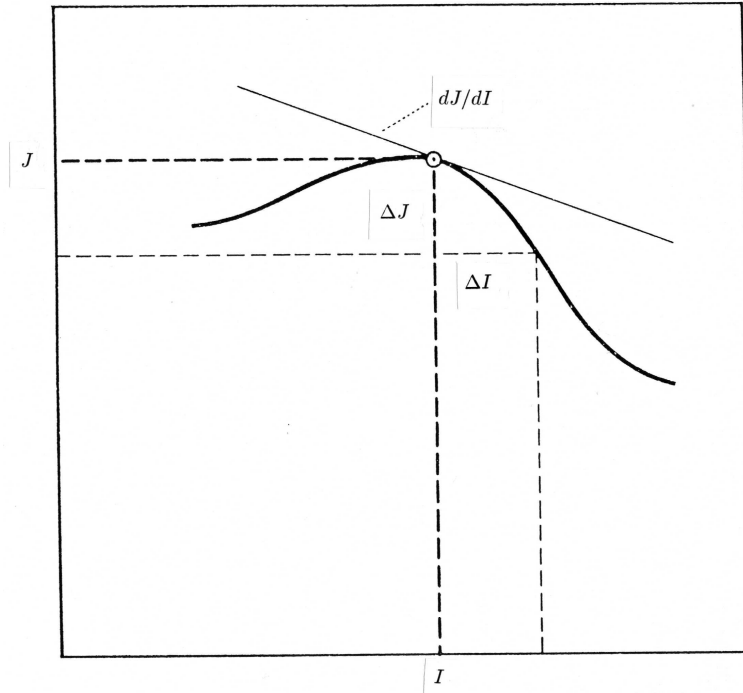


Figure 2: Effect of finite and infinitesimal changes on a hypothetical function $J = f(I)$. $\Delta J / \Delta I \neq dJ/dI$.

or, more generally, for any parameter

$$\frac{\delta J}{J} \bigg/ \frac{\delta P}{P} \approx R_P^J. \quad (1)$$

Let us make two points. Firstly, it is important that we consider small changes (what we call the method of modulation), in order that a good estimate of the slope should be obtained. We shall use this method throughout our treatment. If necessary this can be done by interpolation between points if the shape of the curve is ascertained.

Secondly, by this procedure we can relate two measurements through a coefficient whose value applies to the system *in the state it is*. The ratio can be re-expressed as:

$$\text{fractional change in flux} = R_I^J \cdot \text{fractional change in inhibitor.}$$

We may call R_I^J the *Response Coefficient* and its value may be thought of as an overall measure of the control exerted by the inhibitor at the value it has.

We will now show how this Response Coefficient can be usefully separated into two parts, the first, measured by the Controllability which is

concerned with the response to inhibitor changes of the *enzyme in isolation*, and the second, measured by the Flux Control Coefficient which is concerned with the response of the *whole system* to changes of the enzyme.

The Flux Control Coefficient, symbolised C_i^J , was named Sensitivity, symbolised Z , in the original article.

The Controllability Coefficient

The term Controllability Coefficient has not been extensively adopted, and it is now generally regarded as an Elasticity (introduced later). The distinction being made here by Kacser & Burns was between the response of an isolated enzyme to a chemical whose concentration was determined externally to the metabolic system under consideration, i.e. a parameter, and the response of an isolated enzyme to a metabolite whose concentration was set by the metabolic system itself, i.e. a variable. The former case is their Controllability Coefficient, given the symbol κ , and the latter is the Elasticity Coefficient, given the symbol ε . Nowadays, if it is necessary to distinguish between these two types of elasticity coefficient, the elasticity to a parameter (the controllability coefficient) can be notated as ${}^\kappa\varepsilon$ (or, alternatively, π [5]).

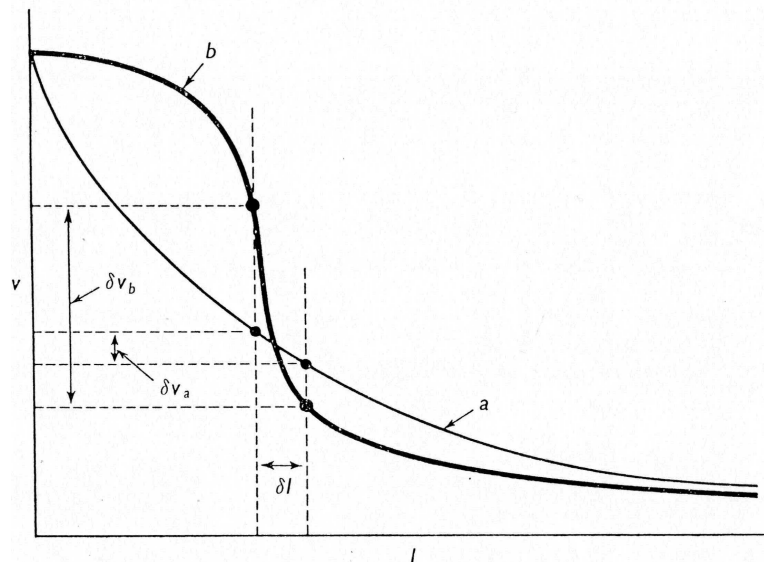


Figure 3: Effect of small changes in inhibitor on the rate, v . (a) Competitive, (b) allosteric.

When we consider the possible mechanisms which may be involved in the inhibition example, it is immediately clear that the value of this Response Coefficient depends, in part, on the nature and the extent of the interaction of the inhibitor with the enzyme. If this interaction is of the familiar competitive kind we would expect to get the relationship (a) (Fig. 3). On the other hand, should the enzyme be an allosteric one with a high Hill coefficient, the relation will be as in (b). Clearly the difference between these two possible situations must have something to do with the size of the response. This is the aspect which is usefully referred to as the Controllability. We are here considering the interaction of enzyme and inhibitor isolated from the complete system, as is usual in most enzymological exercises, and that is why we have described the rate as v and not as a system flux, J . It is, however, important that such determinations are not carried out at some arbitrary or traditional concentrations of the substrates and products. Instead they must be held constant at precisely those steady-state levels which obtain in the organism. The necessity for this is particularly evident when a competitive inhibition example is considered since the response in rate, for a given δI , will depend on the absolute value of the substrate concentrations. We therefore have another important control measure. It consists of isolating, or virtually isolating, the step from the rest of the system so that the surrounding pools are not free to move. Within this milieu, we then determine the change in rate consequent upon a small change in a parameter, P , such as the inhibitor, I , used in the example [Note: on a particular enzyme denoted i]

$$\frac{dv_i}{v_i} \bigg/ \frac{dI}{I} = {}^{\kappa}\epsilon_I^i$$

or operationally,

$$\frac{\delta v_i}{v_i} \bigg/ \frac{\delta I}{I} \approx {}^{\kappa}\epsilon_I^i.$$

In general, for any parametric effector acting directly on the enzyme, we have:

$$\frac{\delta v_i}{v_i} \bigg/ \frac{\delta P}{P} \approx {}^{\kappa}\epsilon_P^i. \quad (2)$$

This is ${}^{\kappa}\epsilon_P^i$, the *Controllability Coefficient*. Like the Response Coefficient, it is a differential applied to the particular constellation of enzyme, substrate and effector values, but unlike the Response Coefficient it defines only the 'local' response of the step in isolation without allowing it to influence or be influenced by events in the rest of the system. The Controllability, then, is a measure of the extent to which the effector has the potential to influence the flux.

Having determined the Controllability we do not, however, know what the net change in the system will be, since this depends on the response of

the rest of the system. This, as we have seen, is mediated via the 'links', the metabolites which are shared among the enzymes. The effect of a change in inhibitor when the metabolites are free to move will be that they settle to new values when a new steady state will be achieved. It is immediately obvious that, in general, this movement of metabolite pools will also result in a new constellation of metabolites around the inhibited enzyme which has, in the first place, initiated these changes. The outcome of all this is then to place the enzyme into a metabolite-inhibitor environment *different* from that which applied to the determination of the Controllability. The *system flux change* consequent upon a change in inhibitor will therefore be different — and in general lower — than the *rate change* determined in isolation.

The Flux Control Coefficient

We must therefore attempt to assess this system response. We have already had a hint how this is to be done. Since the system adjusts by transmitting the pool changes arising at the affected enzyme, it is really changes at that step *when it is embedded* in the system which require discussion. For this, no reference to the immediate cause of the change is called for. The change in the rate, caused by the change in *inhibitor*, can always be thought of as equivalent to some change in the *concentration of the enzyme*. If, for example, some inhibitor change causes a 1% reduction in rate, this is equivalent to a 1% reduction in enzyme concentration. We can therefore ask: how is the effect of a small change in enzyme concentration modified by the interactions of the rest of the system? For this we must measure system fluxes and measure the results of enzyme modulations.

As before, we can determine the respective fractional changes. The symbol e_i here refers to enzyme concentration, [Note: of a particular enzyme denoted i] but its more general meaning is discussed in Appendix A

$$\frac{dJ}{J} \bigg/ \frac{de_i}{e_i} = C_i^J$$

or

$$\frac{\delta J}{J} \bigg/ \frac{\delta e_i}{e_i} \approx C_i^J \quad (3)$$

C_i^J can be described as the *Flux Control Coefficient*² of the system flux with respect to enzyme concentration. It represents the system's sensitivity to modulations of one of its components, and is a measure of the control importance of the step *per se* whether the step is or is not controllable by any external effector.

²In a previous publication (Kacser & Burns, 1968) this coefficient was symbolised by C but no other coefficients were defined. In another treatment Higgins (1965) defined a similar coefficient of 'Control Strength' also designated by the symbol C .

In the original publication of 1973 and many that followed, this control coefficient was symbolised by Z and called the Sensitivity Coefficient. The footnote to the above paragraph was therefore more significant than appears in the light of the current terminology. The change in terminology ought not to obscure the fact that Metabolic Control Analysis is a form of sensitivity analysis, such as is used for equivalent purposes in other scientific fields.

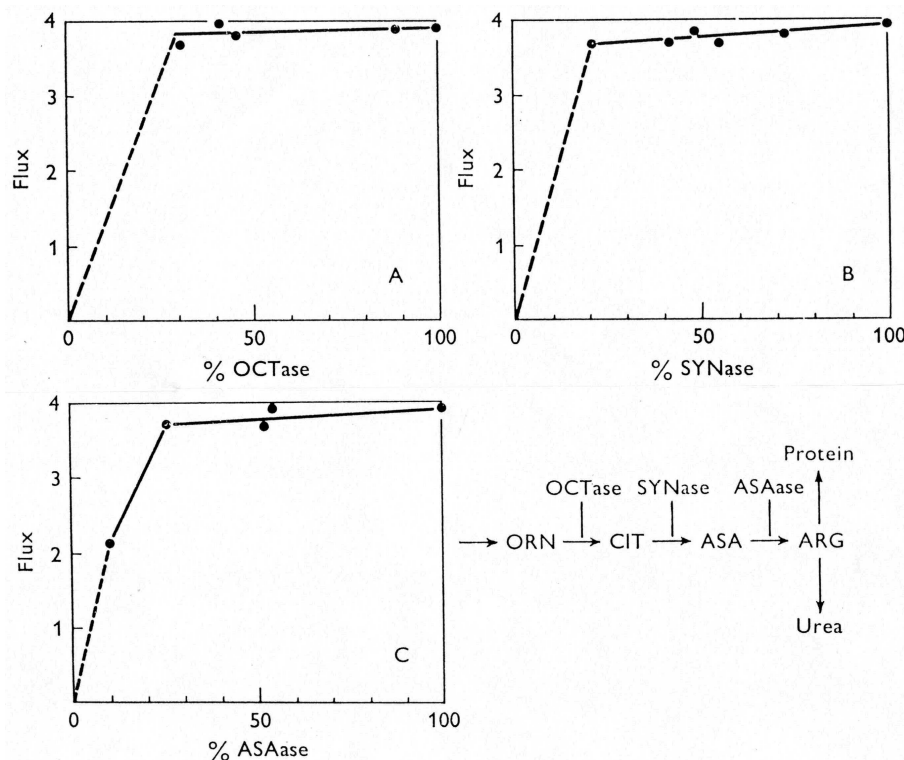


Figure 4: Effect on flux through arginine of different concentrations of the last three enzymes in the pathway. The wild type (homokaryon) level is given, in each case, as 100%. Each graph represents different heterokaryons and each point represents a different nuclear ratio in the mycelium of *Neurospora crassa* [6].

