STUDIES ON THE CONTROL OF TIME-DEPENDENT METABOLIC PROCESSES

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Abstract

Sensitivity analysis studies how changes in the parameters affect the system's variables. Its application to metabolic systems (Metabolic Control Analysis, MCA) was traditionally developed under certain assumptions:

i) the steady state is stable (the effect on the steady state values only is studied).

ii) each reaction is catalyzed by one enzyme, the rates being proportional to the corresponding enzyme concentration.

iii) the parameters are changed by a small (strictly speaking infinitesimal) amount.

In the present work MCA is extended to deal with the instantaneous values of time-dependent metabolite concentrations and fluxes. Their summation and connectivity relationships are derived. In some cases it is more convenient to characterize the time courses by time-invariant variables (such as period and amplitude in oscillating systems). Summation relationships for time-invariant variables are also derived. Stability analysis shows that a linear chain of four enzyme-catalized reactions, where the third metabolite is a negative effector of the first enzyme constitutes a 'minimal' oscillator. The model is used to gain insight in the control of oscillations.

The control exerted by enzyme concentrations and other parameters that are not proportional to the rate is appropriately described by parameter-unspecified coefficients (C_v). A proof of the theorems of steady-state MCA in terms of C_v is given. By a similar procedure an attempt is made to derive the theorems in terms of C_v for time-dependent systems, which is only successful for the particular case of constant π-matrix.

The effect that a simultaneous change in all the enzyme concentrations by the same factor α (Coordinate-Control Operation, CCO) has on the variables of time-dependent metabolic systems is investigated. This factor α can have any arbitrary large value. The metabolic variables are classified according to the relationships they fulfil when the CCO is applied. A method is given to test these relationships in experimental systems and quantify deviations from the predicted behaviour.

Declaration

This thesis was composed by myself and describes my own work except where otherwise stated in the Acknowledgements or in the text.

Luis Acerenza

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Abbreviations

CCO	:	Co-ordinate Control Operation
D-plot	:	Direct co-ordinate control plot
MCA	:	Metabolic Control Analysis
PPS-plot	:	Point to Point Sensitivity co-ordinate control plot
R-plot	:	Rescaling co-ordinate control plot
RPS-plot	:	Reference Point Sensitivity co-ordinate control plot

Chapter 1

General Introduction

1.1) Some thoughts concerning the status of Biology today.

The scientific community is witnessing the greatest rate of production of scientific information ever attained. the Biological Sciences being responsible for the main quantitative contribution. The experimental approach to the biological world reveals a non-ending number of fascinating structures (e.g. the uncountable number of species). Every different molecule in each living organism is a potential object of study. The exploration of a non-negligible proportion of the natural structures appears to be an almost infinite task. Much of the effort of biologists has been and is invested in the acquisition of biological facts. Following this direction. techniques were developed to analyse and modify the composition of living organism ranging from bacteria to mammals. Many structural features are known today. The responses to a variety of stimuli have been recorded and classified.

Together with the acquisition of knowledge two main strategies have emerged. namely Artificial Selection and 'Artificial Induction'. Artificial Selection is one effective way to modify populations genotypically and hence phenotypically. Among its most outstanding achievements is the increase in food and antibiotics production. A common experience. however. is that, as the population becomes more and more extreme. no further progress under selection is possible when e.g. viability and fertility decreases or genetic variance for the character is exhausted. In such situations. a number of generations without selection (to allow recombinants to be formed) or an increase in the mutation rate may

allow further improvement in the population. The time required to achieve a certain average value of a paticular character may be long. Artificial Selection is based in the principle that making an appropiate choice from the elements of the actual population, an increase of a desirable character will appear in a future population. If our interest is to change the present components of the population (e.g. to heal an ill person) the methods of Artificial Selection are not useful by themselves. A different procedure, namely 'Artificial Induction', may be applied consisting in an 'environmental' change that results in a temporary or permanent modification of the organism. In this way the Medical Sciences use pharmaceutical preparations to give an answer to most of our health problems. Therefore, the obvious achievements of this approach do not need further comment. On the other hand, the properties required for a new drug to have a desirable effect are usually unpredictable. Some hints may be obtained from previous work, but most of the search relies on trial and error. Even after a big amount of time, work and money have been invested it is not possible to avoid a list of non-desirable effects. It would be most unfair not to recognize the major contribution of Artificial Selection and Induction to our life quality and expectancy. We must however admit that while biological research has substantially increased our 'knowledge' of living organisms, it has not improved in the same proportion our power to make predictions about situations that have not been explored yet. I feel that to increase predictive power in the biological sciences may probably require a revision of some of our basic concepts and schemes of work.

Behind every experimental design, attempting to answer a particular scientific question, there is a preconception of the system. This perconception (framework or theory) is not the simple sum of previous experimental results. It includes our present view of the

system that is infiltrated by our logic, history, feelings and prejudices. Frequently, new findings do not coincide with expectations. The failure of the predictions follows from the inexactitude of the assumptions. Some aspects of our preconception of the system have to be modified before a new question may be formulated. The introduction of mathematical tools has greatly improved the predictive power of our frameworks. This is particularly clear in the Physical Sciences. To mention one classic example, let us remember that Isaac Newton not only put forward a theory of how bodies move in space and time, but he also developed the mathematics needed to analyse those motions. In contrast, in the Biological Sciences the interaction between experiments and mathematical frameworks is a relatively recent and rare phenomenon. Biologists still feel suspicious about the importance of mathematical frameworks, and the fact that theoretical biology has not many great successes to claim closes the circular chain of negative effects. Most of the scientists that are seduced by the beauty of theoretical approaches tend to work in Astronomy or Physics, while many natural scientists that disregard these approaches are attracted by the Medical or Biological Sciences. This social situation is probably contributing negatively to the rate of development of a strong theoretical biology, but is by no means the only factor causing the delay.

In addition, biological systems have properties, the understanding of which constitutes a great challenge. One of these outstanding properties is high complexity. We can recognize at least two types: structural and functional complexity. The first one is reflected in the fact that even the simplest living organisms are built up of an almost uncountable number of different structures (molecular and supramolecular). It is difficult to find an example of a non-living system with comparable structural features. Biological systems may also exhibit complex behaviour. A biochemical example is metabolic

self-sustained oscillations. Although structural and functional complexity are related, one does not imply the other. Simple structures may come together with complex behaviour or vice versa. It is not difficult to conceive that complex behaviour is a property of the whole system and can not be attributed to any of its individual components. What is striking is the finding that some laws of complexity hold universally, irrespective of the details of the components. The development of a 'science of complexity' has greatly widened our comprehension of many phenomena, including some of our every day experience such as clouds, waterfalls and storms, the understanding of which was usually taken for granted. Importantly, it has also contributed to build up a conceptual link through our tigthly compartmentalized scientific knowledge. Furthermore, this novel conception of complex systems promoted the development of new mathematical tools, which will subsequently contribute to enlarge the ideas that inspire their development. Following similar lines, one can envisage that the reformulation of challenging biological questions could constitute a starting point towards a unified view of the phenomenon of life.

Scientific research is a repeating succession of theory and experiments and, therefore, progress is only possible if both advance in parallel. At the same time, mathematics appears to be one important component to achieve success. But, no matter how self-sufficient this powerful machinery called science may seem to be, it was created and it is driven by human action and thought. Only our imagination and desire for intelectual adventure may lead us

" *to see what everyone has seen and think what no one has thought* ".

(Albert Szent-Györgyi)

1.2) Historical background

Genetics, Biochemistry and Physiology have grown during many years to achieve their status as separate branches of the Biological Sciences. Each subject has its particular questions, methods and ways of reasoning. They share, however, a common goal, i.e. to understand the properties of living organisms. In this section I give a brief account of what I think are some lines of thought tending to make the link among these areas.

The concept of the gene (though not the name) was first put forward by Mendel (Mendel, 1866) when he proposed the hypothesis of particulate inheritance. He showed that the experimental evidence was consistent with his explanatory hypothesis by applying simple mathematical analysis to his 'strategically' obtained data. The existence of a cause-effect link between a genetic determinant (genotype) and its corresponding character (phenotype) was clearly established then. Mendel invented the terms 'dominant' and 'recessive' to describe the situation where the ability to produce certain phenotypes in diploid heterozygotes is or is not expressed. Later, terms such as pleiotropy and epistasis were also coined to describe some special features of the genotype-phenotype relationship. An important part of the study of genetics is the study of allelic substitutions causing qualitative differences in the phenotype. Most of the actual variation in natural populations among organism, however, is not qualitative but quantitative. The continuity of phenotype may result from the dependence of the expression of the genotype on variable environmental conditions or the existence of many segregating loci whose alleles affect to a large extent the phenotype being observed. Quantitative genetics makes use of statistics to answer questions of genetic variation in populations. It is not possible when applying these tools to make predictions of how an individual's phenotypic character

will be modified by a new mutational change in a particular gene. From the point of view of genetics , the organism is a black box where genotypic inputs correspond to phenotypic outputs. A quantitative theory to make the black box transparent is still not available. One early attempt to fill up this gap was made by Kacser (1957). In this essay, he showed how some simple physicochemical systems can display properties which we normally associate with living organisms. Examples are given where the genetic concepts of pleiotropy, epistasis, phenocopy, etc are associated with the kinetic behaviour of simple reaction networks.

In the early sixties a reasonably satisfying picture of the cell was available: ' Nutrients diffuse or are transported into the cell. They are transformed by sequences of metabolic reactions catalyzed by enzymes. Many enzyme activities are regulated by allosteric mechanisms and their synthesis is under genetic 'control'. The 'waste' products are eliminated from the cell.' This view was obtained by applying the analytical approach. The organism is divided and its components are extracted and identified. The small organic molecules (metabolites) are classified according to their chemical properties, and the enzymes to their specific function. It is also established which elements physically interact with each other and what is the nature of the interaction. This is achieved, for example, by studying in isolation which metabolites affect the rate of an enzyme-catalyzed reaction. The outcome of this work is the structure of the system, and its compact representation: the metabolic map. Some extreme modes of metabolic functioning may be inferred from this structural information. Although anatomy and physiology are closely related, the knowledge of the anatomy of an organ is not enough to understand its function. Similarly, the static nature of the metabolic map is not a sufficient basis to make quantitative statements about metabolic behaviour. With the pioneering papers of Umbarger (1956) and Yates and Pardee

(1956) it was first established that some 'control structures' used for a long time in the design of mechanical, electrical and electronic devices were present in metabolic networks. These 'control structures' are particular types of interactions that result in e.g. the input of a system to be affected by its own output (i.e. feedback). Moreover, their existence suggested that metabolic systems could potentially exhibit instabilities resulting in oscillations or even non-periodic changes on the metabolite concentrations. Higgins (1965) termed the study of the changes in time and position of the cellular concentrations as 'cellular dynamics'.

The kinetic study of enzyme catalyzed reactions is a major part of enzymology. The enzyme in the presence of its substrates, products and effectors catalyzes the appearence of products. The reaction rate is measured for the different values to which the concentrations are fixed by the kineticist. As a result a rate law is formulated. This approach reveals outstanding catalytic features of enzyme molecules. It is important to acknowledge, however, that the way the enzyme is assayed does not correspond to the functioning conditions within metabolism. When the enzyme is embedded in the metabolic network most of the metabolite concentrations are internally adjusted by the system, i.e. they are not fixed by the experimenter. The systems behaviour is not attributable to any one component but is the result of the components together with their interactions acting as an irreducible functional entity. It has been argued that in a chain of reactions there is always a 'slow reaction' ('master reaction' or 'rate-limiting step') which determines the rate of the whole (Blackman, 1905; Burton, 1937; Burton, 1939; Denton and Pogson, 1976 and Cohen, 1976). This idea was sometimes assumed to be self-evident without even a clear definition being stated. What is obvious is that in a linear chain of enzyme catalyzed reactions, if the activity of any one enzyme is abolished, the flux through the pathway vanishes whatever

the activities of the other enzymes are. In fact, the large number of examples in which a particular mutation has been found to give rise to a growth-factor requirement by blocking a single reaction in a biosynthetic pathway inspired the 'one gene-one enzyme hypothesis' (Beadle and Tatum, 1941 and Horowitz, 1945). In this respect, all enzymes are equally important. An important observation, however, was that the dramatic decrease of the activity of some enzymes, e.g. reduction to 5% in the activity of argininosuccinase in *Neurospora crassa* (Donachie, 1962), has no measurable effect on the growth rate. Moreover, theoretical arguments show that if the activity of an enzyme is changed by a small fractional amount (i.e. modulation, see Kacser and Burns, 1968) the change in the flux depends also on the activity of the other enzymes (Kacser and Burns, 1973). Equal modulations of different enzymes result in different changes in flux. The quantitative nature of these phenomena appears to be undeniable. Oversimplified assumptions, e.g. the existence of a 'master reaction', tend to ignore some of these aspects. To measure the quantitative effect that enzyme activity has on fluxes, sensitivity coefficients (nowadays called control coefficients, Burns et al., 1985) were defined. They represent the relative change in the variable (e.g. flux) per relative change in the modulated parameter (e.g. enzyme concentration). This type of coefficient was independently introduced in a biochemical context by Higgins (1965), Kacser and Burns (1968) and Savageau (1971). Some mathematical relationships involving the control coefficients, namely summation and connectivity relationships, have been derived (Kacser and Burns, 1973 and Heinrich and Rapoport, 1974). They reflect how the values of the coefficients depend on each other and on some properties related to the enzymatic components. The introduction of this type of theoretical framework constitutes an outstanding contribution to our understanding of metabolism and its relationships with genes and environment.