In some circumstances we can directly determine these Flux Control Coefficients. An example of this comes from the arginine pathway in *Neurospora* investigated by Dr R. Tateson in our laboratory [6]. Part of this pathway is shown in Fig. 4. The flux of arginine can be determined by estimating the amount of urea produced and the amount of arginine incorporated into protein in a given time interval. We would like to know how sensitive the flux is to modulations in the enzymes. They are all clearly

necessary since elimination of any one (by genetic blocks) reduces the flux to zero. But how important are they at the level they are? In *Neurospora* we can modulate the concentration of any enzyme by means of suitable heterokaryons. In this case mixtures of two nuclear types, one of which contained a functionally inactive gene for one of the three enzymes, were constructed. Depending on the nuclear proportions of the two types of nuclei, mycelia with different (and lower) concentrations of the enzyme were obtained. Fig. 4 shows the result of such experiments with three series of heterokaryons. For the moment two aspects will be considered. In the first place it will be noted that, at the normal 'wild type' level, all three enzymes have a small Flux Control Coefficient as indicated by the slope of the curves at the 100% enzyme points. Secondly, although we have not many points at the lower parts, for technical reasons, it is clear that the curves must come down to the zero point. The curves must therefore increase their slopes and, at very low levels of enzymes, the relationship approaches proportionality. The coefficient will then approach unity. This means that there will be an equal fractional response in flux to a given fractional change in the enzyme. At that point, then, an enzyme might be said to be 'fully controlling' the flux or to be a 'bottleneck':

$$\frac{\delta J}{J} \bigg/ \frac{\delta e_i}{e_i} = C_i^J \rightarrow 1(\text{controlling}).$$

What if the enzyme sits in a position when its coefficient is 0.5? Should we say that it is half controlling the flux or is half a bottleneck? At this point we realise that we must not fall into the semantic trap of using language where only a precise quantitative index can represent the situation.

It should be noted that the three curves were constructed each with the other two enzymes at the normal 100% levels. For reasons which will appear later, it is not possible in this situation for all three enzymes to have a coefficient of unity simultaneously. Only one can be in this position at a time. It is of course possible, indeed likely, that control is, so to speak, shared between many enzymes insofar as they all have coefficients between zero and one.

Many experiments have since been published where Flux Control Coefficients have been measured. Taken together, they confirm that near-zero and small coefficients are very common, whereas values approaching 1 have been rare. Some of these experiments will be cited later and more can be found in recent reviews, e.g. [7, 8].

Before we go into more subtle aspects of control, and in particular into an analysis of the various factors that contribute to it, it is worth pointing out again that the coefficient is defined as a differential at one point and in practice therefore requires small changes to define it when the complete

curve is not available. By contrast, a large step, say from 100% to 1% of activity will almost inevitably lead to a severe reduction in flux but will not give any information about the Flux Control Coefficient either at 100% or 1%.

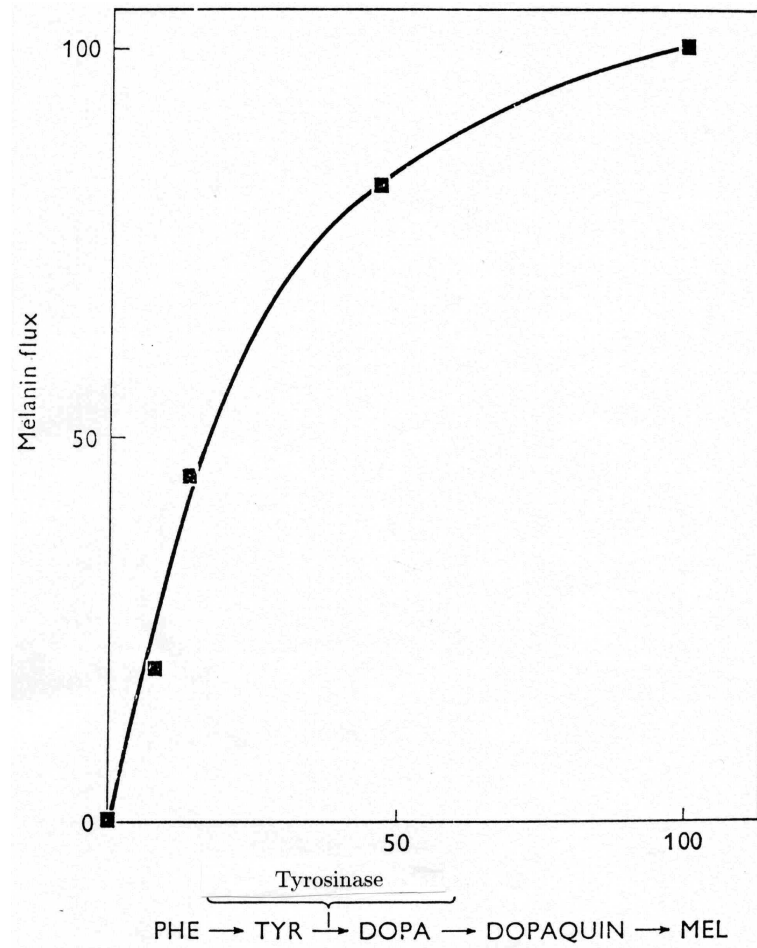


Figure 5: Effect on flux to melanin of different tyrosinase activities. The wild-type (black) level is given as 100% and so is the corresponding flux. The other points are obtained from genetic substitutions at the albino locus of the mouse. (From Andrews, Butfield & Kacser, in preparation.)

Another example comes from the work of Dr Kathryn Andrews investigating the melanin flux in the mouse (Andrews & Kacser, in preparation). The flux can be estimated in the skins of young mice. The pathway is a 'short' one and its probable structure is given in Fig. 5. By suitable genetic substitutions the specific activity of tyrosinase can be varied as shown. In contrast to the arginine pathway enzymes, variation in this enzyme results

in points on the 'lower' part of the flux curve and the coefficient for 'normal' (wild-type) levels is not very small.

These direct means of measuring Flux Control Coefficients are not always feasible and we shall discuss important indirect means in a later section.

Further experimental evidence comes from the work of Niederberger et al. [9] where tetraploid yeast was used and the flux was monitored as a function of each of the last five enzymes in tryptophan synthesis. By means of null mutants, a series of strains containing between zero and four doses of each of the genes specifying the relevant enzymes could be obtained. As before, the flux control coefficients were found to be very small at the 100% enzyme concentrations.

Similar flux-enzyme curves have also been observed by: titration of glycolytic flux in liver homogenates with added enzymes [10]; variable induction of tryptophan 2,3-dioxygenase in tryptophan catabolism in rat hepatocytes [11] and of β -galactosidase in the lactose catabolism of E. coli [12]; and variable expression of one of the elements of the glucose transporter system in E. coli on transport and catabolic flux after placing it under the control of a variably-inducible promoter [13]. The Flux Control Coefficients observed in these ways have varied between 0 and 0.8, with lower values predominating at normal enzyme contents.

The Partitioned Response

We therefore have defined two measures of control each concerned with separate aspect, The Controllability Coefficient, $\kappa \epsilon_p^i$, and the Flux Control Coefficient C_i^J . The net response depends upon both, and it can be shown by a strict theoretical treatment (Appendix B) that the Response Coefficient is the product of the two control measures:

$$R_p^J = C_i^J \kappa \epsilon_p^i. \quad (4)$$

This relationship reveals that *in vitro* studies can be misleading in predicting the effects in the *in vivo* situation without a knowledge of Flux Control Coefficients. The Flux Control Coefficient is a system property independent of whether any effectors act on the enzyme or what their strength is. The Controllability is a local property of the enzyme in its environment independent of what changes it might set into motion in the rest of the system. For both we have indicated experimental procedures which can, in principle, measure their coefficients. There are, of course, the equivalent algebraic operations consisting of partial differentiation of the relevant rate

expressions (Appendix A). These will be shown to be useful in the further analysis of control.

Groen et al. [14] have made brilliant use of this relationship in their study of mitochondrial respiration, which was the first paper to use the inhibitor titration method for the determination of Flux Control Coefficients. Since then, inhibitor titration has been one of the most extensively applied techniques in experimental Metabolic Control Analysis (e.g. see [7, 8]).

The Flux Summation Theorem

While it is useful to determine the value of the Flux Control Coefficient for particular steps (in order to see how narrow the neck of the bottle is), there is a more important consequence of our sensitivity analysis. This concerns the distribution of coefficients in a pathway.

We shall discuss this with reference to a simple sequence of transformations catalysed by enzymes. Consider a chain of enzymes E_1 to E_n

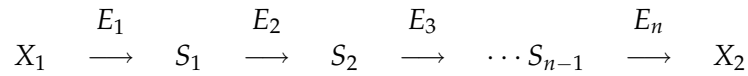


Figure 6:

with any values of V_{max} , K_m , etc., and measure the flux to X_2 ($dX_2/dt = J$) at steady state. All the pools will be at their steady-state values since their net rates of formation and removal are equal. If, in a thought experiment, we were to increase all the enzyme concentrations simultaneously by the same small fractional amount, α ,

$$\frac{\delta e_i}{e_i} = \alpha \quad (5)$$

the balance of all the rates would remain the same, *i.e.* the pools would *not* move. The fractional change in the flux however, would be exactly α :

$$\frac{\delta J}{J} = \alpha \quad (6)$$

This we may call the ‘co-ordinate property’ of the pathway. How is this brought about? Provided α , the common fractional change is small, this total change, $\delta J/J$ can be considered as the sum of all the individual changes, $\sum(\delta J/J)_i$, which would be caused by the alterations to each of the separate

enzymes. For the i th enzyme the definition of the Flux Control Coefficient gives:

$$\left(\frac{\delta J}{J}\right)_i \bigg/ \frac{\delta e_i}{e_i} = C_i^J$$

There are, of course, as many Flux Control Coefficients as there are enzymes in the chain (n). We can write, using relation (5):

$$(\delta J/J)_i = C_i^J \alpha$$

The summation gives

$$\frac{\delta J}{J} = \sum_{1 \rightarrow n} \left(\frac{\delta J}{J}\right)_i = \alpha \sum_{1 \rightarrow n} C_i^J$$

From (6) it follows that

$$\sum_{1 \rightarrow n} C_i^J = 1 \quad (7)$$

The sum of all the Flux Control Coefficients is equal to unity. This means that in such a chain of enzymes the Coefficients are distributed so that they are all smaller than unity and only *one* could approach full control importance with a coefficient nearly unity. Equally possible is that none of the enzymes is of major importance. ‘Pacemakers’ or ‘bottlenecks’ do not therefore necessarily exist in a particular system. It is interesting to note that this conclusion is consistent with the general evidence from inborn errors of metabolism. Most of these are fully recessive which means that the usual 50% reduction in enzyme activity in the heterozygote has almost no effect on the flux.

The Summation Theorem, demonstrated with reference to a simple chain where only one system flux exists, can be shown to apply generally to systems of any complexity (Appendix B). Any flux in such a system will satisfy the condition that the sum of its coefficients over all the enzymes in the system is equal to unity. This allows for a variety of relations such as feedback, coupling and branching. In the case of branching, increasing an enzyme in one branch can result in a decreased flux in the other branch so that we obtain negative coefficients. This will, of course, complicate the analysis of control distribution in such systems.

Although it is true that, in pathways with branches and cycles, certain of the Flux Control Coefficients can be negative, and that this allows the theoretical possibility that one Flux Control Coefficient could be greater than unity there are no experimental examples of such large positive coefficients at present. However, there have been reports of two flux control coefficients in branched systems both being near 1, associated with a coefficient of -1 [15, 16].

One corollary to the Summation Theorem is that, in a system containing a reasonable number of enzymes, almost all the enzymes will appear to be ‘in excess’ in the sense that, choosing any one, its quantity or activity can be reduced (sometimes considerably) without appreciable effect on the flux. This form of words has caused some confusion, particularly in the minds of those who imagine that all enzymes should have activities which are ‘just sufficient’ for the task. We have shown that it is impossible for such a situation to exist.³

Kacser & Burns in their paper ‘The molecular basis of dominance’ [17] discuss in some detail why most mutants show recessivity, that is the heterozygotes of mutant with wild-type have a phenotype not very different from that of the wild-type homozygotes. This arises directly as a systems property of metabolic pathways if the enzyme in such homozygotes has a very small Flux Control Coefficient, as is generally found. Previous explanations of dominance, such as Fisher’s, have assumed it is an evolved property, but this is not consistent with the observation of dominance in artificial diploids of an organism that is normally strictly haploid [18]. On the other hand, these results are perfectly consistent with arguments presented by Kacser & Burns. For these reasons, the impact of ‘The Control of Flux’ has been as significant in genetics as in biochemistry.

A second corollary arises from the situation when, in a given system, say, one enzyme quantity is drastically reduced. In such a case, this step may change from one having little control to one of importance, *i.e.* the Flux Control Coefficient has increased. Since the summation property applies equally at the new position, the Flux Control Coefficients of all the other (unchanged) enzymes must have changed so that the sum remains equal to unity. This demonstrates that the Flux Control Coefficient of one enzyme is a system property only in part determined by its own parameters.

In genetics terminology, such a situation would be described as showing epistasis, which is an inherent property of multi-enzyme systems [19].

Control Analysis of Pathways

We can now return to the analysis of the net response which was seen to be dependent on the relationship

$$R_p^J = C_i^J \kappa \epsilon_p^i$$

³The implication of this for the problem of Dominance and its evolution will be dealt with in a separate publication. [Note: ref. [17]; see also the added commentary above.]

Since

$$R_P^J \approx \frac{\delta J}{J} \bigg/ \frac{\delta P}{P},$$

we can write

$$\frac{\delta J}{J} = C_i^J \kappa \varepsilon_P^i \frac{\delta P}{P}. \quad (8)$$

This formulation shows that the flux *response* is dependent on the numerical values of three factors. The first, C_i^J , indicates how sensitive the system is to change at this step [i] and the summation property imposes restraints on its magnitude. Many authors may have had such a concept in mind when they have attempted to divide enzyme steps into ‘bottlenecks’, ‘pacemakers’, ‘rate limiters’ on the one hand and their presumed opposites on the other. With quantitative specification of the coefficients such a simple dichotomous classification is not very meaningful. It is true that in a limiting case, when the Flux Control Coefficient of one step is very large while the others have very small values, the choice of an appropriate term may be found to be satisfyingly descriptive. In general, however, one expects a distribution of the values with none of them necessarily dominating and this is quite independent of the Controllability which may operate. Much of the interpretation of results in the literature is confounded by the search for the alleged rate-limiting step (or whatever terminology local tradition specifies).

This was particularly well illustrated by the history of research on the control of mitochondrial oxidative phosphorylation and liver gluconeogenesis. The fruitless arguments about the rate-limiting steps in both these pathways were revealed for what they were when the Amsterdam group measured the Flux Control Coefficients in both [14, 20].

Any value for the Controllability, $\kappa \varepsilon_P^i$, the second factor, may be associated with a low Flux Control Coefficient and hence results in a very small response. On the other hand, control can be exerted in such a situation when the Controllability Coefficient [*Note: external elasticity coefficient*] has a very high value (such as may be the case in allosteric systems). In this case an effectively controlling enzyme need not be a ‘bottleneck’ although it *may* become one once it has been acted on by certain concentrations of the effector (see, however, the later section on feedback inhibition). It is clear that the inverse is equally true. Only a quantitative analysis can elucidate the situation.

Finally, since the response is dependent on the magnitude of the effector change, $\delta P/P$, a judgement as to whether control is actually exerted must depend on exact physiological information of possible movements of P .

Metabolic Controllability: the Elasticity Coefficient

The analysis so far has dealt with the effects on system properties such as fluxes, exerted by substances which are controlled, or in principle controllable, by setting some parameter external or internal to the system. In many cases, however, inhibitors are substances generated *in* the metabolism and are therefore *variables*. Any changes in such metabolite levels, be they substrates, products or effectors, are of course only brought about by changes, albeit remote ones, in one or more parameters. Such changes affect many pools simultaneously. Thus one cannot describe the influence of metabolites on system fluxes in the same manner as was used for parameters. Nevertheless, many investigators assert that pools may exert 'control'. We will now consider the role of such pool-mediated controls and their relation to Flux Control Coefficients.

In the same way as we were able to determine the Controllability Coefficient of some parametric inhibitor on the rate of a reaction, we can apply this procedure to, e.g., the substrate. Remembering that the enzyme should be isolated from the rest of the system, but with all interacting molecules held at their *in vivo* concentrations, we can modulate the substrate, for example, and obtain a coefficient [Note: for enzyme *i*]:

$$\frac{dv_i}{v_i} \bigg/ \frac{dS}{S} = \epsilon_S^i$$

or

$$\frac{\delta v_i}{v_i} \bigg/ \frac{\delta S}{S} \approx \epsilon_S^i . \quad (9)$$

This may be called the *Elasticity Coefficient* and for any reaction there are as many coefficients as there are metabolites and effectors which interact with the enzyme. It will be noted that the method of determining these elasticities is not unlike part of a Michaelis constant or inhibition constant determination except that the molecular milieu is the *in vivo* one and the movement of the pool is over a small distance. Although we have defined both Controllability and Elasticity by means of direct operations which may have considerable practical difficulties, their importance for analysing control is unimpaired by this. (We shall discuss alternative methods in a later section.) In what follows it will be seen that the *definitions* of Elasticities allow us to make use of other measurements in a meaningful manner.

Of particular interest are the Elasticities of an enzyme with respect to effectors which are metabolically somewhat remote from the enzyme's action. In the case of, e.g., end-product inhibition, it is not sufficient to demonstrate that the extracted enzyme is inhibitable under assay conditions. Metabolically significant statements must rely on the Elasticity estimates made under conditions equivalent to the internal milieu. Furthermore the range of end-product concentrations investigated should be re-

lated to the known *in vivo* concentration changes. The finding of a high end-product Elasticity could warrant the use of the term ‘regulatory enzyme’ or ‘key enzyme’ but, as before, such classificatory nomenclature is much less desirable than the quantitative description of its Elasticity. A high Elasticity will often be manifested as pool movements around the inhibited enzyme satisfying the Cross-over Theorem (Chance et al. 1958). Having found a high Elasticity does not, however, mean that the enzyme necessarily plays an important role in ‘regulating’ the flux. The value of its Flux Control Coefficient is an equally important factor. We shall discuss further aspects of this in a later section.

The substrate Elasticities are important in being concerned with the linking pools between adjacent enzymes. In fact we shall show that they apportion the Flux Control Coefficients, and hence the control, in a chain of such enzymes. Consider two enzymes within a pathway linked by a common pool S_1 .

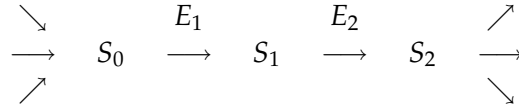


Figure 7:

The rest of the system can have any structure or complexity provided S_1 does not interact with any other enzymes in the system. We can perform another thought experiment and make simultaneous and opposite changes in the concentrations of E_1 and E_2 such that the steady-state values of S_0 and S_2 (and hence the flux) remain unaltered. The only change is in the level of S_1 . The effect of the movement in S_1 on the *rates* of the two enzymes is determined by their Elasticities with respect to S_1 , namely $\varepsilon_{S_1}^1$ and $\varepsilon_{S_1}^2$. An argument (Appendix B) based on this leads to the following:

$$C_1^J \varepsilon_{S_1}^1 + C_2^J \varepsilon_{S_1}^2 = 0 \quad (10)$$

This means that there is a simple relationship between the Flux Control Coefficients of two adjacent steps in a pathway and their Elasticity Coefficients with respect to the common pool.

This equation has since become known as the Connectivity Theorem, though that name was not used in this paper.

It enables one to estimate the relative values of the two Flux Control Coefficients without the direct method previously discussed. Equation (10) can be written as:

$$C_1^J / C_2^J = -\varepsilon_{S_1}^2 / \varepsilon_{S_1}^1 \quad (11)$$

(The negative sign arises because the two Elasticity Coefficients are normally of opposite sign, *i.e.* product 'inhibits', substrate 'activates' the rates.)

Thus by measuring the local response of enzymes (Elasticities), the *ratio* of their effects when acting within the system (Flux Control Coefficients) can be ascertained. This can clearly be extended to determine the relative Flux Control Coefficients ($C_1^J:C_2^J:C_3^J:C_4^J:\dots$) of a number of successive steps of a pathway so long as pools internal to the pathway affect only their adjacent enzymes. The relative Flux Control Coefficients of a pathway can thus be related to measurements on the separate enzymes. Unlike the Controlability which measures the potential of one enzyme to control a flux, the Elasticities enable us to *connect* different steps in a pathway.

This idea has since been developed further both theoretically and experimentally, as will be explained in notes to later sections. Groen's study [20] of hepatocyte gluconeogenesis illustrated this by being the first to use elasticity estimates to determine the Flux Control Coefficients on the gluconeogenic flux.

In summary, we have argued that certain aspects of control, viewed at the level of the whole system, can be described quantitatively by means of the system coefficients R_p^J and C_i^J and we have indicated experimental means of measuring them. These will be seen as particular applications of Higgins' general 'reflection coefficients' (Higgins, 1963). On the other hand we have also considered control as it affects the rate of the individual enzymes, considered in isolation from the rest of the system. This is described quantitatively by the two types of 'local' coefficients ϵ_s^i and ${}^\kappa\epsilon_p^i$. The results of introducing these local coefficients is that it becomes possible to advance a general theory which relates the control behaviour of the whole system to the properties of its components. As will be shown in the next section this directly leads to the establishment of *criteria* of control in terms of familiar and readily accessible measurements.