1.3) Methodological Background

Historically, science had become increasingly subdivided into different disciplines. We are now seeing the reverse process namely that the boundaries between the different areas of knowledge are becoming increasingly diffuse. Understanding of metabolism is now closely related to the knowledge, apart from Biochemistry, of Genetics, Physiology, Physical Chemistry, Chemistry, Physics, Mathematics, etc. For the development of a quantitative account of metabolism the use of some mathematical and physicochemical tools is of particular importance. In this section I shall give an overview of these tools and stress their usefulness in the present thesis.

1.3.1) Thermodynamics and kinetics

Classical thermodynamics, as developed during the 19th century, is mainly concerned with equilibrium situations. From the first and second laws, outstanding results such as Gibbs phase rule and the law of mass action were obtained. The second law of thermodynamics in its most general formulation applies to both equilibrium and nonequilibrium situations. The early conception, however, was that while equilibrium represented order and permanence, nonequilibrium appeared to be a non-desirable perturbation (e.g. to grow big cristals by cooling a solution a slow decrease in temperature is required to maintain near equilibrium conditions). Many examples are available today that show how the flow of energy and matter in a nonequilibrium process may be used to maintain functional and structural order. When a constant temperature gradient is applied to a mixture of two gases the concentration of one of them increases near the hot wall, while the other concentrates at the cold wall. The entropy in this steady state situation is lower than it would be in the

uniform mixture at equilibrium. This phenomenon appears even if the temperature gradient is small, i.e. the conditions are near equilibrium. Another well known example is a container heated from below. For small temperature gradients heat passes through the liquid by conduction. When a critical value of the temperature gradient is attained regular convection cells appear spontaneously. This new structure involving coherent behaviour is called the Benard cells. Certain chemical reactions (e.g. Belousov-Zabotinski reaction) present spatial and temporal cell organization. Glycolytic oscillations, i.e. oscillations in time of the metabolite concentrations involved in the glycolitic pathway, discovered by Chance and co-workers (Chance et al., 1964a and 1964b) was the first biochemical example where such temporal patterns were unambiguosly recognized. The extension of thermodynamics to near and far from equilibrium situations was essential as a common language to describe phenomena like the ones mentioned above (Onsager, 1931 and Glansdorff and Prigogine, 1971). Moreover, it suggests that life is not an 'improbable' event struggling against the laws of physics, but a consequence of these laws appropriate to specific non linear interactions and far from equilibrium conditions.

Thermodynamics is not the most suitable tool when the purpose is to study a particular chemical mechanism. In contrast, kinetics has proven to be very useful for obtaining and analysing reaction mechanisms. Chemical kinetics may be divided in two main areas: i) the empirical analysis of reaction rates and ii) the theories of chemical kinetics. The first one studies how the rate of a process depends on the species present in the reaction mixture. This information is summarized in an equation called a rate law. Apart from the participating concentrations, it involves kinetic constants which also determine how the rate depends on the temperature, viscocity and other physical properties. The rate law is experimentally determined,

for which several procedures are available. A further objective of empirical kinetics is to propose a plausible mechanism, i.e. a set of 'elementary' reactions which will conform to the observed rate law. This kinetic approach may be applied to systems of different nature and complexity. For example, the kinetics of gas phase reactions have been extensively studied. The mechanisms of some 'simple' gas phase reactions, such as $H_2 + I_2 = 2\ HI$, first described a century ago, are still a matter of debate. Enzymes are biological catalysts, i.e. they increase the rate of cellular reactions without being consumed or produced in the overall process. A basic mechanism of enzymatic action was proposed by Michaelis and Menten (see e.g. Cornish-Bowden, 1976) to explain the hyperbolic relationship between rate and substrate concentration. Many other kinetic features of enyme -catalysed reactions have been established since then. One of the most outstanding findings is that the catalytic power of an enzyme may be reversibly modulated by the presence of specific molecules located in a 'distant position' to the enzyme in the metabolic map. Allosteric activators and inhibitors belong to this type of molecule. This phenomenon contributes to the highly interactive nature of metabolic reactions. It must be pointed out, however, that most of enzyme kinetics is concerned with the study of isolated enzyme catalyzed reactions. The kinetic analysis was also applied to intact living systems. Monod (Monod 1942, Lwoff and Ullmann, 1978) was the first to obtain an hyperbolic rate equation between bacterial specific growth rate and the concentration of an essential growth substrate. A detailed mechanism to give account of this rate law includes in principle all the metabolic processes occuring in the cell.

The second area of chemical kinetics is the development of theories. Its main goals are to gain insight into the factors that influence reaction rates and to make calculations from first principles of the rate of reactions. Neither of these two objectives have yet been

satisfactorily attained. The first theory to give some useful results was the Collision Theory. It assumes that molecules can be treated as hard spheres and that there are no interactions between molecules until they touch (Trautz, 1916 and Lewis, 1918). This theory is altogether too crude and gave reasonable predictions only for reactions that involve very simple molecules. In the 1930s' it was superseded by the Activated-Complex Theory (Eyring, 1935 and Evans and Polanyi, 1935). This theory is based in the assumption that the reacting molecules cross a potential energy barrier. The state of the molecule with maximum potential energy along the reaction coordinate corresponds to the activated complex. Another important postulate is that the reactants are always in equilibrium with the activated complexes. A consequence of the latter assumption is that the rate constant is amenable to a thermodynamic formulation in terms of entropy and enthalpy of activation. This thermodynamic description of the activated-complex formation has been used to gain understanding of some factors contributing to enzymatic catalysis (Leinhard, 1973). Stochastic theories of reaction rate consider the chemical reaction as a stochastic process, i.e. a process about which only probabilistic predictions are possible. One of this type is Kramers' Theory (Kramers, 1940). In this theory the molecules are supposed to become activated through their collisions with other molecules of the surrounding medium, which acts as a constant temperature heat bath. After many exchanges of energy during such collisions a molecule may acquire sufficient energy to cross a time-independent potential barrier. The interaction of the reactant molecules with the heat bath is analogous to the Brownian motion of the particle in a viscous medium. During many decades, the use of Kramers' Theory was overwhelmed by the use of the Activated-Complex Theory. Recent experimental and theoretical studies of enzyme catalysis, however, suggest that the catalytic constant can be better described using Kramers' Theory of reaction rate (Gavish, 1986). An important piece of evidence is the

experimental dependence of the catalytic constant (kcat) with the solvent viscocity (η): kcat α η^p ($0 \leq p \leq 1$) (Gavish, 1986), which is in agreement with the theoretical predictions of Kramers' Theory.

It is my intention in this thesis to study properties of living systems and, therefore, the analysis of nonequilibrium situations is essential. Although some thermodynamic concepts such as distance from equilibrium are very helpful in the analysis of particular situations, thermodynamics is chiefly a conceptual reference. In contrast, the kinetic approach is appropriate to describe the dynamical behaviour of metabolic networks and is used throughout the thesis. Combined with sensitivity and stability analysis it constitutes the main tool of this work.

1.3.2) Differential equations and stability.

The traditional kinetic description of metabolic reactions is by the use of deterministic differential equations. These equations relate the change of each metabolite concentration with time to the rates of production and consumption of the metabolites. The solution of the system of differential equations is the time course of the concentrations. For different sets of parameter values the solution may exhibit different temporal behaviours. When there exists a unique stable steady state, the variables show temporary changes that asymptotically approach constant values, i.e. the steady state values. If the unique steady state is not stable, oscillatory or even chaotic regimes in the metabolite concentrations may be obtained (Decroly and Goldbeter, 1982). The stability of the steady state may be analysed by the technique of 'linearized stability analysis' (see e.g. Stucki, 1978). As a consequence of the non-linearities in the rates the system may have multiple steady states. In such a situation for

different initial conditions (and the same parameter values) different final behaviours can be attained. Degn (1968) was the first to find bistability in an enzymatic system. He suggests that the inhibition of horseradish peroxidase by its oun substrate (O_2) is a possible mechanism to explain the existence of two stable steady states. Another form under which double steady states may appear is birrithmicity. In this case the existence of two unstable steady states gives rise to two different periodic regimes. Birrithmicity has been found in very simple metabolic models with two (Moran and Goldbeter, 1984). or three variable metabolite concentrations (Decroly and Goldbeter, 1982). These theoretical findings suggest that it is not unlikely that real metabolic networks may exhibit this type of behaviour. Birrithmicity has recently been demonstrated in chemical oscillatory reactions (Alamgir and Epstein, 1983 and Lamba and Hudson 1985). It has, however, not yet been observed in biological systems.

The study of the phenomenon of chaos helped to clarify the difference between determinism and predictability. The description by differential equations mentioned above may be classified as deterministic, because for a given set of initial conditions and parameter values there is a unique solution. If this solution is chaotic an additional property emerges. The result obtained with slightly different initial conditions diverges from the original solution in such a way that after a short period of time almost all resemblance disappears (i.e. high sensitivity on initial conditions). The initial conditions can not be measured with an infinite precision. Even if we assume that the system of differential equations is a perfect model of the process we want to describe, and that the parameters are known, small errors in the initial values of the variables may give very different predictions of how the values evolve in time. In this case the future is unpredictable, though determined. A four steps metabolic

chain where the second and third reactions are activated by their products can display chaotic behaviour (Decroly and Goldbeter, 1982). This regime is obtained when the pathway is fed with a constant input flux (it is not forced by a time-dependent input). Metabolic networks are, therefore, potential generators of chaotic regimes even in a constant environment. It is important to point out that there is not enough evidence to affirm whether chaotic biochemical behaviour is physiological, pathological or an unimportant curiosity. In contrast, chaos is used as a valuable tool for the determination of the number of independent variables that describe the system (Shuster, 1984). This method was applied to the chaotic response of the glycolitic pathway obtained under a sinousoidal glucose input flux (Markus et al., 1984, Markus and Hess, 1990). The experimental results show that three independent variables are needed to describe the dynamic properties of the metabolic system. Therefore, a plausible model must be constructed with, at least, three autonomous differential equations. This number is smaller than the total number of variable metabolite concentrations involved in the system.

The concentrations in a chemically reacting system fluctuate due to the random structure of the intermolecular interactions. The magnitude of the fluctuations is usually related to the inverse of the volume. Thus, for macroscopic systems the fluctuations are often negligible and deterministic differential equations provide an accurate description. There are situations, however, in which even in macroscopic systems fluctuations are important. If the system posseses multiple steady states and it is initially close to an unstable steady state, then the transition to one of the stable steady states can be driven by fluctuations (Mangel, 1978). A convenient description of fluctuating reacting systems is the use of stochastic differential equations. These equations are similar to the deterministic ones mentioned above except for the addition of random terms. This

approach was used, for example, in the study of kinetic processes in micellar systems. Here, the reacting molecules are characterizaed by lengths which are not orders of magnitude smaller than the space within which the chemical reaction takes place (Hatlee and Kozak, 1980).

The time evolution of metabolic systems is a central topic in the present thesis. The description by differential equations is appropriate to this subject and used throughout. I do not intend to ask questions in which fluctuations play an important role and, therefore, stochastic terms are not included. In all the situations considered the concentrations are homogeneous in space and diffusion terms can be omitted. Linearized stability analysis is used to characterize the stability of the steady state. This technique allows a classification into simple models according to the type of dynamical behaviour that may or may not be expected. Minimal models that present, for example, oscillations are recognized here by this method. Stability analysis is also used to divide the parameter space into regions each corresponding to a particular behaviour. Finally, sensitivity analysis of the variables (e.g. metabolite concentrations, fluxes, period and amplitude of oscillation) in different regions is performed.