Applications to Control Criteria

The above analysis connects Flux Control Coefficients to the Elasticities of enzymes. This makes it possible to introduce such quantities as specific activity (V_{max}), pool levels, degree of inhibition, etc. To establish these relationships to elasticities requires us to make assumptions about or have information on the form of the rate expression which applies to the steps under consideration.

A method which avoids any assumptions about the form of the rate expression has been developed by Kacser & Burns [21]. This has now been called the 'double modulation method' and has been incorporated into the 'top-down' procedure that has been applied by Brand,

Brown and colleagues [16, 22] to the control of respiration. Theoretical analysis of the method continues [23]. In the modulation method, measurements of metabolite pool changes and flux changes in situ are made consequent on two independent changes (e.g. inhibitions by unspecified mechanisms). This makes it possible to obtain values for the elasticities without knowing what rate equations operate. A further development has recently been proposed [24] that allows all the elasticities and control coefficients to be obtained from a set of such in vivo modulation measurements.

For the case where Fig. 7 represents a chain of unimolecular transformations, a suitable form of the rate expression (allowing for reversibility and saturability) is, for the first enzyme, e.g.

$$v_1 = \frac{V_1/M_1(S_0 - S_1/K_1)}{1 + S_0/M_1 + S_1/M_1^*}, \quad (12)$$

where S_0 and S_1 refer to the pools and K_1 refers to their equilibrium constant. V_1 and M_1 are the maximal velocity (V_{max}) and Michaelis constant (K_m) measured in the 'forward' direction and M_1^* is the Michaelis constant for the backward direction. The suffix '1' for the constants refers to the number of the enzyme in the chain. (For expressions of more complex reactions see Cleland, 1963.)

The symbol M , to stand for the more familiar K_m , was used in the original to simplify the formulations, and has been retained here for the same reason.

Let us first consider the limiting case, amenable to immediate analysis, where we can assume that all the enzymes concerned are 'unsaturated' (when the value of the denominator of (12) approaches unity). Here the appropriate rate expressions simplify and for two successive steps are:

$$v_1 = (V_1/M_1)(S_0 - S_1/K_1) \quad (13a)$$

$$v_2 = (V_2/M_2)(S_1 - S_2/K_2). \quad (13b)$$

There were superfluous division signs between the bracketed terms in the above equations in the original.

The operation of modulating S_1 to determine the Elasticity Coefficients has its algebraic equivalent in differentiating these expressions with respect to S_1 (Appendix A). This yields

$$\varepsilon_{S_1}^1 = \frac{-S_1/K_1}{S_0 - S_1/K_1}, \quad (14a)$$

and

$$\varepsilon_{S_1}^2 = \frac{S_1}{S_1 - S_2/K_2}. \quad (14b)$$

The signs of these two equations were transposed in the original.

It is thus seen that the Elasticity Coefficients are related to the pool configuration around the enzymes at steady state. Using equation (11) we obtain

$$C_1^J / C_2^J = -\varepsilon_{S_1}^2 / \varepsilon_{S_1}^1 = \frac{S_0 - S_1 / K_1}{(S_1 - S_2 / K_2) / K_1}.$$

We can therefore express the *ratios* of Flux Control Coefficients as ratios involving the steady-state pools. Repeating this for successive pairs of enzymes, we obtain the ratio of Control Coefficients for a sequence of steps in terms of pools

$$C_1^J : C_2^J : C_3^J : \dots \equiv (S_0 - S_1 / K_1) : (S_1 - S_2 / K_2) / K_1 : (S_2 - S_3 / K_3) / (K_1 K_2) : \dots \quad (15)$$

Disequilibrium

The first thing to note is that the difference terms represent the degree to which the step is out of equilibrium. (For equilibrium this difference approaches zero.) One can express this in another way, given by the Disequilibrium Ratio, ρ , which for the first step, e.g., is

$$\rho_1 = (S_1 / S_0) / K_1 = (\text{Mass Action Ratio} / \text{Equilibrium Constant}).$$

Relation (15) can be rewritten in terms of the ratios:

$$C_1^J : C_2^J : C_3^J : \dots \equiv 1 - \rho_1 : \rho_1(1 - \rho_2) : \rho_1\rho_2(1 - \rho_3) : \dots \quad (16)$$

Disequilibrium has frequently been used as a criterion (Krebs, 1947; Hess & Brand, 1965; Williamson, 1965; Newsholme & Gevers, 1967; Krebs, 1969) for classifying steps into non-controlling ('equilibrium') and potentially controlling ('non-equilibrium') categories. Since the deviation from equilibrium can take any value between these extremes, it is difficult to see where the two classes should be separated, although an arbitrary limit of 0.2 has been suggested (Rolleston, 1972) on thermodynamic grounds. More important, however, is the fact, shown in the above relations that the Disequilibrium Ratios do not themselves represent the correct functions for comparison. Relation (16) represents the proper formulation. We are thus able to assess the relative Flux Control Coefficients in terms of pool ratios and equilibrium constants only. It will be noted that since only pool *ratios* are involved many of the difficulties attached to determinations of absolute concentrations are avoided.

One result clearly follows. If any of the steps is *at* equilibrium ($\rho_i = 1$), the term corresponding to its Flux Control Coefficient becomes zero and

hence the step becomes completely insensitive. A further consequence is that all steps 'to the right' act, from a control point of view, as if that step were not present (all terms multiplied by $\rho_i = 1$). This conclusion is, of course, in accordance with the commonly held view that equilibrium reactions are 'non-controlling'. We must, however, sound a cautionary note. No step in a system carrying a flux can be exactly at equilibrium and therefore its exact ρ value must be considered within the above relation (16) in comparison with the other steps.

This is perhaps best illustrated by two simple examples. Taking three successive steps we can show using relation (16):

- (1) The step nearest to equilibrium (largest ρ) is not necessarily the least sensitive to control (smallest C_i^J).

Step no.	1	2	3
Assumed ρ	0.9	0.01	0.1
Calculated C_i^J ratios	0.10 :	0.89 :	0.008

Step no. 1, which is 90% towards equilibrium, is 12 times more sensitive than no. 3 which is further away from equilibrium (10%).

- (2) The step most out of equilibrium (smallest ρ) is not necessarily the one most sensitive to control (largest C_i^J).

Step no.	1	2	3
Assumed ρ	0.3	0.3	0.01
Calculated C_i^J ratios	0.70 :	0.21 :	0.09

Step no. 3 has the highest disequilibrium (1%) of the three but is the least sensitive to control.

(In both examples the last step has the smallest C_i^J . This is, of course, *not* an invariable result, although there is a 'position effect' tending this way.)

Although these are constructed counter-examples, they are intended to demonstrate that the intuitively held view must be modified by reference to the more rigorous relations derived here.

Analysis of the mammalian serine synthesis pathway [25] provides an experimental illustration that the largest control coefficient can be found on the final step, and also that near-equilibrium steps can have a non-zero control coefficient.

Maximal Rates

Instead of formulating the ratios of Flux Control Coefficients in terms of pools we can replace the terms in relation (15) by maximal velocities. Using the rate equations (equation 13) for the condition when they are equal to the common pathway flux, J , this yields the equivalent form:

$$C_1^J : C_2^J : C_3^J : \dots \equiv \frac{M_1}{V_1} : \frac{M_2}{V_2 K_1} : \frac{M_3}{V_3 K_1 K_2} \dots \quad (17)$$

Again it can be seen that maximal velocities are not in themselves a proper measure of Control. The values of the equilibrium and Michaelis constants can be seen to be equally important. With these additional measurements, however, we have a feasible criterion in relation (17).

Criteria for control involving Disequilibrium Ratios and maximal velocities (V_{max}) have often been advocated. No reference to the degree of saturation of the enzymes is made in such discussions. We have shown that, for the case of a chain of unsaturated enzymes, these experimental quantities, although relevant, are not themselves criteria. We must now enquire what role saturation plays.

It can be shown that the simple formulation for the Elasticity Coefficient previously established (equation 14) is modified by an additional factor (Appendix C). Thus for the second step:

$$\varepsilon_{S_1}^2 = \frac{S_1}{S_1 - S_2/K_2} \quad (\text{unsaturated}) \quad (14b)$$

$$\varepsilon_{S_1}^2 = \frac{S_1}{S_1 - S_2/K_2} \left(1 - \frac{J}{V_2} \right) \quad (\text{saturable}). \quad (18)$$

By additional determination of the system flux, J , and the maximal velocity, V_2 , it is possible to make a correction to estimates based on the unsaturated theory.

One particular limiting case is of some interest. When the maximal velocity of a reaction is found to be very near to the measured system flux, their ratio, J/V_i , approaches 1 and the correction factor approaches zero. This means that the Elasticity Coefficient becomes very small and the Flux Control Coefficient of the enzyme becomes large by comparison with others. This is because the two coefficients are inversely related (equation 11). In this case, therefore, the V_{max} of a single step is a criterion for a high Flux Control Coefficient and this has been recognised by a number of authors (e.g. see Krebs, 1969). It is worth pointing out that the condition $V_{max} \approx J$ carries with it the implication that the enzyme is highly saturated *and* its Disequilibrium Ratio, ρ , extremely small. Either of these conditions separately, however, is not a valid criterion.

In the absence of such extreme conditions, the general formulation of type (18) must be used and will give the desired information.

Although the previous sections have referred to the calculation of the ratios of Flux Control Coefficients, if the ratios of all the Control Coefficients are expressed in terms of their elasticity ratios, the addition of the Summation Theorem requirement that all the Flux Control Coefficients add up to 1 is sufficient to uniquely determine their values. This was first applied experimentally to hepatocyte gluconeogenesis by Groen et al. [20]. A systematic procedure for expressing the values of the Flux Control Coefficients in terms of the Elasticity Coefficients, the matrix method was proposed by Fell & Sauro in 1985 [26] and was claimed to be extensible to complex pathways that included feedback inhibition, cycles and branches. This led to much work on the mathematics of these relationships, [27, 28, 29, 30, 31] culminating in the proof by Reder [32] that such calculations were generally possible in metabolic pathways of any complexity provided they attained a stable steady state and were fully connected by mass flows. See the notes to the section on Limits and Limitations for further discussion, particularly concerning two or more metabolic processes connected solely by catalytic or regulatory effects and exceptions to the summation theorem.

Feedback Inhibition

Having discussed the role of the enzymes' immediate metabolites in the control of flux, we must now turn to those interactions involving molecules not metabolised by the enzyme. The classical case is that of feedback inhibition.