1.3.3) Minimal and 'mimical' models.

Two conceptions (or strategies) of metabolic modeling by differential equations can be detected in the literature. The first one takes into account all the known components and interactions existing in the system. One differential equation is written to describe the change in each variable concentration. The rate laws representing the quantitative interaction between the components, and the values of the constants involved are also assumed to be known. Due to the

non-linearities of these rate equations only a numerical solution is possible. The outcome of the numerical simulation is a table where the concentrations corresponding to the time intervals explored are given. The model is said to be succesful when the experimental data coincide with the result of the numerical simulation. One of the main goals of this approach is, therefore, to mimic reality. I propose the term 'mimical' to describe this type of model. Examples of mimical models have been used by Garfinkel (see e.g. Garfinkel and Hess, 1964) and Wright (see e.g. Wright and Albe, 1990). The second strategy focuses its attention on a small number of properties of the system. The model is constructed with the aim of answering a particular question related to these properties. For example, in certain conditions metabolic systems exhibit oscillations. Thus, one may ask, what are the structural requirements to obtain this type of behaviour. Possible answers include positive and negative feedbacks. If it is known that the system presents a negative feedback, further questions may be addressed. There are usually many models with different numbers of variables that exhibit the property of interest. The criterion in this case is to choose the 'minimal' model i.e. the 'smallest' model that gives the answer we search. Kacser has used minimal models to exemplify the existing relationships between the control coefficients (system changes) and elasticity coefficients (isolated rate changes). These relationships give insight on the contribution that the properties of the components have to the behaviour of the whole system. Importantly, he pointed out that each control and elasticity coefficient of the minimal model could represent the resulting response of a group of reactions in a more detailed model (Kacser, 1983). Goldbeter has extensively used minimal models to show how complex patterns may arise in regulated enzymatic systems. In a model consisting only of two variables his group showed the existence of birrithmicity, multiple thresholds and tristability (Goldbeter and Moran, 1987). Mizraji and co-workers have used models including time delays to

analyse the stability of metabolic pathways controlled by end product. They assume that the kinetics of the intermediate steps of the pathway are unknown and the lack of information is covered by using a time delay. (see e.g. Mizraji et al. 1988).

Mimical models rely on a complete knowledge of the structure and properties of the components of the metabolic network. Therefore, the acquisition of new facts promotes, automatically, the growth of the model. In principle, all possible behaviour of the real system can be reproduced by its formal twin, if the latter is sufficiently accurate. At this point, the advantage of operating on the model over performing the equivalent experiments may be questioned. It may be argued that manipulating the model may show some unexpected behaviour that can be later reproduced in the experimental system. But, is the model contributing to our understanding much more than the direct analysis of the experimental data ? Moreover, it is usually suggested that these models reproduce the *in vivo* behaviour. It is, of course, a very difficult task to determin the kinetic parameters of enzyme-catalized reactions *in vivo*. As a consequence, most of them are obtained in *in vitro* conditions. The environment presented to an enzyme by the cell, where all the other proteins, membranes, etc are present, is very different from the conditions where the pure enzyme acts in a test tube. In addition, the *in vitro* conditions, in which the enzymes are assayed differ from one reaction to another. Taking into account these considerations it is difficult to sustain the hypothesis that the quantitative responses of the model mimic those of the living organism. Minimal models are less pretentious than mimical models. They are based on a small number of facts and they are built up to address a particular question. Consequently, they are usually not suitable to answer many other questions. In contrast, they can give a satisfactorily answer even if many facts are not known and, hence, they have the advantage to be able to overcome our ignorance. If new

facts relevant to the question of interest are acquired the present model may be modified to obtain a more realistic one.

I would like to point out, briefly, one consequence of aiming to mimic nature as far as it is possible. In the description of metabolic networks by differential equations the immediate protagonists are the concentrations of the different molecular species. These macroscopic concentrations result from the average behaviour of a large collection of molecules, each showing random motions. The molecules are constituted of atoms and these of subatomic particles. One may, in principle, argue that the quantum mechanical transitions in some atoms are relevant to the catalytic events occuring in the enzymes. Following this argument, a microscopic quantum mechanical treatment of the metabolic system would be a better approach than the macroscopic description. Such a model, even if physically right, would be impossible to handle because of its size. Thus, it is important to make an adequate choice of the level of description (e.g. microscopic or macroscopic) according to the phenomenon of interest, if a tractable model is to be obtained (see discussion in Mizraji et al., 1987).

Minimal and mimical models, considered above, are the extreme conceptions of a spectrum of intermediate solutions. Both have advantages and disadvantages. In principle, depending on the aim underlying the model building, it could be preferable to be closer to one extreme or the other. In the present stage of our knowledge, I am inclined to choose minimal models. In this thesis, minimal models are used either to answer metabolic questions or to exemplify general metabolic properties. Importantly, they provide the substance to perform numerical experiments, which may be very useful to discover new relationships.

Finally, I would like to relate an anecdote. In January 1988, I arrived in Edinburgh to work with Henrik Kacser. Then, one of the things he let me know was about the existence of the 'Edinburgh organism'. This organism was composed of three enzyme catalized reactions and two variable metabolite concentrations (S_1 and S_2), i.e. X_0 ---> S_1 ---> S_2 ---> X_3 . This structure lacks most of the structural and functional features that even the simplest living organisms have. It is, however, sufficiently complex to show the essence of some properties of living organisms. Using this model many answers to general questions related to the control of metabolic networks could be tested. In the meeting 'Control of Metabolic Processes' (Il Ciocco, Luca, Italy, 1989) organized by Athel Cornish-Bowden, the Edinburgh organism was, of course, present in Kacser's talk. This time its objective was to reveal some consequences of enzyme-enzyme interactions on the control properties of metabolic pathways. The Edinburgh organism was not alone in this meeting. A close friend, the 'Bordeaux organism' (as Mazat named it), i.e. X_0 ---> S ---> X_1, was an important protagonist in Jean-Pierre Mazat's talk exemplifying geometrical aspects of metabolic control. These two organisms have been successfully used for several years and ,therefore, share a thoroughlly deserved reputation. In that meeting, I presented another organism. I named this new born the 'Edinburgh-Montevideo organism', because , although it was born in Edinburgh, it was also inspired in an oscillator including a time delay that Eduardo Mizraji introduced to me in Montevideo some years ago. It consists of four enzyme-catalysed reactions and three variable metabolites. In addition, the third metabolite is a negative effector of the first enzyme. This organism shows stable and unstable steady states. It constitutes a minimal oscillator with negative feedback. Depending on the environmental conditions it exhibits either smooth transients to the steady state or self-sustained oscillations. It was a very useful tool for the development of the sensitivity analysis of

time-dependent metabolic systems included in this work. To conclude, I must say that I am grateful to the three 'organisms' mentioned above and many others of the same group for the achievements obtained in this thesis (For another anecdote concerning minimal models see Gleick, 1989).

1.3.4) Sensitivity Analysis

The description of metabolic systems by differential equations renders the variables (e.g. metabolite concentrations) as functions of time. This solution, whether analytical or numerical, depends on the parameter values. When the aim is to change the system's outcome in a certain way, the question of which parameters to manipulate arises. Quantification of the role of the parameters in the systems outcome is the traditional task of sensitivity analysis. This type of analysis is at present used in different areas of science and engineering (see e.g. Tomovic and Vukobratovik, 1972; Rabitz et al., 1983).

Basic concepts of sensitivity analysis were first applied to metabolic systems by Higgins (1965). He brought the sensitivity coefficients of sensitivity analysis into a metabolic context. The development of a sensitivity analysis appropriate to metabolic systems was independently initiated by several groups. The three main approaches were those pioneered by: i) Savageau (1972), ii) Kacser and Burns (1973) and Heinrich and Rapoport (1974) and iii) Crabtree and Newsholme (1985). Each of them show particular formal features and aims, although the differences are probably less than the common aspects they share (Cornish-Bowden, 1989 and 1990). There has been debate concerning their power, accuracy, limitations, easy handling and other advantages and disadvantages (see TIBS (1987) Vol 12). Unfortunately, these discussions are not easy to grasp to many of the biologists engaged in traditional biochemistry. It is a

matter of fact, however, that the most extensively used by theoreticians and experimenters is the analysis introduced by Kacser and Burns (1973) and Heinrich and Rapoport (1974) (based on Science Citation Index 1988, 1989 and 1990). This framework is nowadays called 'Metabolic Control Analysis' or 'Metabolic Control Theory' and abbreviated MCA and MCT respectively (in what follows MCA). The main part of the present thesis is concerned with the development of some aspects of MCA. At this point, I could give a list of objective arguments to justify my choice in favour of MCA, but I feel I would be diluting one important point: " MCA provides me with a 'confortable' language to develop intuition and materialize ideas ". This is, of course, a subjective argument, even though I think it is valid when evaluating equivalent tools for scientific inquiry.

Next, I shall give an outlook on some ideas of steady-state MCA. More technical aspects are left for next section. The starting point of MCA is to classify the metabolic quantities dividing the system into parameters or variables. Kinetic constants, external effectors (nutrients, waste products, etc) and enzyme concentrations are usually considered as parameters while internal metabolites and fluxes are the variables. For steady-state systems the only way to achieve a permanent change in the value of a variable is by modulation of one or more parameters. The control coefficient (Burns et al., 1985) of a variable V, with respect to a parameter p, represents the relative change in V ($\delta V/V$) divided by the small relative change in p ($\delta p/p$) when the other parameters are not altered. The effect of environmental factors on metabolism can be quantified by evaluating the control coefficient involving external effectors. The smaller the values of these coefficients, the more independent is the system's state on environmental fluctuations. If the parameter considered is an enzyme concentration, the control coefficient may represent, for example, the

effect of a change in the corresponding gene expression. The control coefficients with respect to enzyme concentrations satisfy some relationships called summation theorems. The flux summation theorem (Kacser and Burns, 1973) states that the sum of the control coefficients for any one flux with respect to all the enzyme concentrations in the system equals one. This result constrains the value that the control coefficients can take. In a linear chain of reactions, and if substrates increase and products decrease the rates, the flux control coefficients are all positive (Kacser, 1983). Thus, no coefficient can be greater than one, because the unit amount of control is shared by all the enzymes. Furthermore, the greater the number of enzymes, the smaller is the value expected to be obtained when a control coefficient is determined. The definition of control coefficient and the summation theorem have clarified the concept of 'rate limiting' enzyme. Due to the usual proportionality between rate v, and enzyme concentration E, an enzyme modulated in isolation by a relative amount ($\delta E/E$) produces an equal relative change in the rate ($\delta v/v$). If the same relationship exists between enzyme concentration and flux when the enzyme is embedded in a metabolic network, we describe it as a 'rate limiting' enzyme. According to the summation theorem, in a linear chain of reactions, if any one enzyme is rate limiting all the others have no effect on the flux. Moreover, the existence of a rate limiting enzyme, is by no means inevitable, and the usual situation is probably that the control of the flux is shared by two or more enzymes. The flux summation theorem is also a very important component in a metabolic description of some genetic phenomena such as dominance (Kacser and Burns, 1981). Other relationships constraining the values of the control coefficients such as the concentration summation theorem (Heinrich and Rapoport, 1974), connectivity theorems (Kacser and Burns, 1973 and Westerhoff and Chen, 1984), branch theorems (Fell and Sauro, 1985 Sauro et al., 1987 and Small and Fell, 1989) and substrate cycles (Fell and Sauro, 1985,

Hofmeyer et al., 1986 and Sorribas and Burtons, 1986) have also been derived.

The modulation of a parameter causes an immediate change in the rate (or rates) in which it occurs. Consequently, this change tends to modify the concentration of the flanking substrates and products. The perturbation spreads across the system affecting metabolite concentrations and fluxes. The control coefficients describe the metabolic network as a whole and therefore take into account all these changes. On the other hand, the component blocks of metabolism are the enzyme-catalyzed reactions. One task of the enzymologist is to study their kinetic properties in 'isolation'. The procedure followed by the enzyme kineticist is different from the one used to determine control coefficients. While in the determination of the control coefficients the internal metabolite concentrations are adjusted by the system (i.e. are variables), in the 'isolated' enzyme assay they are adjusted by the experimenter (i.e. are parameters). Thus, in the conditions of an enzyme assay a new coefficient can be defined , the elasticity coefficient (Kacser and Burns, 1973). It represents the relative change in the rate ($\delta v/v$) per relative change in concentration ($\delta S/S$), when all the other concentrations affecting the rate are maintained constant. One important result of MCA is that the control coefficients can be expressed in terms of the elasticity coefficients. These relationships show how small changes in the whole system can be dissected into the changes of its components. Moreover, they allow the prediction of to what extent changes in the properties of the isolated components affect the system's behaviour. Several methods have been proposed to obtain the control coefficients in terms of the elasticity coefficients. The first one was introduced by Kacser (1983). It is based in the use of the equations that relate the small change in a parameter with the resulting changes in the variables (i.e. modulation equations). Although this is a general method the difficulties to apply it increase rapidly with the size of the model.