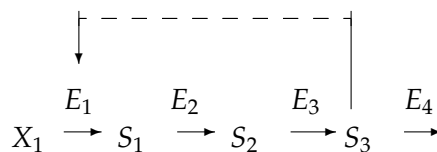


Figure 8:

Just as in the last section the Elasticity Coefficients with regard to shared pools formed the basis for relating the Flux Control Coefficients of adjacent enzymes, now the pool S_3 forms the control link between three enzymes E_1 ,

E_3 and E_4 (Fig. 8). By an extension of the previous argument for equation (10) it is easy to show that (Appendix B):

$$C_1^J \epsilon_{S_3}^1 + C_3^J \epsilon_{S_3}^3 + C_4^J \epsilon_{S_3}^4 = 0 \quad (19)$$

The three Flux Control Coefficients are connected by the Elasticities to the common pool S_3 . The presence and nature of the inhibition function, however, produce complexities and make insight into the problem somewhat opaque. Let us consider again the two types of inhibited enzymes, this time affected by the internal pool S_3 , differing only in their inhibition curves. A steady state of the system with the pool at S_3^* can be maintained with either of these enzymes. In each case the level of the inhibited enzyme is the same, but their Elasticity Coefficients with respect to S_3 are very different, as can be seen from the slopes at that point (Fig. 9).

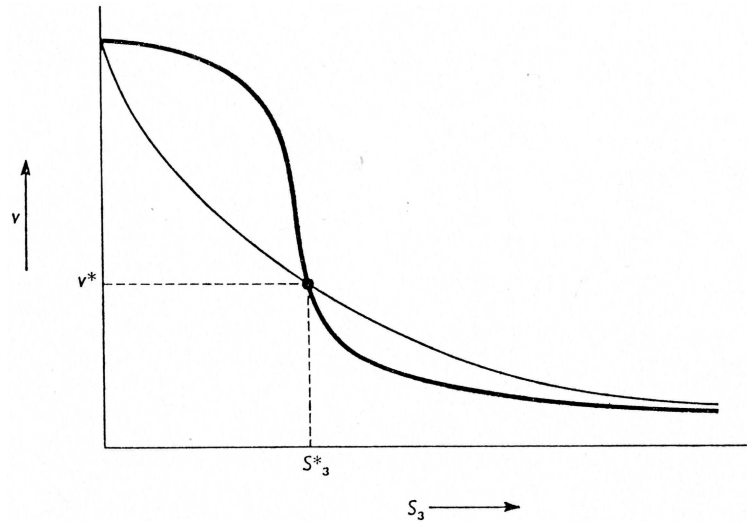


Figure 9: Effect of a pathway product, S_3 , on the rate of the first enzyme E_1 (cf. Fig. 8). (a) Competitive inhibition, (b) allosteric inhibition.

Let us consider in greater detail the interesting case when the pool lies in this 'control range' of an allosteric enzyme (curve b), i.e. in the range of high Elasticity. A common question of importance is to enquire how a system such as that in Fig. 8 responds to alterations in 'demand' and what role the feedback plays in this. Demand can be represented by an effectively irreversible last step and variation in demand represented by changes in the concentration of this last enzyme (E_4), which can be thought of as having been brought about by some change in physiological state.

Consider, therefore, an increase taking place in the last enzyme (E_4). The resulting tendency for S_3 to decrease would be counteracted by the

sharp de-inhibition of E_1 in the control range. This has the effect of virtually 'locking' the pool within narrow limits over a wide range of last-enzyme variation. The flux 'extracted' from the system will therefore be almost proportional to the level of this last enzyme, since flux varies directly with enzyme concentration in a constant pool environment. This means, of course, that the last enzyme has a Flux Control Coefficient of almost unity, i.e. it 'controls' the flux. Since the sum of the Control Coefficients in a chain has been shown to be equal to unity, it immediately follows that the rest of the enzymes, *including the 'controlled' one*, have very low control coefficients and therefore no 'control'. This apparent paradox of 'a controlled enzyme exerting no control' is in fact semantic and is resolved when it is restated as: The enzyme has a high Elasticity Coefficient with respect to the controlling pool, but its Flux Control Coefficient with respect to the system flux is low (Appendix C).

The reason for these rather complex relations is to be found in the fact that Elasticities (to pools) and Control Coefficients are interrelated contrary to the independence of the Controllabilities (to parameters) and Control Coefficients.

This emphasises the necessity to distinguish clearly the two types of control, the one by internal variable, the other by external parameter. It also raises the great difficulties in interpreting certain types of experiment, where an intermediate pool is *de facto* converted into a parameter by supplying it from outside (e.g. see Williamson, Browning & Olson, 1968). The result of such an operation is really to alter the *structure* of the system.

Limits and Limitations

In conclusion it is important to consider the range of applicability of the foregoing analysis. Throughout the treatment we have considered only the steady state, applicable either to a constant volume or an expanding system. The former has fixed internal and external parameters and time-invariant values for pools and fluxes. It could correspond to, for instance, an adult metazoan or a liver slice.

The expanding system also has fixed external parameters (nutrients) and fixed enzymic parameters, but the *quantities* of enzymes, pools and fluxes increase. In an exponentially growing system, however, volume increases exponentially, as well as enzymes, pools and fluxes, so that their concentrations remain time-invariant (e.g. see Stebbing, 1972). Such a system could correspond to some early stages of growing organisms or a culture of micro-organisms, where time averages are found, since concentration changes of both enzymes and pools may occur periodically throughout the cell cycles, and mycelial organisms where a single expanding phase with constant concentrations is approximated. Expanding systems, how-

ever, differ in the number of real fluxes since every steady-state pool balances not only its fluxes of formation and removal but also its flux to expansion into the exponentially growing volume. Certain additions to the set of flux equations must therefore be made to describe such a system (Appendix A). The Summation Theorem must take this into account when applied to such systems.

Our treatment does not deal with transients, the trajectories of the system from one steady state to another. Experiments designed to measure these transitions and interpret their results in control terms (e.g. see Higgins, 1965) are therefore not served by our analysis. There are probably relationships between our various coefficients and the relaxation times of such events, but we have no explicit statements to make about them.

Since then some progress has been made in dealing with the transition times, τ , of metabolic systems and their dependence on enzyme concentrations. Meléndez-Hevia et al. [33] showed that a different Summation Theorem ($\sum C_i^T = -1$) applies.

It has also been shown that Flux Control Coefficients are obtainable from observations of the pool transients [34]

Similarly the major changes which higher organisms undergo during differentiation and development can only be marginally illuminated by the analysis (Kacser, 1963). During restricted periods, however, when enzyme changes may be slow compared to the metabolic relaxation times, a strict steady-state treatment is still applicable.

The complex structure of metabolic systems provides the possibility that, in certain parts, there may occur constellations of effective time constants which will not result in a steady state of some pools, but will produce an oscillatory solution under certain conditions (See e.g. [35]). Such circumstances are, strictly speaking, excluded from our treatment but we believe certain time-average values would allow us to apply the general results of our analysis.

A major problem, hardly touched on so far, and yet fundamental to every theoretical and experimental approach, is the question of system limits (Kacser & Burns, 1968). It is evident that the whole metabolism is one system and to draw arbitrary lines around a part is unjustified. Yet often theoretical approaches start at a constant source of supply and, a few enzymes — or even a few dozen enzymes — later, end in a 'product'. Experimentally too, we sever or ignore vast portions of the map and happily make our measurements. How justified are we in adopting these procedures and are there any rules that tell us where to draw the lines? In some special circumstances criteria for delimiting sub-systems can be given but, in general, this remains a most intractable problem. A condition which allows us to assign a 'beginning' to a sub-system (apart from externally controlled substance) would be if the pool in question is effectively held

constant by mechanisms outside the sub-system. One such mechanism has already been referred to when a pool acting as a feedback signal to a steep control enzyme was described as 'locked'. Over a considerable range of variation of any sub-system distal to this pool, the pool can be considered as a parameter. Similarly, if a pool is participating in many reactions with considerable fluxes through it, one of these fluxes, particularly if it is relatively small, may cause little alteration in the concentration. The 'end' (or 'ends') of a sub-system may sometimes be identified if there occurs an effectively irreversible step to a product which represents another phase (precipitation, gaseous product, etc.).

Such specific circumstances, however, may not be frequent enough to serve as a general recipe for dealing with the problem. Is our analysis, then, restricted to such rare cases? It will be recalled that the elasticity analysis yielded *ratios* of Control Coefficients by estimating Elasticities only. These can be determined from the isolated enzymes or from steady-state pools and these ratios are therefore not dependent on defining any sub-system. It therefore requires only to determine the absolute value of one Control Coefficient to calculate the values of all the others for which adjacent Elasticities are available. Sub-system limits are, therefore, not relevant and hence not restrictive to this treatment.

On the other hand the Summation Theorem, if it is to be applied to a small portion of the system, does depend on identifying a 'beginning' and an 'end'.

This also applies to the methods referred to earlier, such as the matrix method, where the values (rather than the ratios) of the Control Coefficients are obtained from the Elasticities, because these calculations assume the applicability of the Summation Theorem to the system. In addition, a number of other circumstances have been identified where the Summation Theorem may not be applicable or may need modification. These include channelling (i.e. direct transfer of an intermediate from one enzyme to the next without it entering the bulk phase) [36], enzyme-enzyme interaction in general [37] and group transfer pathways [38]. Some of these questions are affected by the form of the definition of the flux control coefficient [5], which will be mentioned again in the notes to Appendix A.

The identification of units of metabolism that have a beginning and end has been taken further in Modular Control Analysis [39, 40]. A module will be a set of connected reactions involving mass flows; when its environment remains constant, it obeys the Summation and Connectivity Theorems. Separate modules, either at the same level (another metabolic pathway) or at a different hierarchical level (synthesis and degradation of the enzymes of the pathway) may interact via catalytic and regulatory interactions. Modular Control Analysis

deals with how the Control Coefficients of isolated modules are modified to become overall Control Coefficients in a system consisting of several interacting modules.

For determinations of absolute Control Coefficients the previously discussed method of genetic modulation is suitable, as also is a method of using a specific enzyme inhibitor and applying relation (4). As far as Elasticities are concerned extraction and reconstruction of the *in vivo* milieu involve methodological and practical difficulties and these must be borne in mind when such experiments are considered. In particular a knowledge of the effective concentration surrounding the *in vivo* enzyme is required. Where organelles such as mitochondria are involved this may not prove excessively difficult. When evidence of compartmentation, not associated with organelles, exists, however, the problem becomes extreme. There is a means of circumventing these difficulties by *in vivo* Elasticity determinations which consists essentially in producing suitable pool modulations generated by alterations (e.g. inhibitions) to other components remote from the enzyme in question [Note: since published as [21].]

A considerable number of experiments are performed, both *in vivo* and *in vitro*, which change the concentration of a metabolite by a very large step. Sometimes this is achieved through hormones or nutritional changes and sometimes by direct presentation of the metabolite. We have already commented on the methodological implications of changing variables into parameters. Here we would like to restrict consideration to the quantitative aspects.

The first thing to observe is that such large step experiments are outside the scope of our modulation analysis. The various quantifiable coefficients (C_i^J , $\kappa \varepsilon_p^i$ and ε_s^i) are strictly differentials and in practice can only be estimated by modulation experiments involving small changes. Large steps could still be used if the whole curve (or a significant portion) is determined and, by interpolation, the small change in slope is estimated. What cannot be done is to compare usefully the metabolic values of two widely differing configurations. The reason for this is evident when it is realised that the Control Coefficients, for example, change in value with large changes of any parameter since they themselves are systemic properties.