This problem can, however, be overcome by introducing the modulation equations into a matrix form (Westerhoff and Chen, 1984). Fell and Sauro (1985) developed a different 'matrix method'. The control coefficients are obtained in terms of the elasticity coefficients by solving matrix equations generated directly from the theorems (see also Sauro and Fell, 1987). Recently, Small and Fell (1990) showed how the matrix method can be used to calculate the sensitivity of control coefficients to the change in elasticity coefficients. Reder (1988) has developed a structural approach to MCA. The advantage of this method lies in the fact that the structure of the metabolic system depends neither on the environment nor on the internal state of the system. Moreover, it leads to a generalization of the theorems. It is important to acknowledge, however, that the mathematical elegance and rigour of Reder's work makes it very difficult to understand by experimental biochemists. Theoretical biochemists will have to 'translate' this powerful approach to a more suitable language (e.g. modulation equations) to make it amenable to experimental application. Other methods to calculate control coefficients in terms of elasticity coefficients are given in Cascante et al. (1989a and b), Giersch (1988) and Hofmeyer (1990).

In the final part of this section I shall describe some assumptions made in the traditional approach to MCA, and how this thesis could at least partially overcome them. MCA as proposed by Kacser and Burns (1973) studies metabolic pathways where all the variables (metabolite concentrations, fluxes, etc) have constant values in time, i.e. the steady state. Most of the subsequent contributions to the field (including all the ones previously mentioned in this section) also deal with this particular state. Many biological systems exhibit a quasi-steady state behaviour during considerable intervals of time, for which the steady state treatment is a good approximation. On the other hand, fluctuations of the environment, both temporary and

permanent, will affect the internal composition, giving rise to transient changes in the values of the variables. Furthermore, as is well known, the widely distributed allosteric mechanisms in metabolism are potential generators of temporal and spatial patterns (see e.g. Rapp, 1979; Boiteux et al., 1980). Although, abundant theoretical and experimental evidence is available concerning unstable steady states and transients, metabolic control analysis for these situations has not been developed. A few contributions in this field have been made (Higgins et al., 1973, Kohn et al., 1979 and Kohn and Chiang, 1982), but no complete theoretical treatment is available.

In MCA two assumptions were made regarding the kinetic properties of the enzymes. The first is that the rate equations of all enzyme-catalysed reactions are first order with respect to the total enzyme concentration (i.e. $v_j \propto E_j$), namely additivity. This hypothesis is based on the kinetic data collected from a wide range of purified enzymes. There are, however, exceptions , e.g. enzymes that show monomer-dimer associations (see e.g. Kurganov 1978). The second assumption is that the catalytic properties of any one enzyme does not depend on the concentrations of the other enzymes, namely independence. There is structural evidence that enzyme-enzyme complexes are formed (see e.g. Ovadi et al., 1983), but there is not general agreement yet about what the kinetic consequences of the interaction are (Cornish-Bowden, 1991). The traditional summation and connectivity theorems of MCA (Kacser and Burns, 1973 and Heinrich and Rapoport, 1974) are only valid if all the enzymes satisfy the assumption of additivity and independence. Thus, if these assumptions break down, the derivation of more general relationships is required .

The coefficients of MCA apply, strictly speaking, to infinitesimal changes. The main reason to introduce infinitesimal changes was to search for a constant coefficient, independent of the size of the

modulation (Kacser and Burns, 1968). Due to the non-linear relationship between variables and parameters, the ratio between the relative change in the variable and the relative change in the parameter depends on the size of the latter change, when finite (i.e. non-infinitesimal) changes are considered. Another advantage of the use of infinitesimal changes is that they are additive. This means that the overall change in the variable caused by simultaneous changes in several parameters can be written as the sum of the changes in the variable resulting from the independent modulation of those parameters. The additivity of infinitesimal changes is essential to the derivation of the theorems of MCA. Experimental changes, in metabolic systems, are always finite. In practice, however, if sufficiently small, they are amenable to MCA. On the other hand, it must be recognized that some responses due to either environmental, physiological or developmental processes are the consequence of large changes. Goldbeter and Koshland (1982) define a sensitivity amplification factor that is based on finite changes to quantify the amplification properties of simple biochemical systems (e.g. enzyme subject to covalent modification and substrate cycles). Large metabolic changes is one of the areas where less theoretical understanding has been achieved.

One aim of the present thesis is to extend MCA of the steady state to time-dependent metabolic systems. This implies to develop a formal framework such that, if the system approaches a stable steady state, the definitions and relationships take the form of the traditional steady state counterparts. Moreover, it is desirable that the extension could be used to analyse temporal behaviours which result from the existence of non-stable steady states. The present work fulfils these requirements. Some contribution to the analysis of large changes is made. A general approach to the problem appears to be very difficult. Some insight may be obtained, however, by considering particular large

changes. Some consequences of the breakdown of the proportionality between rate and enzyme concentartion are also analysed in steady states and time-dependent systems.

1.4) Technical Background to MCA of the steady state

In this section I will introduce some technical aspects related to steady-state MCA. The definitions, relationships and procedures described, will lay the basis to understand the following chapters of this thesis. Here, I do not intend to make an exhaustive revision, because a number of reviews covering the field are now available (Westerhoff et al., 1984, Porteous, 1985, Derr, 1985, Kacser, 1987, Kacser and Porteous, 1987 and Small, 1988). Historically, control coefficients were defined before the elasticity coefficients in the context of MCA. This is easy to conceive, because while control coefficients are essential to the description of the systemic changes, elasticity coefficients are useful to give an interpretation of how these changes are originated. The order followed in this section is not the chronological one. I will first analyse the isolated enzyme-catalyzed reaction, and in a second stage, how a group of these rates linked by their substrates and products behaves. This order has the advantage of following the increase in complexity.

A 'system' can be defined as the set of measurable quantities that the observer (or experimenter) select from those available. It is the part of the universe on which the interest is focused: the rest of the universe is known as the 'surroundings' (or environment). The measurable quantities may be classified in two groups: parameters and variables. Parameters are the quantities whose values are under direct control of the experimenter. These values are either constant or forced

to change in time in a pre-determined way. In the case of steady state systems parameters are fixed to a constant value. The variables are the quantities whose values depend on the values of the parameters.

1.4.1) The isolated enzyme-catalized reaction

I shall first consider an isolated enzyme-catalized reaction. In the absence of catalyst the rate at which the reaction takes place is negligible, i.e. only the enzyme-catalyzed rate is measurable. Two substrates (X_1 and X_1') are reversibly converted into products (X_2 and X_2'). An effector (X_m) of the enzyme (E_1) is also present.

$$X_1 + X_1' \xrightarrow{E_1 / X_m} X_2 + X_2' \qquad (1.1)$$

The mechanism of this reaction will involve, apart from the species shown in the scheme other species such us enzyme-substrate complexes. It is assumed that the concentrations of free substrates (x_1 and x_1'), products (x_2 and x_2') and effector (x_m), and the total enzyme concentration (E_1) are maintained constant in time, i.e. are parameters. It may be shown that under these conditions the variables (i.e. enzyme-substrate complexes, free enzyme concentrations, etc) approach a unique asymptotically stable steady state (Wyman, 1975). In this state all the variables have constant values which can be explicitly expressed in terms of the parameters. A rate law relating the steady-state rate as a function of the parameters can be written:

$$v_1 = v_1 (x_1, x_1', x_2, x_2', x_m, E_1, ks) \qquad (1.2)$$

where ks represents all the kinetic constants involved. The change in any one of the parameters, causes a change in the rate. The effect that a small absolute change in a parameter, for example x_1, has on the steady state rate, when all the other parameters are not altered, can be described by the partial derivative of the rate with respect to the parameter, i.e. $\partial v_1 / \partial x_1$. Similarly, if we are interested in relative changes, the log-log derivative may be introduced:

$$\pi_{x_1}^{v_1} = \frac{\partial \ln v_1}{\partial \ln x_1} = \frac{x_1}{v_1} \frac{\partial v_1}{\partial x_1} \qquad (1.3)$$

$\pi_{x_1}^{v_1}$ is called the 'π-elasticity coefficient' of the rate v_1 with respect to the parameter x_1. There is, in principle, one elasticity coefficient for each parameter in the rate equation. Activators, i.e. parameters the increase of which produces an increase in the rate, such as substrates or positive allosteric effectors, give rise to positive elasticity coefficients, while inhibitors of the rate, such as products or negative effectors result in negative elasticity coefficients. Substrates (products) may produce negative (positive) elasticity coefficients in the special case of substrate inhibition (product activation). The range of values that the elasticity coefficients can take depends on the rate equation (see e.g. Westerhoff et al., 1984 and Sauro, 1986). One very frequent feature of the steady state rate of an enzyme-catalyzed reaction is its proportionality to total enzyme concentration. If this is the case, Eq (1.2) can be written as follows:

$$v_1 = E_1 \, f (x_1, x_1', x_2, x_2', x_m, ks) \qquad (1.4)$$

It may be easily shown that when proportionality holds the elasticity coefficient with respect to enzyme concentration equals one, thus, in the case of eq(1.4) we can write:

$$\pi_{E_1}^{v_1} = \frac{E_1}{v_1} \frac{\partial v_1}{\partial E_1} = 1 \qquad (1.5)$$

Deviations from proportionality may appear, for example, when the enzyme presents monomer-oligomer associations.

1.4.2) Bienzyme system

In addition to the reaction in scheme (1.1), we consider another reaction,

$$X_2 \xrightarrow{E_2} X_3 \qquad (1.6)$$

The rate equation for this enzyme-catalized reaction is given by:

$$v_2 = E_2 \; g(x_2, x_3, ks) \qquad (1.7)$$

where x_2 and x_3 are the free substrate and product concentrations, E_2 the total enzyme concentration and ks rate constants. The product of reaction scheme (1.1) is the substrate of reaction scheme (1.6). Thus, we can study both reactions combined in a multienzyme system. The overall scheme is:

$$X_1 \xrightarrow{E_1 \,/\, X_m} S_2 \xrightarrow{E_2} X_3 \qquad (1.8)$$

X_1' ⟶ X_2'

where the symbol X_2 has been substituted by S_2 to show that the concentration of this substance is now a variable. The concentration of

S_2, is not under direct control of the experimenter, but may be changed only indirectly by modulation of the parameters. We shall assume that for the values of the parameters considered the concentration of S_2 reaches a steady-state value s_2. This value depends in principle on all the parameters, i.e.

$$s_2 = s_2 (x_1, x_1', x_2', x_3, x_m, E_1, E_2, ks) \qquad (1.9)$$

At this state, as a consequence of mass conservation, the rate of transformation from X_1 to S_2, equals the rate from S_2 to X_3. This overall rate is called the flux, J , and may be calculated by substituting s_2 into any of the two rate equations (eqs(1.4) and (1.7)).

$$J = E_1 \; f (x_1, x_1', s_2, x_2', x_m, ks) \qquad (1.10a)$$

$$J = E_2 \; g (s_2, x_3, ks) \qquad (1.10b)$$

A coefficient may be defined to study the sensitivity of the steady-state concentration and flux to the change in a parameter, for example E_1, when all the other parameters are not altered.

$$C_{E_1}^{s2} = \frac{E_1}{s_2} \frac{\partial s_2}{\partial E_1} \qquad (1.11a)$$

$$C_{E_1}^{J} = \frac{E_1}{J} \frac{\partial J}{\partial E_1} \qquad (1.11b)$$

They represent the relative change in the variable per unit relative change in the parameter. The substrate control coefficient (eq(1.11a)) is characteristic of multienzyme systems, because when we are dealing with a single enzyme-catalized reaction all the concentrations are parameters (Section 1.4.1). On the other hand, the flux control

coefficient (eq(1.11b)) has a similar formal appearance to the π-elasticity coefficient (eq(1.5)). There is, however, an important difference between them. While the rate (v_1) is only 'immediately' affected by the change in E_1 (eq(1.4)), the flux (J) is both affected by the immediate change in E_1 and by the 'cumulative' effect due to the change in the variable concentration s_2 (eq(1.10a)). This is the reason why, although the individual rates are proportional to the corresponding enzyme concentrations, the flux is a non-linear function of them. As a consequence, even though $\pi^{v1}_{E1} = 1$, C^{J}_{E1} is usually different from one.

The control and elasticity coefficients fulfil some relationships. For example, differentiating eq(1.10a) with respect to E_1 (taking into account that s_2 depends on E_1 too, see eq(1.9)) and scaling by the appropriate factors we obtain:

$$C^{J}_{E_1} = \pi^{v1}_{E_1} + \varepsilon^{v1}_{s2}\, C^{s2}_{E_1} \qquad (1.12a)$$

ε^{v1}_{s2} is defined as π^{v1}_{x1} in eq(1.3), i.e. $\varepsilon^{v1}_{s2} = (s_2 / v_1)(\partial v_1 / \partial s_2)$. The change in notation from π to ε is to emphasize that while X_1 is a parameter of the system, s_2 is a variable. The usefulness of this notation will become apparent in Chapter 3. Eq(1.12a) expresses the flux control coefficient in terms of the π and ε-elasticity coefficients and the substrate control coefficient. The effect of E_1 on J is due to two contributions, one associated to each term in the right hand side of equality (1.12a). The first term represents the change in v_1 before the metabolite concentration s_2 has changed, i.e. the immediate change. The second term represents the contribution to the change in flux caused by the change in s_2, i.e. the cumulative change. This contribution is, in turn, expressed as a product of two factors: i) the effect that the change in E_1 has on s_2, and ii) the effect that a

change in s_2 has on J. ε^{v1}_{s2} is a product elasticity coefficient and usually negative, while E_1 is an enzyme upstream s_2 and, hence, C^{s2}_{E1} usually positive. The product of these two factors has a negative contribution to the increase in flux. This means that the substrate's movement tends to compensate or buffer the immediate effect of the enzyme concentration change. As a consequence, the flux control coefficient, although it is positive, it is smaller than one due to the buffering effect (note that $\pi^{v1}_{E1} = 1$, eq(1.5)). Similar conclusions may be obtained from the analysis of eq(1.12b), that describes the effect of E_2 on the flux.

$$C^{J}_{E_2} = \pi^{v2}_{E_2} + \varepsilon^{v2}_{s2}\, C^{s2}_{E_2} \qquad (1.12b)$$

This equation is obtained by differentiation of eq(1.10b) with respect to E_2 and appropriate scaling. Eqs(1.12a) and (1.12b) are the direct consequence of applying the chain rule of partial differentiation. By more elaborate procedures other relationships may be obtained. Summation relationships involve control coefficients only. The flux summation theorem (Kacser and Burns, 1973) corresponding to scheme (1.8) is:

$$C^{J}_{E_1} + C^{J}_{E_2} = 1 \qquad (1.13)$$

and the concentration summation theorem (Heinrich and Rapoport, 1974) is:

$$C^{s2}_{E_1} + C^{s2}_{E_2} = 0 \qquad (1.14)$$

From the analysis of eq(1.12a) we concluded that the change in one

enzyme does not result in an equal relative change in the flux; the flux control coefficient with respect to E_1 is smaller than one. C^{J}_{E2} is also smaller than one. According to eq(1.13), however, if we simultaneously change both enzymes by the same relative amount (or if we add the contributions to the change in flux caused by equal and independent relative changes in both enzymes) the flux changes by the same relative amount. Moreover, eq(1.14) shows that in this situation the concentration of s_2 does not change.

The concentration connectivity theorem (Westerhoff and Chen, 1984) can be obtained adding member by member eqs(1.12a) and (1.12b) and substracting eq(1.13). The result is:

$$\varepsilon^{v1}_{s2}\ C^{s2}_{E1} + \varepsilon^{v2}_{s2}\ C^{s2}_{E2} = -1 \qquad (1.15)$$

The terms on the left hand side of eq(1.15) are the ones appearing in eq(1.12a) and (1.12b) respectively. As was discussed above, they represent the negative contribution to the change in flux due to the movement of s_2 when the enzyme is modulated. Eq(1.15) shows that the sum of the buffering terms corresponding to the enzymes flanking the substrate equals -1. The flux connectivity theorem may be obtained as follows. Differentiating eqs(1.10b) and (1.10a) with respect to E_1 and E_2 respectively we first obtain:

$$C^{J}_{E1} = \varepsilon^{v2}_{s2}\ C^{s2}_{E1} \qquad (1.16a)$$

$$C^{J}_{E2} = \varepsilon^{v1}_{s2}\ C^{s2}_{E2} \qquad (1.16b)$$

If we multiply eqs(1.16a) and (1.16b) by ε^{v1}_{s2} and ε^{v2}_{s2} respectively, and add member by member the resulting equations, taking into account eq(1.14) we finally obtain:

$$C^{J}_{E_1} \varepsilon^{v_1}_{s_2} + C^{J}_{E_2} \varepsilon^{v_2}_{s_2} = 0 \qquad (1.17a)$$

This is the flux connectivity theorem (Kacser and Burns, 1973). Eq(1.17a) may be rearranged giving:

$$\frac{C^{J}_{E_1}}{C^{J}_{E_2}} = \frac{\varepsilon^{v_2}_{s_2}}{-\varepsilon^{v_1}_{s_2}} \qquad (1.17b)$$

This equation shows that the relative values of the enzyme control coefficients of adyacent reactions can be expressed as the ratio of the elasticity coefficients of those reactions with respect to the common metabolite. It is an interesting property, and quite unexpected, that the ratio of systemic changes (i.e. control coefficients) can be expressed as the simple ratio of the changes in isolation (i.e. elasticity coefficients). It must, however, be recognized that, although the elasticity coefficients represent changes when the reaction is isolated, their values depend on the steady state values of the metabolite concentrations and these, in turn on all the parameters of the system. In this sense, the elasticity coefficients are systemic properties too.

The control coefficients can be expressed in terms of the elasticity coefficients. This may be achieved by operating on eqs(1.12a), (1.12b), (1.16a) and (1.16b). A similar procedure was introduced by Kacser (1983). The results are:

$$C^{s_2}_{E_1} = \frac{1}{\varepsilon^{v_2}_{s_2} - \varepsilon^{v_1}_{s_2}} \qquad (1.18a)$$

$$C^{s_2}_{E_2} = \frac{-1}{\varepsilon^{v_2}_{s_2} - \varepsilon^{v_1}_{s_2}} \qquad (1.18b)$$

$$C^{J}_{E_1} = \frac{\varepsilon^{v_2}_{s_2}}{\varepsilon^{v_2}_{s_2} - \varepsilon^{v_1}_{s_2}} \qquad (1.18c)$$

$$C^{J}_{E_2} = \frac{-\varepsilon^{v_1}_{s_2}}{\varepsilon^{v_2}_{s_2} - \varepsilon^{v_1}_{s_2}} \qquad (1.18d)$$

These equations show how the response of the system to changes in the enzyme concentration can be expressed in terms of the properties of the enzymes studied in isolation. The analysis of these equations may give insight concerning the origin of the values of the control coefficients. Let us assume the usual situation where $\varepsilon^{v_1}_{s_2}$ (product elasticity coefficient) is negative and $\varepsilon^{v_2}_{s_2}$ (substrate elasticity coefficient) is positive. In this case, according to eqs(1.18), $C^{J}_{E_1}$, $C^{J}_{E_2}$ and $C^{s_2}_{E_1}$ are positive while $C^{s_2}_{E_2}$ is negative. As described by eq(1.12a) an increase in E_1 produces an identical immediate relative change in v_1, before s_2 and v_2 move. Subsequently s_2 increases. As a consequence, v_2 increases while v_1 decreases (from the value achieved after the immediate change) until v_1 and v_2 are equal to each other and to the final value of the flux. If $-\varepsilon^{v_1}_{s_2}$ and $\varepsilon^{v_2}_{s_2}$ are large then small changes in s_2 produce relatively large changes in the rates. Therefore, the change in s_2 necessary to balance the rates in order to reach the new steady state is small. This is shown in eqs(1.18a) and (1.18b). The minus sign in eq(1.18b) indicates that an increase in E_2 decreases the value of s_2 , although the absolute values of the control coefficients are the same. The sum of the flux control coefficients (eqs(1.18c) and (1.18d)). is equal to one. If the elasticity coefficients, $\varepsilon^{v_1}_{s_2}$ and $\varepsilon^{v_2}_{s_2}$, are equal in absolute value the change in s_2

to attain the new steady state will produce an increase in v_2 equal to the decrease in v_1. Therefore, the final flux will be half way between the initial flux and the value of v_1 immediately after the change in enzyme concentration. In this case, the relative change in the flux due to a unit relative change in the concentration of any one of the enzymes is 0.5 as stated by eqs(1.18c) and (1.18d). But, on the other hand, if the increase in s_2 due to an increase in E_1 has a greater effect on, for example, rate v_2, i.e. $\varepsilon^{v2}_{s2} > -\varepsilon^{v1}_{s2}$, then the final value of the flux will be closer to the value of v_1 immediately after the increase in E_1 than to the initial flux. Following a similar reasoning, it may be shown that when changing E_2 the final value of the flux is closer to the initial flux. Consequently, $C^J_{E1} > C^J_{E2}$. This inequality can also be deduced from eqs(1.18c) and (1.18d). Eqs(1.18) show that the control coefficients can be calculated from the values of the ε-elasticity coefficients. The ε-elasticity coefficients can be obtained, by at least three different procedures: i) direct modulation of the concentrations in the isolated enzyme-catalized reaction, ii) double modulations in the system (Kacser, 1983) and iii) calculation of the normalized partial derivatives of the rate laws evaluated with the steady state concentration values. The latter procedure involves the knowledge of the rate laws and the values of the kinetic constants obtained in the *in vivo* conditions. This detailed knowledge, related to the main aims of enzyme kineticists, is not necessary to MCA. The only properties relevant to the values of the control coefficients are the values of the elasticity coefficients. On the other hand, if the aim is to analyse in what conditions the elasticity coefficients may be small or large, then the use of the rate laws is essential.

So far, we analysed the effect that changes in the enzyme concentrations have in the variables. The theorems described are only valid when the rates are proportional to the corresponding enzyme concentrations (i.e. $\pi^{vj}_{Ej} = 1$ for all E_j). If we consider parameters

that do not affect proportionally the rate, e.g. the substrate concentration x_1, two other relationships hold:

$$C^{s2}_{x_1} = C^{s2}_{E_1}\ \pi^{v1}_{x_1} \qquad (1.19a)$$

$$C^{J}_{x_1} = C^{J}_{E_1}\ \pi^{v1}_{x_1} \qquad (1.19b)$$

This was originally called the partitioned response (Kacser and Burns, 1973). Eqs(1.19a) and (1.19b) show that the response of the variable to a small change in the parameter (x_1) can be expressed as the product two factors: i) π^{v1}_{x1} represents the effect that a change in x_1 has on the isolated rate (scheme (1.1)), and ii) C^{s2}_{E1} or C^{J}_{E1} represent how the change in the enzyme concentration (parameter fulfilling proportionality) affects the systems' variables. It may be immediately shown that if in eqs(1.19) x_1 is replaced by E_1 the value of the elasticity coefficient is one resulting in trivial identities. The response equations reveal an important property. The effect of an external effector on an isolated rate, even though large, can only produce a substantial effect in a metabolic variable if the variable is reasonably sensitive to changes in the concentration of the enzyme affected by the external effector.