An instructive example is given in Fig. 10. This represents the results of a computer experiment in which the effect of changing the initial substrate is varied. The system consists of a chain of five enzymes whose rate expressions include saturation terms as in equation (12). By the general method described elsewhere (Burns 1968, 1969) the flux was computed for a range of values of the initial substrate, X . The computation also generated the Control Coefficient of the flux with respect to each enzyme at all settings of X . Fig. 10 shows the plot of flux against the substrate of the pathway. It is seen that the flux responds in a reasonably monotonic fashion. The Control

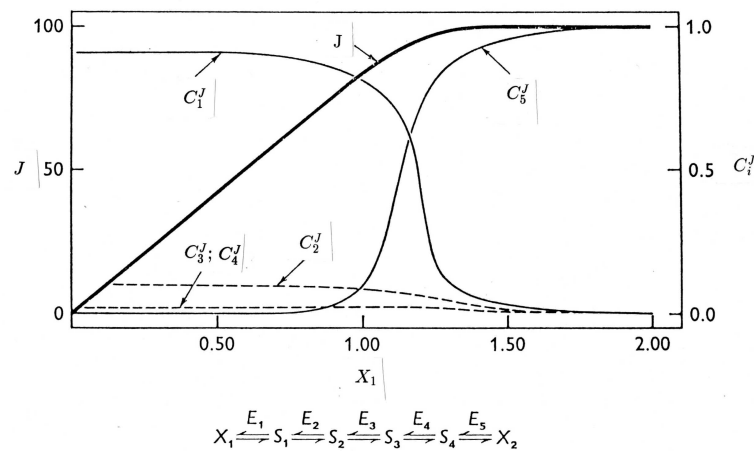


Figure 10: Effect of changes in the pathway substrate, X , on the flux. Changes in the Flux Control Coefficients of the five enzymes are also given. Computer simulation of a chain of saturable enzymes. (From Bacon, Burns & Kacser, in preparation.)

Coefficients for some of the enzymes, however, change in a dramatic way. It can be seen that E_1 has almost all the control for part of the X range ($C_1^J \approx 1$) then declines rapidly and control is 'transferred' to E_5 . This is connected with the greater saturability of this enzyme. Such a switch could not have been predicted from a measurement of the flux change only. Thus an arbitrary large change in X may fall anywhere and even a knowledge of the Control Coefficients at the start will give no indication of their distribution at the new point. This kind of situation may well apply to many of the reported experimental investigations. If large steps are therefore produced, modulation analysis at *both* points must be carried out.

In some cases a naturally occurring situation is found to involve changes of considerable magnitude and widespread consequences. Thus, e.g. Bulfield (1972) has shown that the *obese* and *adipose* alleles in the mouse, apart from exhibiting about twice the rate of lipogenesis of their normal alleles, show simultaneous alterations in at least fourteen enzymes. Although certain genetical conclusions can be drawn from these investigations, he points out that it is impossible to draw any meaningful conclusions concerning rate control from such comparisons.

There have since been a number of significant developments in using the control coefficients to predict the effects of large changes in enzyme activities [41, 42]. The theory developed in these papers also clarifies why a two-fold change in lipogenesis requires an increase in the activity of so many enzymes, and explains from a Metabolic Con-

trol Analysis perspective why it will generally be difficult to obtain large increases in flux by modulating single enzyme. Out of these insights have come proposals as to how metabolism can be modified to achieve a selective increase in a particular metabolic flux, the 'Universal Method' [43].

Conclusions

The purpose of the foregoing analysis has been twofold. In the first place it has attempted to set the problems of control in biochemical systems on a conceptually sound basis. As we have shown, there are a variety of phenomena and procedures which must be distinguished, and distinguished clearly, if experimental evidence is not to founder on semantic confusion. Controllability is distinct from the sensitivity to control. Direct parametric influences are distinct from those acting, perhaps at many removes, through the system. Systemic properties are distinct from those measurable on isolated components.

Elucidation of the conceptual issues, however, is not enough in an area where logical argument has to be supplemented by quantifiable relations. The analysis, therefore, has to be carried out within the framework of kinetic systems theory and the concepts must be given precise quantitative definitions. When this is done relationships are uncovered which allow a clearer understanding of the complex processes within the organisms.

An important conclusion was that the 'pacemaker' (or similar term) has little meaning (except in extreme circumstances) and should be replaced by the assignment of a quantity, the Flux Control Coefficient, to each of the enzymes. There will, in general, be a distribution of such values of coefficients in a pathway rather than two extreme classes.

Our theory also shows rigorously how these coefficients may be calculated from easily accessible data such as disequilibrium ratios, equilibrium constants, maximal velocities and Michaelis constants. The relevant formulations derived from our theory were compared to a variety of criteria which had been suggested on mainly intuitive grounds.

Alternative to these methods we showed how direct determinations of Flux Control Coefficients can be made by modulation of enzymes. The method of modulation was equally applicable to the determination of Elasticities which stand central in our theory.

The second purpose was to marry theory to experiment and observation. Experiments suggest theories and the interpretation of results is always carried out within the context of such a theory and its assumptions. We have attempted to show that the area of control requires a quantitative theory and have presented its outline. Equally important is the feedback from theory to experiment. We have suggested what new types of experi-

ment might be done — and what experiments we could do without. Our theory generates its own methodology and the technique of modulation is basic to this approach, making it possible to match operational and algebraic procedures.

The feedback from theory to experiment has been well illustrated by the subsequent development of Metabolic Control Analysis, in which many biochemical, genetic and molecular biological techniques that were originally developed for different purposes have been modified and applied in new ways to measure the coefficients described here (see e.g. [7, 8]).

Appendix A. Mathematical Representation

We begin with a mathematical formulation capable of representing the steady state properties of fairly general biochemical systems. The system may be growing or non-growing, the enzyme concentrations may be fixed or subject to regulation by the levels of small metabolites, the reactions may be bimolecular or of higher order and may be subject to inhibition or activation by remote metabolites.

Consider the system at any moment to have concentrations S_1, S_2 , etc., of intermediary metabolites and to exist in a volume \mathcal{V} , not necessarily constant. In unit volume of this space the net rates at which the different enzymes, considered separately, produce or remove their substrates and products are represented at any instant by values v_1, v_2 , etc. For a metabolite with concentration S_1 the total amount present in the system at any moment is $\mathcal{V}S_1$. The net rate of its production in the whole volume can be found by adding or subtracting terms of the form $\mathcal{V}v_1, \mathcal{V}v_2$ etc., in accordance with the information from the metabolic map concerning which enzymes remove or produce which metabolites. Assuming, for example, that the metabolite with concentration S_1 is produced by the first and third enzymes and removed by the seventh, we can write:

rate of increase in total amount = net rate of production in whole volume.

or

$$\frac{d(S_1\mathcal{V})}{dt} = \mathcal{V}v_1 + \mathcal{V}v_3 - \mathcal{V}v_7$$

This can be written in the form

$$\frac{dS_1}{dt} = v_1 + v_3 - v_7 - \left(\frac{1}{\mathcal{V}} \frac{d\mathcal{V}}{dt}\right) S_1. \quad (\text{A } 1)$$

For the steady-state condition, in which we are primarily interested, the pool concentrations become stationary, $dS_1/dt = 0$, and the system settles

to a steady exponential growth rate, G , with $G = 1/\mathcal{V}(d\mathcal{V}/dt)$. Thus, at the steady state, the condition for balance at the first metabolite gives

$$v_1 + v_3 - v_7 - GS_1 = 0 \quad (\text{A } 2)$$

In this steady state condition any rate per unit volume, v , is in principle related to the enzyme concentration and to the concentrations of all metabolites interacting with enzyme by a suitable rate equation (e.g. see Cleland, 1963). Suppose, for example, that the reaction rate v_1 is catalysed by an enzyme of concentration E_1 , which interacts with internal metabolites S_1 and S_5 , the rate equation could be formally written as :

$$v_1 = f(E_1, S_1, S_5, P_1, P_2, X_1, X_2)$$

or $= v_1$, for convenience. Such a rate equation also involves genetically determined parameters P_1, P_2 , etc. (e.g. Michaelis constant, inhibition constant, etc.) as well as the environmentally set parameters X_1 and X_2 (levels of nutrients, effectors, etc.).

In general the enzyme concentrations $E_1, E_2 \dots$ will themselves be related to the concentrations of any pools which act as signals within the enzyme control loop. This relation can be taken account of by replacing the enzyme concentrations, occurring in the rate expression, with suitable 'regulatory' functions. Thus the inclusion of enzyme regulation in the mathematical formulation only involves using more complicated 'general' rate expressions and does not alter their number or their disposition in balance conditions such as (A 2). The condition for balance at S_1 can now be written as an algebraic equation involving these general rate expressions

$$v_1 + v_3 - v_7 - GS_1 = 0 \quad (\text{A } 3)$$

This is a balance equation involving the parameters mentioned above and the unknown steady-state values of the variable pools S_1, S_2 , etc. There are as many such equations as there are pools since a similar balance must apply for each pool. The solution of this complete set of simultaneous algebraic equations, involving general rate expressions, thus represents the way in which steady state fluxes, J , and pools are related to the underlying genetic and environmental parameters. The steady state fluxes, J , are values which the rates, v , take when a steady state is achieved. It is usually the case that the equations cannot be solved but nevertheless the representation just stated provides the basic theory of control outlined in this paper.

The terms $-GS_1, -GS_2 \dots$, which occur in the above balance equations represent the effect of exponentially increasing volume, the value of G being zero when no such increase occurs. They are formally equivalent to the rates, v_1, v_2 , etc., and can be thought of as *fluxes to expansion*.

Coefficients and Differentiation

The control coefficients introduced at various points in the paper are all measures of the relations between a fractional change and a fractional response:

$$\text{fractional response} = \text{coefficient} \cdot \text{fractional change}$$

The coefficients are related to partial differentiation and this will be used in our subsequent theoretical treatment.

Local coefficients

The local coefficients are concerned with the response of isolated reactions. They were defined in the limit as:

For 'Controllability'

$$\frac{dv_i}{v_i} = \kappa \epsilon_P^i \frac{dP}{P}. \quad (\text{A } 4)$$

For 'Elasticity'

$$\frac{dv_i}{v_i} = \epsilon_S^i \frac{dS}{S}. \quad (\text{A } 5)$$

The relation of the coefficients to the partial differentiation of *rate expressions* is thus seen to be of the form

$$\kappa \epsilon_P^i = \frac{P}{v_i} \frac{\partial \mathbf{v}_i}{\partial P} \quad (\text{A } 6)$$

$$\epsilon_S^i = \frac{S}{v_i} \frac{\partial \mathbf{v}_i}{\partial S}, \quad (\text{A } 7)$$

where ∂ represents the operation of partial differentiation. [Note: and \mathbf{v}_i represents the rate function.]

System coefficients

These coefficients are concerned with the response of *system* flux, J , to a parametric change. The flux, J , involves the solution of the *complete set of simultaneous equations* (A3). This solution can be formally written as a function involving all the parameters of all the enzymes and all the external parameters.