1.4.3) Multienzyme system

The theorems exemplified for the bienzyme system represented in scheme (1.8) can be extended to systems of any number of reactions. In what follows the more general versions of the theorems and some of their consequences are given. Parts of proofs are also introduced to gain understanding on their operational meaning. A system with m variable metabolite concentrations and n reactions is described by a set of m differential equations:

$$\frac{d\, s_i}{d\, t} = \sum_{j=1}^{n} n_{ij}\, v_j \qquad i = 1, \ldots, m \qquad (1.20)$$

where v_j are the rates and n_{ij} the stoichiometric coefficients. At the steady state $d\, s_i / d\, t = 0$ ($i = 1, \ldots, m$).

$$\sum_{j=1}^{n} n_{ij}\, v_j = 0 \qquad i = 1, \ldots, m \qquad (1.21)$$

The solution of eqs(1.21) are the steady state concentrations of metabolites: S_i^{ss} ($i = 1, \ldots, m$). In what follows the superscript ss stands for steady state. As was mentioned above, we assume that the rates are proportional to the corresponding enzyme concentration, i.e. $v_j \propto E_j$. Consequently, the change in any enzyme concentration E_j by a factor α ($E_{j,\alpha} = \alpha\, E_j$) produces a change in the rate v_j by the same factor:

$$v_{j,\alpha} = \alpha\, v_j \qquad (1.22)$$

where $v_{j,\alpha}$ is the final value of the rate. If we simultaneously change all the enzyme concentrations by the same factor α the new set of equations are:

$$\sum_{j=1}^{n} n_{ij}\, v_{j,\alpha} = 0 \qquad i = 1, \ldots, m \qquad (1.23)$$

Introducing eq(1.22) into eqs(1.23) we obtain:

$$0 = \sum_{j=1}^{n} n_{ij}\, v_{j,\alpha} = \sum_{j=1}^{n} n_{ij}\, \alpha\, v_j = \alpha \sum_{j=1}^{n} n_{ij}\, v_j \qquad (i = 1, \ldots, m) \qquad (1.24)$$

The sum in the last member of eq(1.24), i.e. $\sum n_{ij}\, vj$, must be equal to zero because α is different from zero. This is the same set of equations as for the initial state, given in eqs(1.21). We can, therefore, conclude that the change of all the enzyme concentrations by the same factor α does not affect the steady state values of the metabolite concentrations. In contrast, all the steady state fluxes (J_j^{ss}) are modified by the factor α (eq(1.22)). In theory, these conclusions are valid irrespective of the values of α . When small changes ($\alpha \approx 1$) are considered, they may be used to derive the flux and concentration summation theorems (Kacser and Burns, 1973 and Heinrich and Rapoport, 1974) given respectively by:

$$\sum_k C^{J_j^{ss}}_{E_k} = 1 \qquad (1.25)$$

$$\sum_k C^{S_i^{ss}}_{E_k} = 0 \qquad (1.26)$$

Some of the implications of the flux summation theorem, when a linear chain of reactions (i.e. unbranched pathway) is considered, were already described in a previous section, and are not repeated here. In the case of a branched pathway some flux control coefficients are negative, when the enzyme modulated is in a different branch from the flux measured. Whatever the signs and magnitudes of the control coefficients are, if any one control coefficient is changed by some means, other or others must change to fulfil eqs(1.25) or (1.26).

The changes in all the enzyme concentrations by the same factor produce, as was mentioned above, a change in the flux by that factor without alteration of the metabolite concentrations. Conversely, the system may be subject to another type of modulation of the enzyme concentrations that changes one or more metabolite concentrations without modification of the flux. Let us assume that a metabolite S_i is

produced at a rate given by the rate law $v_j = E_j\ f(S_i, \ldots)$ and is decomposed by another rate $v_{j+1} = E_{j+1}\ g(S_i, \ldots)$. If S_i changes by an amount δS_i then v_j and v_{j+1} will also change. As the rates are proportional to the corresponding enzyme concentrations, it is always possible to change these concentrations in such a way to compensate the change in the rate due to the change in S_i, i.e.

$$E_j\ f(S_i, \ldots) = (E_j + \delta E_j)\ f(S_i + \delta S_i, \ldots) \qquad (1.27a)$$

$$E_{j+1}\ g(S_i, \ldots) = (E_{j+1} + \delta E_{j+1})\ g(S_i + \delta S_i, \ldots) \qquad (1.27b)$$

In this situation, the change in S_i does not produce a change in the rates and, therefore, the rest of the system remains unaltered. From these considerations, the flux and concentration connectivity theorems (Kacser and Burns, 1973 and Westerhoff and Chen, 1984) may be derived:

$$\sum_k C^{J_j^{ss}}_{E_k}\ \varepsilon^{v_k}_{S_l^{ss}} = 0 \qquad (1.28)$$

$$\sum_k C^{S_i^{ss}}_{E_k}\ \varepsilon^{v_k}_{S_l^{ss}} = -\delta_{il} \qquad (1.29)$$

δ_{il} is the Kronecker δ; it equals one if $i=l$ and zero if $i \neq l$.

1.4.4) Transition time

So far, the control of steady state metabolite concentrations and fluxes has been considered. Now we consider another variable, the transition time τ (Easterby 1981, 1984 and 1986). This quantity is a measure of the time required for a linear chain of reactions to relax to the steady state when starting with an empty system. It has the

peculiarity that can be defined from the values of the steady state flux (J^{ss}) and metabolite concentrations (S_i^{ss}) only.

$$\tau = \frac{\sum S_i^{ss}}{J^{ss}} \qquad (1.30)$$

As a consequence, the transition time control coefficient, can be expressed in terms of the flux and concentration control coefficients (Melendez-Hevia et al. 1990),

$$C_p^{\tau} = C_p^{\sum S_i^{ss}} - C_p^{J^{ss}} \qquad (1.31)$$

where,

$$C_p^{\sum S_i^{ss}} = \frac{1}{\sum S_i^{ss}} \sum S_i^{ss} C_p^{S_i^{ss}} \qquad (1.32)$$

Using eqs(1.30) to (1.32) in conjunction with eqs(1.25), (1.26), (1.28) and (1.29) summation and connectivity theorems for the transition time were derived.

$$\sum_k C_{E_k}^{\tau} = -1 \qquad (1.33)$$

$$\sum_k C_{E_k}^{\tau} \varepsilon_{S_i^{ss}}^{v_k} = \frac{-S_i^{ss}}{\sum S_i^{ss}} \qquad (1.34)$$

The C_{Ek}^{τ} can be expressed in terms of the elasticity coefficients and metabolite concentrations using either eqs(1.31), (1.32) and the ones relating C_{Ek}^{J} and $C_{Ek}^{S_i}$ to the elasticity coefficients or directly from eqs(1.33) and (1.34). A similar summation as eq(1.33) was obtained by Heinrich and Rapoport (1975) using a somewhat different definition of transition time.

Chapter 2

Control Analysis of Time-Dependent Metabolic Variables *

2.1) Introduction

The main purpose of this chapter is to extend Metabolic Control Analysis to systems whose variables are functions of time. First, a description of the metabolic system is given. It is assumed that the time derivative of the metabolite concentrations can be written as a linear combination of rate laws, each one propotional to the corresponding total enzyme concentration. Under these conditions the consequences of changing all enzyme concentrations by the same arbitrary factor are studied. When infinitesimal changes are considered, these arguments are used in the derivation of concentration and flux summation relationships. The control coefficients involved in these relationships are an extension of the traditionally used in steady state MCA (see section (1.4)), and a new type of coefficient (time coefficient, T) has to be defined. Next, we compare two situations were all the rates are identical, differing only in the concentration of one metabolite. The conclusions obtained are used to show how to construct connectivity relationships. Up to this point all the derivations are made using modulation equations. A mathematical proof for the summation and connectivity relationships, in matrix form, is also given. Finally we illustrate, by an example, how the control coefficients can be expressed in terms of the elasticity coefficients.

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* see footnote on page 79

In this paper we lay the conceptual and mathematical foundations for the control treatment of metabolic systems that exhibit time-dependent phenomena of biological relevance. While the usual approach to such questions involves the simulation of particular systems with assumed parameters (which are rarely known *in vivo*), our treatment enables us to make some general statements which are independent of detailed mechanistic considerations. Although the restriction of a steady state analysis is overcome in the present chapter, the assumption of proportionality between rate and enzyme concentration is maintained. The breakdown of this assumption is considered in next chapter.

2.2) The system

We consider a metabolic system ($\mathcal{S}$) whose dynamics is described by a system of differential equations:

$$\frac{d\,\mathbf{s}}{d\,t} = \mathbf{N}\,\mathbf{v} \qquad (2.1)$$

where $\mathbf{s}$ is the column vector of concentrations of metabolites S_i , t the independent variable time, $\mathbf{v}$ the column vector of the rates v_j , and $\mathbf{N}$ the stoichiometry matrix. The element n_{ij} of this matrix is the stoichiomtry coefficient of the metabolite S_i in the reaction j, and is positive, negative or zero if S_i is product, substrate or is not transformed in the reaction respectively. In $\mathbf{v}$ we assume that each individual rate law is of the form:

$$v_j = E_j\ f_j (\mathbf{s} \,.\, k_j) \qquad (2.2)$$

Here E_j is the total enzyme concentration of step j , and the function f_j depends on the concentrations of some intermediates of the pathway and of the parameters k_j . f_j is independent of enzyme concentrations and time. k_j includes kinetic constants, external effectors or other parameters related to the step j . We also assume that the stoichiometry matrix is constant.

The solution of equation (2.1) is

$$\mathbf{s} = \mathbf{s}\,(\,\mathbf{k}\,,\,\mathbf{s_0}\,,\,t_0\,,\,t\,) \qquad (\,2.3\,)$$

($\mathbf{s_0}$, t_0) is the vector of initial conditions of metabolite concentrations and time, and $\mathbf{k}$ is the vector of parameters of the system. Without loss of generality we assume $t_0 = 0$. Combining equations (2.2) and (2.3) we obtain the flux for each step , J_j , as a function of time:

$$J_j = E_j\ f_j(\ \mathbf{s}\ (\ \mathbf{k}\ ,\mathbf{s_0}\ ,\ 0\ ,\ t\ )\ ,\ k_j\ ) \qquad (\,2.4\,)$$

We use the symbol v_j in equation (2.2) and J_j in equation (2.4). Although both quantities have the same value when evaluated in the system, in v_j we consider the metabolite concentrations as parameters (isolated reaction), while in J_j they are affected by change of enzyme concentrations and all the other parameters. A further distinction will be made in section (2.4).

2.3) The change in time scale

We compare the system described previously ($\mathcal{S}$) with another metabolic system ($\mathcal{S}_\alpha$). The only difference between them is that all

the enzyme concentrations of $\mathcal{O}_\alpha$ ($E_{j,\alpha}$) are obtained by multiplying those of $\mathcal{O}$ by the same arbitrary (not necessarily small) constant α .

$$E_{j,\alpha} = \alpha E_j \qquad (2.5)$$

The dynamics of the new metabolic situation is described by a new system of differential equations:

$$\frac{d\, s_\alpha}{d\, t_\alpha} = N\, v_\alpha \qquad (2.6)$$

s_α and v_α are the vectors of metabolite concentrations and rates respectively. Even though t_α is measured by the same time scale as t , it is useful to mark the time in the system $\mathcal{O}_\alpha$ by a different symbol. The solution of equation (2.6) can, in principle, be written with the same notation of equation (2.3) as follows:

$$s_\alpha = s_\alpha(k_\alpha , s_0 , 0 , t_\alpha) \qquad (2.7)$$

where we take the same initial condition as in $\mathcal{O}$ (s_0 , $t_0 = 0$).

From equations (2.2) and (2.5) we immediatly obtain: $v_\alpha = \alpha\, v$. Substituting this relation into equation (2.6) , the equation takes the form:

$$\frac{d\, s_\alpha}{d(\alpha\, t_\alpha)} = N\, v \qquad (2.8)$$

where $\alpha\, t_\alpha$ is a new time scale (for a similar concept see Selwyn, 1965). The right members of equations (2.1) and (2.8) are identical. Then, the only effect of changing all enzyme concentrations by a factor α , is to affect the time scale , without qualitative modification of the dynamics of the metabolic system. Taking into account these

considerations we can write the solution $\mathbf{s}_\alpha$ in terms of the solution $\mathbf{s}$ given by equations (2.7) and (2.3) respectively.

$$\mathbf{s}_\alpha = \mathbf{s}\,(\,\mathbf{k}\;,\,\mathbf{s}_0\;,\;0\;,\;\alpha\,t_\alpha\,) \qquad (\,2.9\,)$$

Finally , comparing equations (2.3) and (2.9) we obtain the relation between t_α and t so that the solutions of equations (2.1) and (2.6) have the same value. That is if

$$t_\alpha = t\,/\,\alpha \qquad (\,2.10\,)$$

then

$$\mathbf{s}_\alpha\,(\,t\,/\,\alpha\,) = \mathbf{s}\,(\,t\,) \qquad (\,2.11\,)$$

In other words, if for a time t , the system $\mathcal{S}$ has a particular set of values of metabolite concentrations, then the system $\mathcal{S}_\alpha$ exhibit the same values of concentrations at the time t/α. This statement is true for any point of the temporal evolution of the system.

The flux through reaction j for $\mathcal{S}_\alpha$ is

$$J_{j,\alpha} = E_{j,\alpha}\,f_j\,(\,\mathbf{s}_\alpha(\,\mathbf{k}_\alpha\,,\,\mathbf{s}_0\,,\;0\;,\;t_\alpha\,)\;,\;k_j\,) \qquad (\,2.12\,)$$

Combining equations (2.5), (2.10), (2.11) and (2.12) we obtain:

$$J_{j,\alpha}\,(\,t\,/\,\alpha\,) = \alpha\;\;J_j\,(\,t\,) \qquad (\,2.13\,)$$

Equation (2.13) tell us that, if for any time t, the fluxes in $\mathcal{S}$ present

certain values, then $\mathcal{S}_\alpha$ has values of fluxes that are α times those of $\mathcal{S}$ at a time t/α (where all metabolite concentrations have the same values as in $\mathcal{S}$).

If α is greater (smaller) than one, the system $\mathcal{S}_\alpha$ evolves α times faster (slower) , that is:

$$\left(\frac{d\,\mathbf{s}}{d\,t} \right)_\alpha = \alpha \frac{d\,\mathbf{s}}{d\,t} \qquad (2.14)$$

Then, as is stated by Equation (2.11), to obtain the same values of metabolite concentrations in both systems we have to look in $\mathcal{S}_\alpha$ at a smaller (greater) time t/α. But, at that smaller (greater) time , as $\mathcal{S}_\alpha$ evolves α times faster (slower), the fluxes are α times greater (smaller) than in the reference system $\mathcal{S}$, as appears in equation (2.12).

If $\mathcal{S}$ and $\mathcal{S}_\alpha$ are at a stable steady state, the variables (metabolite concentrations, fluxes, etc) have constant values in time. In this case equations (2.11) and (2.13) can be written: $\mathbf{s}_\alpha = \mathbf{s}$ and $J_{j,\alpha} = \alpha\, J_j$ respectively. In other words, in a stable steady state situation, for any time, both systems have the same values of metabolite concentrations, and all the fluxes in $\mathcal{S}_\alpha$ are α times the corresponding ones in $\mathcal{S}$.

These results are related to the conclusions derived from the Summation relationships (section 2.5).

2.4) Control, elasticity and time coefficients

In the last section we considered the consequences of a simultaneous change in all enzyme concentrations by a factor α . This factor can be, in principle, any real number and the enzyme

concentrations in both metabolic situations ($\mathcal{O}$ and $\mathcal{O}_{\alpha}$) can differ by a large finite amount. From now on, however, we are going to deal with infinitesimal differences in enzyme concentrations, as is usual in control analysis. The first question we are going to pose is: How does an infinitesimal relative change in a particular enzyme concentration (at the initial conditions) affect the value of one variable Y (metabolite concentration or flux) at any time ? The quantitative answer to this question is given by the value of the control coefficient of the variable, at that time, with respect to the enzyme concentration. We define two types of control coefficient for time dependent metabolic systems. The " unscaled control coefficients "(${}^{u}C^{Y}_{E_k}$) tell us what is the absolute change in the value of the variable per relative change in one enzyme concentration. And what we simply call " control coefficients " ($C^{Y}_{E_k}$) account for the relative change in the variable per relative change in enzyme concentration. For the variable Y the unscaled control coefficient with respect to enzyme concentration E_k can be mathematically defined ($\equiv$) as follows:

$$ {}^{u}C^{Y}_{E_k} \equiv E_k \left(\frac{\partial Y}{\partial E_k} \right)_{E_j, t \quad (j \neq k)} \qquad (2.15) $$

The subscripts under the parenthesis indicate that while changing E_k , all the other enzyme concentrations (parameters in general) and time are held constant. The control coefficients (scaled) can be obtained from the unscaled control coefficients dividing by the variable:

$$ C^{Y}_{E_k} \equiv \frac{E_k}{Y} \left(\frac{\partial Y}{\partial E_k} \right)_{E_j, t \quad (j \neq k)} \qquad (2.16) $$

These, apart from their time dependence, are the ones used in classical control analysis at steady state (Burns et al, 1985). It is important to note that the coefficients defined by equations (2.15) and (2.16)

depend on the properties of the whole metabolic system.

Next, we introduce another type of coefficient: the elasticity coefficients. These tell us what the relative change of a local (isolated) rate is, given by equation (2.2), when the concentration of one of the metabolites which explicitly appears in that rate equation changes by an infinitesimal amount, holding all the other variables constant. In this case we define unscaled and scaled elasticity coefficients with respect to the metabolite concentration. The " unscaled elasticity coefficients " are defined in mathematical terms as follows:

$$u\varepsilon_{S_i}^{v_k} \equiv \frac{1}{v_k}\left(\frac{\partial v_k}{\partial S_i}\right)_{S_j} \quad (j \neq i) \qquad (2.17)$$

The scaled elasticity coefficients, that from now on we simply call " elasticity coefficients ", are obtained from the unscaled ones by multiplying by the corresponding metabolite concentration:

$$\varepsilon_{S_i}^{v_k} \equiv \frac{S_i}{v_k}\left(\frac{\partial v_k}{\partial S_i}\right)_{S_j} \quad (j \neq i) \qquad (2.18)$$

These, apart from their time dependence, will be recognized as identical to the elasticity coefficients used in steady state analysis. From equations (2.17) and (2.18) it is clear that the elasticity coefficients can be defined only if the rates are non zero. The mathematical function that gives the elasticity coefficients in terms of the metabolite concentrations can be obtained by making the partial derivative of v_k (given by equation (2.2)) with respect to S_i and then scaling with the appropriate factor. The elasticity coefficients are defined for isolated rates, and in that sense they represent local variations in the metabolic system. But, the actual values of the

elasticity coefficients depend on the values of the metabolite concentrations that are functions of time. Through these concentrations, the elasticity coefficients are affected by the properties of the whole system.

The definitions of control and elasticity coefficients given in equations (2.16) and (2.18), respectively, constitute an extension of the steady state coefficients (Burns et al., 1985). In general, the time-dependent control and elasticity coefficients have different values for different times; but if the metabolic system is one that approaches a stable steady state, then in the limit, they are identical to the coefficients defined for steady state.

Finally, we define a new type of coefficient: the "time coefficients". They tell us how a relative infinitesimal change in the time of observation of the metabolic system affects the value of the variable (metabolite concentration or flux) under consideration, when all the parameters (enzyme concentrations, etc) are held constant. As before, we define unscaled and scaled time coefficients with respect to the variable. Their mathematical definition is given, respectively, by:

$$ {}^{u}_{T}{}^{Y} \equiv t \left(\frac{\partial Y}{\partial t} \right)_{E_k} \qquad (2.19) $$

and

$$ {}_{T}{}^{Y} \equiv \frac{t}{Y} \left(\frac{\partial Y}{\partial t} \right)_{E_k} \qquad (2.20) $$

These coefficients are directly related to the time evolution properties of the variable. It is clear from equations (2.19) and (2.20), that if the system is in a stable steady state, when all the variables have

constant values, their time coefficients are zero. This is the reason why they do not appear explicitly in control analysis of steady state systems. It has to be pointed that if we do not choose the initial time equal to zero (see section (2.2)), the definitions given in equations (2.19) and (2.20) have to be modified, substituting the factor t by ($t - t_0$).

We have introduced the unscaled coefficients, which are not used in steady state analysis. These are required when the connectivity relationships are considered. Furthermore, unscaled control and time coefficients, can be used when, in the time evolution of the system, the variable at a particular time is zero.

2.5) Summation relationships

Here, using some results of section (2.3), we derive summation relationships for time-dependent metabolic systems. We symbolize by Y any variable of the system $\mathcal{S}$. This variable depends, in general, on the parameters, initial conditions and time. In this section we consider Y as a function of all enzyme concentrations and time only.

$$Y = Y (E_1 , ... , E_n , t) \qquad (2.21)$$

We compare the system $\mathcal{S}$ with another metabolic system $\mathcal{S}_\beta$. They differ in the values of all enzymes concentrations by infinitesimal amounts . The relative difference between the enzyme concentrations is a constant β (the same for all enzymes) . That is:

$$E_{k,\beta} = E_k + d E_k \qquad (k = 1 , ... , n) \qquad (2.22)$$

where

$$d E_k = \beta E_k \qquad (k = 1, \ldots\ldots, n) \qquad (2.23)$$

In comparing the systems $\mathcal{S}$ and $\mathcal{S}_\beta$ we will take a different time in each one, t and t_β respectively, their inverse being different by an infinitesimal amount d(1/t).

$$1 / t_\beta = 1 / t + d(1/t) \qquad (2.24)$$

We choose the relative differences between the inverses of the times of observation, t and t_β, for each time, such that they are a constant equal to β: $d(1/t) = \beta (1/t)$. Taking into account that $d(1/t) = -(dt)/t^2$, this leads to:

$$dt = -\beta t \qquad (2.25)$$

From equations (2.22) and (2.23) it follows that $E_{k,\beta} = (1 + \beta) E_k$. Similarly, from equations (2.24) and (2.25) we obtain: $t_\beta = t / (1 + \beta)$. Then equations (2.23) and (2.25) are equivalent conditions to equations (2.5) and (2.10) respectively when infinitesimal differences are considered (with $\alpha = 1 + \beta$). From equation (2.11), we know that any metabolite concentration S_i has the same value in $\mathcal{S}$ and $\mathcal{S}_\beta$. Similarly if we consider equation (2.13), all the fluxes J_j differ in the same relative amount β . Then if equations (2.23) and (2.25) are fulfiled, the differences between the metabolite concentrations dS_i, and the differences between fluxes $d J_j$, are:

$$dS_i = 0 \qquad (2.26)$$

and

$$d J_j = \beta J_j \qquad (2.27)$$

The infinitesimal difference in any variable Y (see equation (2.21)), between ϕ and ϕ_β, is given in terms of the differences in enzyme concentrations and time by the following equation:

$$d Y = \sum \frac{\partial Y}{\partial E_k} d E_k + \frac{\partial Y}{\partial t} d t \qquad (2.28)$$

We can introduce equations (2.23) and (2.25) into equation (2.28) obtaining:

$$d Y = \beta \left(\sum_k E_k \frac{\partial Y}{\partial E_k} - t \frac{\partial Y}{\partial t} \right) \qquad (2.29)$$

In the right member of equation (2.29) appear the unscaled control and time coefficients defined by equations (2.15) and (2.19) respectively, and therefore equation (2.29) can be written as follows:

$$d Y = \beta \left(\sum_k {}^{u}C^{Y}_{E_k} - {}^{u}T^{Y} \right) \qquad (2.30)$$

This equation can be written in terms of the scaled control and time coefficients defined in equations (2.16) and (2.20), dividing both members by Y:

$$d Y / Y = \beta \left(\sum_k C^{Y}_{E_k} - T^{Y} \right) \qquad (2.31)$$

If Y is the metabolite concentration S_i , we combine equation (2.26) with equation (2.30) and (2.31), and as β is a non-zero constant, we obtain:

$$\sum_k {}^{u}C^{S_i}_{E_k} = {}^{u}T^{S_i} \qquad (2.32)$$

and

$$\sum_k C^{S_i}_{E_k} = T^{S_i} \qquad (2.33)$$

We call equation (2.32) the " unscaled summation relationship " for metabolite concentration coefficients. The " summation relationship " (scaled), obtained from the unscaled one, dividing by the metabolite concentration, appears in equation (2.33)

Now we consider the case when Y is the flux through reaction j , J_j. Combining equation (2.27) with equations (2.30) and (2.31) we obtain, respectively,

$$\sum_k {}^{u}C^{J_j}_{E_k} = J_j + {}^{u}T^{J_j} \qquad (2.34)$$

and

$$\sum_k C^{J_j}_{E_k} = 1 + T^{J_j} \qquad (2.35)$$

Equations (2.34) and (2.35) are the unscaled and scaled summation relationships for flux coefficients.

All the summation relationships, obtained in this section, are independent of the value of the infinitesimal constant β . They are

links between the control and time coefficients, and impose constraints on the values these coefficients can attain.

If $\mathcal{O}$ and $\mathcal{O}_\beta$ represent stable steady state situations, as the metabolite concentrations and fluxes are constant in time, all the time coefficients are zero. In this case equations (2.33) and (2.35) take the form of the well known summation theorems of steady state control analysis (Kacser & Burns, 1973; Heinrich & Rapoport, 1974).