Considering first the case where the enzyme concentrations E_1, E_2, \dots can be treated as parameters, this function would be of the form

$$J = f(E_1, E_2, E_3, \dots, P_1, P_2, \dots, X_1, X_2, \dots) \quad (\text{A } 8)$$

or

$$= \mathbf{J}, \text{ denoting the function}$$

The system coefficients were defined in the limit as:

For 'Response'

$$\frac{dJ}{J} = R_{P_i}^J \frac{dP_i}{P_i} \quad (\text{A } 9)$$

For 'Sensitivity'

$$\frac{dJ}{J} = C_i^J \frac{dE_i}{E_i} \quad (\text{A } 10)$$

The relationship of the coefficients to the partial differentiation of the solution of the set of simultaneous equations is thus seen to be

$$R_P^J = \frac{P}{J} \frac{\partial J}{\partial P} \quad (\text{A } 11)$$

and

$$C_i^J = \frac{E_i}{J} \frac{\partial J}{\partial E_i} \quad (\text{A } 12)$$

General Control Coefficients

The account so far given of the Control Coefficient, C_i^J , has assumed that the enzyme concentrations can be considered as parameters in the formal function (A 8). This will not always be the case and it is necessary to define the coefficient in a more general way than hitherto. In this situation the balance equations (A 3) will involve general rate expressions and will not refer directly to enzyme concentrations. However, they can be rewritten with a new set of parameters, e_1, e_2 , etc., introduced so that each occurs as a multiplier within its corresponding rate expression, $\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_n$. Further parameters, e_{n+1}, \dots , are formally attached to the terms representing flux to expansion. The purpose of introducing these parameters is that they are related to the various rate expressions in the same way as the enzyme concentrations were related when considered as parameters. Using $\mathbf{v}'_1, \mathbf{v}'_2, \dots$ to refer to the original rate expressions of (A3), the new form of the balance equation is

$$e_1 \mathbf{v}'_1 + e_3 \mathbf{v}'_3 - e_7 \mathbf{v}'_7 - e_{n+1} J \cdot S_1 = 0 \quad (\text{A } 13a)$$

or

$$\mathbf{v}_1 + \mathbf{v}_3 - \mathbf{v}_7 - \mathbf{v}_{n+1} = 0 \quad (\text{A } 13b)$$

where $\mathbf{v}_1 \dots \mathbf{v}_n$ are now functions combining e , and $\mathbf{v}_{n+1} = e_{n+1} G S_1$. The solution of this set of simultaneous equations, which do *not* now refer to enzyme concentrations, but which include e parameters, can be written formally as for the system flux, J :

$$J = f(e_1, e_2, e_3, \dots, P_1, P_2, \dots, X_1, X_2, \dots) \quad (\text{A } 14)$$

When the parameters e_1, e_2, \dots have the value unity, the original steady flux, J , will be unaltered by their presence but we can now consider the *response*

of J to modulation of the e -type parameter occurring in any particular rate expression. For the enzyme reactions it will be recognized that the e -type parameters are equivalent to turn-over numbers. The control coefficients for these abstract parameters are in fact the generalized definition of Control Coefficient which we are seeking. The general Flux Control Coefficient is then defined, in the limit, as:

$$\frac{dJ}{J} = C_i^J \frac{de_i}{e_i} \quad (\text{A } 15)$$

Expressed as a partial derivative of (A14), C_i^J is of the form

$$C_i^J = \frac{e_i}{J} \frac{\partial J}{\partial e_i} \quad (\text{A } 16)$$

Although there was agreement in the new terminology to standardise on the definition of the Control Coefficients in terms of enzyme concentration, there are technical advantages of a more general definition in terms of an e -type parameter. Heinrich & Rapoport [2, 3] assumed a more general parameter acting on enzyme rate in their original development. This was also considered by Reder [32], and there has also been a more recent discussion of the differences between the various definitions [5].

Appendix B. Relations Between Coefficients

A number of general relationships exist between coefficients and were referred to in the paper; these will now be proved. The method of proof is similar throughout and involves the consideration of certain small movements of e -type parameters (A 13). The effects of these movements on the system are written out using the coefficient definitions of (A 4), (A 5) and (A 9),(A 10).

The response of an isolated reaction to movement of its e -type parameter will often be required and is, by definition (A 13), directly proportional. So that for an isolated reaction

$$\frac{dv}{v} = \frac{de}{e} \quad (\text{B } 1)$$

Partitioned response

The relationship $R_P^J = C_i^J \kappa \epsilon_P^i$ of equation (4) can be proved by considering the situation when, in a system, a differential movement of a parameter, P , affecting an enzyme is exactly neutralised by a contrary movement of its e -type parameter. Under these circumstances no pool or flux has moved and

the only changed factors affecting the enzyme are P and e . This can now be viewed in two different ways. Firstly considering the local situation of the enzyme we can write down the fact that the sum of the effects from P and e on the rate is zero, by using relations (A 4) and (B 1), which involve *local* coefficients. Thus

$$\frac{dv_i}{v_i} = \kappa \epsilon_P^i \frac{dP}{P} + \frac{de_i}{e_i} = 0 \quad (\text{B } 2)$$

On the other hand we can write down the same fact, that the net effect is zero, using the relations (A 9) and (A 10) which involve *system* coefficients. Thus

$$\frac{dJ}{J} = R_P^J \frac{dP}{P} + C_i^J \frac{de_i}{e_i} = 0 \quad (\text{B } 3)$$

Elimination of the ratio $(dP/P)/(de_i/e_i)$ from (B 2) and (B 3) then yields the required result of

$$R_P^J = C_i^J \kappa \epsilon_P^i \quad (\text{B } 4)$$

Elasticities and Control Coefficients

Any given pool will usually influence the rate of several enzymes either by being involved as a substrate or a product of them, or as a remote effector. There is a general theorem which connects the Control Coefficients of such a group of enzymes with their Elasticities to the given pool.

This is the theorem now known as the Connectivity Theorem.

Equation (10) is an application of the theorem to the case where a pool influences only its two neighbouring enzymes in a pathway. Equation (19) is an application to the case where a pool influences an enzyme remote in the pathway in addition to its neighbours.

The theorem itself is quite general but will be proved for the case where the given pool, S , influences any three enzymes, E_1 , E_2 and E_3 , in a general metabolic network. Let the enzymes have elasticities with respect to S of ϵ_S^1 , ϵ_S^2 and ϵ_S^3 respectively.

If we imagine a differential movement dS/S in the given pool then it is always possible to choose movements in the e -type parameters of the separate enzymes such that they exactly balance the effects of the S movement. Using (B 1) and (A 5) to express the fact that the sum of the effects of e and S on the *isolated* enzymes are zero, we obtain the following:

$$\left. \begin{array}{l} \text{For } E_1, \\ \text{For } E_2, \\ \text{For } E_3, \end{array} \right\} \begin{array}{l} \frac{de_1}{e_1} + \epsilon_S^1 \frac{dS}{S} = 0 \\ \frac{de_2}{e_2} + \epsilon_S^2 \frac{dS}{S} = 0 \\ \frac{de_3}{e_3} + \epsilon_S^3 \frac{dS}{S} = 0 \end{array} \quad (\text{B } 5)$$

On the other hand these e movements will clearly maintain a new steady state differing from the original only in the pool S . In particular, no change will result in any given flux, J , as a result of these e movements. We can express this fact by writing down that the sum of the effects of the e movements, acting within the whole system, is zero. Using (A 15) this gives

$$\frac{dJ}{J} = 0 = C_1^J \frac{de_1}{e_1} + C_2^J \frac{de_2}{e_2} + C_3^J \frac{de_3}{e_3} \quad (\text{B } 6)$$

Using (B 5) to replace the ratios de/e in (B 6) by the common factor dS/S we obtain the required general relation between Flux Control Coefficients and Elasticities as

$$C_1^J \varepsilon_S^1 + C_2^J \varepsilon_S^2 + C_3^J \varepsilon_S^3 = 0 \quad (\text{B } 7)$$

It should be noticed that the theorem (B 7) is very general since the three enzymes are not necessarily in the same pathway and the Control Coefficient can refer to *any* given flux, J , within a general metabolic network. The applications leading to equations (10) and (19) in the paper are for the restricted case where the enzymes all carry the same pathway flux.

General Summation Theorem

Inspection of the generalised balance equations (A 13) shows that if all the e -type parameters, including those formally attached to expansion fluxes, were simultaneously increased by a small fraction α , then any given system flux, J , would also undergo a fractional increase α and no steady pool levels would be altered. This is because the balance of the rates would not be disturbed by such a change, only the rates being altered.

This part of the proof is related to the analogous summation theorem for Concentration Control Coefficients put forward at this time by Heinrich & Rapoport [2]. This states that the sum of Control Coefficients for the concentration of a particular metabolite over all the enzymes in the system is zero.

We can thus write for any flux, J :

total change = sum of changes due to separate e modulations,

remembering that the total change is α and, using (A 15) to express the effects of the separate e modulations, this becomes

$$\begin{aligned} \alpha &= C_1^J \frac{de_1}{e_1} + C_2^J \frac{de_2}{e_2} + \dots \\ &= \alpha (C_1^J + C_2^J + \dots) \end{aligned}$$

Hence

$$C_1^J + C_2^J + \dots = 1 \quad (\text{B } 8)$$

The theorem is a general one applying to any steady-state in a metabolic system of any complexity. It should be noted, however, that the sum is over *all* e -type parameters, that is it refers to Flux Control Coefficients associated with all enzymic steps in the system and also the Flux Control Coefficients associated with all fluxes to expansion.

There are many other relationships between coefficients that have since been proved, and indeed additional coefficients have been defined (see recent reviews for a summary [7, 8]). The Concentration Control Coefficients defined by Heinrich & Rapoport and the Summation Theorem governing them [2] have been mentioned above. In addition, these authors also proved a relationship between the Flux Control Coefficients and the Concentration Control Coefficients that also involves the Elasticity Coefficients. Fell & Sauro [26], using the type of arguments shown above, developed a method (the matrix method) that claimed to be able to express the Flux Control Coefficients in terms of the Elasticities in any pathway structure, though this required developing additional relationships between certain of the Flux Control Coefficients in branched and cyclic systems (the Branch Point Relationships) and modification of the Connectivity Theorem where pairs of metabolites contain a conserved moiety (such as nicotinamide in NAD^+ and NADH). Many authors have since expanded this work (e.g. [27]), but of particular note is the work of Reder [32], who showed that all these relationships would be valid in pathways of any arbitrary structure by applying more purely mathematical reasoning rather than working from thought experiments on physical systems. Her work also showed that the Summation Theorem and the Branch Point theorems were different aspects of the constraints imposed by the structure of a metabolic network.

Appendix C. Applications

Straight chain of unsaturated enzymes

We will now consider in detail the application of the theory to the particular case of a chain of unsaturated enzymes, E_1, E_2, \dots, E_n , carrying out the overall conversions of an external substance X_1 to another external substance X_2 via successive intermediary metabolites S_1, S_2, \dots . This is indicated in Fig. 11 and is of interest because it is possible to solve the simultaneous equations representing the system explicitly to yield an expression for the pathway flux, J , in terms of enzymic and environmental parameters.

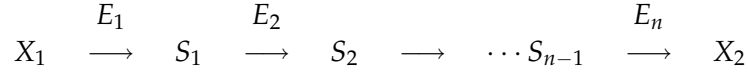


Figure 11:

Explicit solution

Suitable rate expressions to represent the reversible unsaturated enzyme-catalysed reactions at each step were suggested in equations (13). Using these together with the fact that, at the steady state, all the rates, v , must equal the pathway flux, J , we can write the set of equations

$$\begin{aligned} v_1 = J &= \frac{V_1}{M_1} \left(X_1 - \frac{S_1}{K_1} \right) \\ v_2 = J &= \frac{V_2}{M_2} \left(S_1 - \frac{S_2}{K_2} \right) \\ v_n = J &= \frac{V_n}{M_n} \left(S_{n-1} - \frac{X_2}{K_n} \right) \end{aligned} \quad (\text{C } 1)$$

V and M represent the maximal forward velocity and Michaelis Constant of the successive enzymes and K the Equilibrium Constants. The unknown pool levels S_1, S_2 , etc., can be eliminated from (C 1) by dividing the left hand sides by the appropriate V/M terms and also by K_1 for the second equation, $K_1 K_2$ for the third and so on. Addition of all the equations in this form eliminates the S s and leads to

$$J \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \frac{M_3}{V_3 K_1 K_2} + \cdots \right) = X_1 - X_2 / (K_1 K_2 \cdots K_n)$$

Hence the required solution for J is

$$J = (X_1 - X_2 / (K_1 K_2 \cdots K_n)) / \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \frac{M_3}{V_3 K_1 K_2} + \cdots \right) \quad (\text{C } 2)$$

This result shows immediately that all the enzymic, environmental and thermodynamic parameters are intimately involved in determining J and that no single factor need necessarily be controlling.

Flux Control Coefficients

Since in this case we have an expression, (C 2) of type (A 8) for the flux, we can discover the Control Coefficient of J with respect to the different

enzymes by direct differentiation as specified in (A 12). Thus we have, for the first step, and remembering that the V s are equivalent to enzyme quantities,

$$C_1^J = \frac{V_1}{J} \frac{\partial J}{\partial V_1}$$

which yields

$$C_1^J = \frac{M_1}{V_1} \bigg/ \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \dots \right). \quad (\text{C } 3)$$

By the same process the Control Coefficients of other enzymes turn out to involve replacing the term in the numerator by the term in the denominator corresponding to them. For example:

$$C_3^J = \frac{M_3}{V_3 K_1 K_2} \bigg/ \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \dots \right) \quad (\text{C } 4)$$

We can see that

$$C_1^J + C_2^J + \dots = \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \dots \right) \bigg/ \left(\frac{M_1}{V_1} + \frac{M_2}{V_2 K_1} + \dots \right) = 1$$

which confirms the general Theorem (B 8).

Inspection of (C 3) and (C 4) directly confirms the criteria of equation (17) for relative Control Coefficients in terms of maximal velocities,

$$C_1^J : C_2^J : C_3^J : \dots \equiv \frac{M_1}{V_1} : \frac{M_2}{V_2 K_1} : \frac{M_3}{V_3 K_1 K_2} : \dots$$

Alternatively equations (C 1) can be used to write this criterion in terms of pool differences, thus confirming equation (15).

Criteria for Flux Control Coefficients

We now wish to consider more rigorously the basis of the criteria for relative Flux Control Coefficients set forth in the paper and just confirmed in the particular example of an isolated chain of unimolecular unsaturated reactions. In particular we will consider in what way the criteria are affected by the pathway being embedded within a larger metabolic system and by effects such as feedback inhibition within the pathway and enzyme saturation.

The basis of the argument for establishing relative Control Coefficients involves connecting the Control Coefficients of the enzymes with certain of their Elasticities, namely those concerned with their response to the pools which are links within the pathway. This connection depends on a general result, relation (B 7), and does not exclude situations where, for example, the reactions are also coupled to ATP or perhaps inhibited by a metabolite

remote from the pathway. The values of the Elasticities for these other 'out of pathway' substances do not appear in (B 7) and therefore do not affect the results concerning relative Control Coefficients, no matter how complex the rest of the metabolism may be.

Relation of Elasticities to disequilibrium and saturation

Consider a single saturable enzyme within a chain having substrate S_1 and product S_2 within its pathway and possibly other substrates and products coupled to it from outside the pathway. From the point of view of obtaining the Elasticities by differentiation of a rate expression, using relation (A 7), a reasonable representation of a situation in which several metabolites may be involved in a reversible and saturable reaction is:

$$v = \frac{V}{M}(S_1ab - S_2cd/K) \left/ \left(1 + \frac{S_1}{M} + \frac{S_2}{M^*} + \frac{a}{M_a} + \frac{b}{M_b} \dots \right) \right. \quad (\text{C } 5)$$

This will be recognised as a generalisation of equation (12) for a unimolecular reaction which was:

$$v = \frac{V}{M}(S_1 - S_2/K) \left/ \left(1 + \frac{S_1}{M} + \frac{S_2}{M^*} \right) \right. \quad (\text{C } 6)$$

The additional substances denoted by a, b, c and d represent substrates and products outside the pathway which will enter into both the disequilibrium term (i.e. the numerator) and the saturation term of the generalised rate equation (C 5). The terms M_a, M_b , etc., in (C 5) represent the necessary extra Michaelis constants. As mentioned above we do not need to assume that the co-factors a, b, c and d are in any way held constant.

Using (A7) we can now write down the Elasticities as:
for substrate,

$$\varepsilon_{S_1}^v = \frac{S_1ab}{S_1ab - S_2cd/K} - \frac{S_1/M}{1 + (S_1/M) + (S_2/M^*) + (a/M_a) + \dots} \quad (\text{C } 7)$$

for product

$$\varepsilon_{S_2}^v = \frac{-S_2cd}{S_1ab - S_2cd/K} - \frac{S_2/M^*}{1 + (S_1/M) + (S_2/M^*) + (a/M_a) + \dots} \quad (\text{C } 8)$$

These Elasticities can be seen to be simply related to the general disequilibrium ratio, $\rho = (S_2cd)/(S_1ab)/K$, of the reaction, and to the degree of saturation of the enzyme by substrates as measured by the term Q_1 and Q_2 where

$$Q_1 = \frac{S_1}{M} / (1 + (S_1/M) + (S_2/M^*) + (a/M_a) + \dots), \text{ etc.}$$

Using this relation we can write the Elasticities in (C 7), (C 8) as for substrate,

$$\varepsilon_{S_1}^v = \frac{1}{1-\rho} - Q_1 \quad (\text{C } 9)$$

for product,

$$\varepsilon_{S_2}^v = \frac{-\rho}{1-\rho} - Q_2 \quad (\text{C } 10)$$

Clearly if the substrate and the product are small compared with their Michaelis constants then the Q terms will be small and the Elasticities will reflect the disequilibrium situation. However, even if the enzyme is saturated, when the Q terms may approach unity, the equilibrium terms will still dominate when the reaction is close to equilibrium, $\rho = 1$, since in this case they become very large. In the case of the unimolecular equation, (C 6), it is possible to write the Elasticities as their disequilibrium term multiplied by a correction factor which involves the 'flux saturation', J/V .

Thus by using (C6) we can write (C9) as

$$\varepsilon_{S_1}^v = \frac{1}{1-\rho} \left(1 - \frac{J}{V} \right)$$

However, this relation does not hold for the general rate equation of (C 6), in which we are presently interested.

Relative Control Coefficients with feedback inhibition

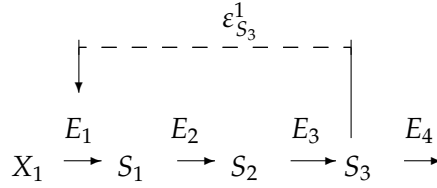


Figure 12:

We will assume that the various steps are either unsaturated or close to equilibrium in which case their Elasticities to substrates and products in the pathway will be given by $1/(1-\rho)$ and $-\rho/(1-\rho)$ respectively. The pools S_1 and S_2 only influence their neighbouring enzymes whereas the pool S_3 also exerts a feedback inhibition on E_1 measured by $\varepsilon_{S_3}^1$, the Elasticity of E_1 to S_3 .

Let $\rho_1, \rho_2, \rho_3, \rho_4$, be the disequilibrium ratios of the four steps and suppose, for convenience and since the *absolute* values are not known, that the value of $C_1^J = 1 - \rho_1$. We can find C_2^J by using the general relation (B 7). Thus

$$C_1^J \frac{-\rho_1}{1 - \rho_1} + C_2^J \frac{1}{1 - \rho_2} = 0$$

Remembering that we set $C_1^J = 1 - \rho_1$, this gives

$$C_2^J = \rho_1(1 - \rho_2)$$

We can now carry the identical operation to link C_2^J and C_3^J which yield $C_3^J = \rho_1 \rho_2 (1 - \rho_3)$.

However, in order to calculate C_4^J we must note that *three* enzymes are connected by S_3 which gives

$$C_1^J \varepsilon_{S_3}^1 + C_3^J \frac{-\rho_3}{1 - \rho_3} + C_4^J \frac{1}{1 - \rho_4} = 0$$

Using the known values for C_1^J and C_2^J this gives

$$C_4^J = \rho_1 \rho_2 \rho_3 (1 - \rho_4) - (1 - \rho_1) \varepsilon_{S_3}^1 (1 - \rho_4)$$

Collecting these together we have the result

$$C_1^J : C_2^J : C_3^J : C_4^J \equiv (1 - \rho_1) : \rho_1(1 - \rho_2) : \rho_1 \rho_2 (1 - \rho_3) : \rho_1 \rho_2 \rho_3 (1 - \rho_4) - (1 - \rho_1) \varepsilon_{S_3}^1 (1 - \rho_4) \quad (\text{C } 11)$$

Clearly, when $\varepsilon_{S_3}^1 = 0$ and there is no feedback this confirms and extends the previous result, equation (16), making clear that it is fairly generally applicable.

When the feedback is operating the result (C 11) allows us to understand more clearly how it modifies the pattern of Control Coefficients which would be expected from the disequilibria alone. Thus we see that if $\varepsilon_{S_3}^1$ is large it will tend to make the flux sensitive to the enzyme E_4 , that is, it will transfer some of the control *out* of the loop. On the other hand the idea that the controlled enzyme should have a high Control Coefficient is partially borne out if we note that this transfer of control will be most effective when $\rho_1 \approx 0$. This will give $C_1^J \gg C_2^J$ and C_3^J even though $C_4^J \gg C_1^J$. In other words E_1 should have a relatively high Control Coefficient *within* the loop if control is to be effective.

This last paragraph embodies one of the most important results of Metabolic Control Analysis, but one which has proved unwelcome because it has contradicted conventional views of metabolic regulation.

E₁ is a regulatory enzyme which is concerned with homeostasis because the feedback effect adjusts the rate of supply of S₃ to the rate of its consumption by E₄. This has the effect of making the enzyme a poorer site for the exercise of control by external effectors that modify the enzyme activity because its Flux Control Coefficient has been reduced. Metabolic Control Analysis therefore makes a clear distinction between regulatory effects and control, whereas there has been an unwarranted assumption that regulatory enzymes are potential controlling enzymes. The issue is discussed at greater length in [44], but a potent illustration of the force of the argument presented here is the failure of a number of genetic engineering experiments to increase pathway flux by increasing the expression of regulatory enzymes subject to feedback inhibition.

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Summary of changes

The following list shows the equivalences between the terms and symbols used in the original paper and the current terminology used in this current version.

Kacser & Burns, 1973		This paper	
Term	Symbol	Term	Symbol
Flux	F	Flux	J
Response Coefficient	R	Response Coefficient	R_P^J
Controllability Coefficient	κ	(external) Elasticity Coefficient	$\kappa \epsilon_P^i$
Sensitivity Coefficient	Z_i	Flux Control Coefficient	C_i^J
Elasticity Coefficient	$v_i \epsilon_S$	Elasticity Coefficient	ϵ_S^i

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