2.6) The invariance of rates

In this section, we compare the original system $\mathcal{O}$, with another metabolic system $\mathcal{O}_\gamma$. They differ in the concentration of one metabolite S_i, and in the values of some parameters and time. S_i appears explicitly in some of the rate laws v_k ($k = 1, \ldots, l$; $l \leq n$) introduced in equation (2.2). We assume that for a particular time and values of the parameters of $\mathcal{O}$, the values of the quantities of $\mathcal{O}_\gamma$, given below with subscript γ, are related to those of $\mathcal{O}$ as follows:

$$S_{i,\gamma} = S_i + dS_i \qquad (2.36)$$

$$E_{k,\gamma} = E_k + dE_k \quad (k = 1, \ldots, l) \qquad (2.37)$$

$$t_\gamma = t + dt \qquad (2.38)$$

E_k are the enzyme concentrations corresponding to rates v_k mentioned above, and t is time. The other parameters are identical in both systems.

For a particular infinitesimal value dS_i, we adjust dE_k ($k = 1, \ldots, l$), so that the rates v_k ($k = 1, \ldots, l$) of $\mathcal{O}_\gamma$ are equal to

those of $\mathcal{O}$, and therefore their relative differences are zero.

$$\frac{d\,v_k}{v_k} = {}^{u}\varepsilon_{S_i}^{v_k}\, d\,S_i + \frac{d\,E_k}{E_k} = 0 \qquad (k = 1, \ldots, l) \qquad (2.40)$$

The unscaled elasticity coefficients that appear in these equations were defined in equation (2.17). Here the relative difference in the enzyme concentrations is multiplied by a factor equal to one, because the rates are proportional to total enzyme concentration (see equation (2.2)). Simultaneously, for the values of dE_k calculated from equations (2.40) we can adjust dt introduced in equation (2.38), such that the derivative of S_i with respect to t ($\dot{S}_i$) , has the same value in both systems, and the difference $d\dot{S}_i$, is zero. This difference can be given in terms of the differences in enzyme concentrations and time as follows:

$$d\,\dot{S}_i = \sum_{k=1}^{l} \frac{\partial \dot{S}_i}{\partial E_k}\, d\,E_k + \frac{\partial \dot{S}_i}{\partial t}\, d\,t = 0 \qquad (2.41)$$

Then, for a particular value of dS_i , if we choose appropriate values of dE_k ($k = 1, \ldots, l$) and dt , all the rates immediatly related to S_i (v_k , $k = 1, \ldots, l$ and $\dot{S}_i$) are the same in $\mathcal{O}$ and $\mathcal{O}_\gamma$. It has to be noted that, for a given dS_i , the values of dE_k ($k = 1, \ldots, l$) and dt are different for each time.

To obtain the invariance of rates immediatly related to S_i, we only adjust the differences in enzymes concentrations corresponding to those rates, maintaining all the other enzyme concentrations and parameters constant. For this reason, all the other rates in $\mathcal{O}$ and $\mathcal{O}_\gamma$ are also the same. In this situation we expect that all the fluxes, metabolite concentrations and their time derivatives are the same in

both systems, S_i being the only variable with different value in both systems.

The quantitative conclusions obtained above, are used in next section to construct connectivity relationships for time-dependent metabolic systems. Even though these conclusions depend on the validity of the considerations made, their consequences (connectivity relationships) are verified by a separate mathematical procedure in Section (2.8).

2.7) Connectivity relationships

The quantitative conclusions obtained from the comparison of systems $\mathcal{S}$ and $\mathcal{S}_\gamma$ described above, are now used to construct relationships that link the control and elasticity coefficients. S_i is the metabolite whose concentration is different in the systems we are considering, being the difference:

$$d S_i = \sum_{k=1}^{l} \frac{\partial S_i}{\partial E_k} d E_k + \frac{\partial S_i}{\partial t} d t \qquad (2.42)$$

As all other concentrations are equal in both systems, we can write for S_j ($j \neq i$):

$$d S_j = \sum_{k=1}^{l} \frac{\partial S_j}{\partial E_k} d E_k + \frac{\partial S_j}{\partial t} d t = 0 \qquad (2.43)$$

Using equations (2.40) we can eliminate dE_k ($k = 1 , \ldots . , l$) from equations (2.41) to (2.43) obtaining equations (2.44) to (2.46) respectively.

$$d S_i \left[\sum_{k=1}^{l} \left(E_k \frac{\partial \dot{S}_i}{\partial E_k} \right) {}^{u}\varepsilon^{v_k}_{S_i} \right] = \frac{\partial \dot{S}_i}{\partial t} \, d t \qquad (2.44)$$

$$d S_i \left[1 + \sum_{k=1}^{l} \left(E_k \frac{\partial S_i}{\partial E_k} \right) {}^{u}\varepsilon^{v_k}_{S_i} \right] = \frac{\partial S_i}{\partial t} \, d t \qquad (2.45)$$

$$d S_i \left[\sum_{k=1}^{l} \left(E_k \frac{\partial S_j}{\partial E_k} \right) {}^{u}\varepsilon^{v_k}_{S_i} \right] = \frac{\partial S_j}{\partial t} \, d t \qquad (j \neq i) \qquad (2.46)$$

The time derivatives that appear in the right members of equations (2.44) to (2.46) are linked by the following equation:

$$\frac{\partial \dot{S}_i}{\partial t} = \sum_{h=1}^{m} \left(\frac{\partial \dot{S}_i}{\partial S_h} \right) \frac{\partial S_h}{\partial t} \qquad (2.47)$$

where the sum is carried over all the metabolite concentrations, including S_i, that appear explicitly in the differential equation corresponding to $\dot{S}_i$ (equation (2.1)).

Equations (2.44) to (2.47) have to be satisfied simultaneously, so we combine them by eliminating the time derivatives, obtaining:

$$\sum_{k=1}^{l} \left(E_k \frac{\partial \dot{S}_i}{\partial E_k} \right) {}^{u}\varepsilon^{v_k}_{S_i} = \frac{\partial \dot{S}_i}{\partial S_i} + \sum_{h=1}^{m} \left[\frac{\partial \dot{S}_i}{\partial S_h} \sum_{k=1}^{l} \left(E_k \frac{\partial S_h}{\partial E_k} \right) {}^{u}\varepsilon^{v_k}_{S_i} \right] \qquad (2.48)$$

The quantities between curved parenthesis, in the right member of the last equation, are the unscaled concentration control coefficients. In the left member appear the analogous coefficients for $\dot{S}_i$. For a particular set of the values of the parameters, equation (2.48) is a function of time only. Then if we interchange the derivatives with respect to time and enzyme, in the metabolite concentration control coefficients of $\dot{S}_i$, we obtain a differential equation in the metabolite concentration control coefficients. Taking into account these considerations, equation (2.48) can be written as follows:

$$\sum_{k=1}^{l} {}^{u}\dot{C}^{S_i}_{E_k}\,{}^{u}\varepsilon^{v_k}_{S_i} = \frac{\partial \dot{S}_i}{\partial S_i} + \sum_{h=1}^{m}\left[\frac{\partial \dot{S}_i}{\partial S_h}\sum_{k=1}^{l} {}^{u}C^{S_h}_{E_k}\,{}^{u}\varepsilon^{v_k}_{S_i}\right] \qquad (2.49)$$

where

$${}^{u}\dot{C}^{S_i}_{E_k} = \frac{\partial}{\partial t}\left({}^{u}C^{S_i}_{E_k}\right) \qquad (2.50)$$

Equation (2.50) is one connectivity relationship for a time dependent metabolic system. It relates the metabolite concentration control coefficients and elasticity coefficients. By the procedure used a number of connectivity relationships equal to the number of metabolite concentrations to the square can be constructed. A mathematical derivation of all the connectivity relationships, in a matrix form, is given in Section (2.8).

If we consider a stable steady state situation, the right members of equations (2.45) and (2.46) vanish. As dS_i is non-zero, the quantities between square parenthesis in both equations must be zero. After appropriate scaling, and using equations (2.16) and (2.18), equations (2.45) and (2.46), for steady state conditions become

$$\sum_{k} C^{S_j}_{E_k}\,\varepsilon^{v_k}_{S_i} = -\delta_{ij} \qquad (2.51)$$

where δ_{ij} is the Kronecker δ ($\delta_{ii}= 1$ and $\delta_{ij}= 0$ $(i \neq j)$) . These expressions are the connectivity relationships for steady state concentration control coefficients (Westerhoff & Chen, 1984).

2.8) Summation and connectivity relationships (Matrix equations).

The definitions of the vectors and matrices used in this section are given in Table (2.1) in pages 80-81.

2.8.1) Preliminary equations

First, we obtain general equations that are used in the derivation of summation and connectivity relationships. The solution of equation (2.1) is given in equation (2.3), and the resulting fluxes in equation (2.4). Substituting the solution in both members of equation (2.1), this equation takes the form:

$$\dot{\mathbf{s}} = \mathbf{N}\ \mathbf{j} \qquad (2.52)$$

where $\mathbf{j}$ is the flux vector, and we symbolize by $\dot{\mathbf{s}}$ the partial derivative of the solution with respect to time . The derivative of the flux vector with respect to $\mathbf{e}$ (enzyme concentration vector) is:

$$\frac{\partial \mathbf{j}}{\partial \mathbf{e}} = \frac{\partial \mathbf{v}}{\partial \mathbf{e}} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\ \frac{\partial \mathbf{s}}{\partial \mathbf{e}} \qquad (2.53)$$

Here we assume that the derivative of $\mathbf{j}$ with respect to $\mathbf{e}$ at fixed $\mathbf{s}$, equals the derivative of $\mathbf{v}$ with respect to $\mathbf{e}$ ($(\partial \mathbf{j} / \partial \mathbf{e})_{\mathbf{s}} = \partial \mathbf{v} / \partial \mathbf{e}$), and the derivative of $\mathbf{j}$ with respect to $\mathbf{s}$ at fixed $\mathbf{e}$, equals the

derivative of **v** with respect to **s** ($(\partial \mathbf{J} / \partial \mathbf{s})\mathbf{e} = \partial \mathbf{v} / \partial \mathbf{s}$). This is true only if the concentrations of metabolites are not linked by conservation equations (see also: Reder, 1988). We differentiate equation (2.52) with respect to **e** and postmultiply both members by **E** (the enzyme concentration matrix), remembering that the stoichiometry matrix **N** is constant. In the left member appears the successive partial derivatives of **s** with respect to time (first) and enzyme concentration (second). Assuming that the concentrations of metabolites and their derivatives are continuous functions of time and enzyme concentration, we can change the order of partial differentiation. After these considerations we obtain:

$$\dot{\mathbf{C}}^{\mathbf{u}}_{\mathbf{E}}{}^{\mathbf{s}} = \mathbf{N}\left(\mathbf{J} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,\mathbf{C}^{\mathbf{u}}_{\mathbf{E}}{}^{\mathbf{s}}\right) \qquad (2.54)$$

For a particular set of the values of the parameters, equation (2.54) is a function of time only. If we assume that the fluxes (elements of **J**) and their derivatives with respect to metabolite concentrations ($\partial \mathbf{v} / \partial \mathbf{s}$) are known functions of time, this equation represents a system of linear differential equations in the metabolite concentration control coefficients ($\mathbf{C}^{\mathbf{u}}_{\mathbf{E}}{}^{\mathbf{s}}$). The solution tells us how the control coefficients depend on the fluxes, and their derivatives with respect to metabolite concentration (these can be seen as totally unscaled elasticity coefficients). Equation (2.54) can be used, even when one or more rates are zero, at some point of the interval of time considered (and elasticity coefficients given in equations (2.17) and (2.18) are not defined).

Taking the derivative of equation (2.52) with respect to time, it is easy to derive the equation that relates the vector of time coefficients ($\mathbf{T}^{\mathbf{u}}{}^{\mathbf{s}}$) with its time derivative ($\dot{\mathbf{T}}^{\mathbf{u}}{}^{\mathbf{s}}$):

$$\dot{\mathbf{T}}^{\mathbf{u}}{}^{\mathbf{s}} = \dot{\mathbf{s}} + \mathbf{N}\,\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,\mathbf{T}^{\mathbf{u}}{}^{\mathbf{s}} \qquad (2.55)$$

2.8.2) Summation Relationships

To prove the summation relationships, we begin by post multiplying equation (2.54) by a unit column vector , μ , obtaining:

$$ {}^{u}\dot{C}^{s}_{E}\,\mu = \dot{s} + N\,\frac{\partial v}{\partial s}\,{}^{u}C^{s}_{E}\,\mu \qquad (2.56) $$

Then we substract, member by member, equation (2.55) from equation (2.56), to eliminate $\dot{s}$.

$$ {}^{u}\dot{C}^{s}_{E}\,\mu - {}^{u}\dot{T}^{s} = N\,\frac{\partial v}{\partial s}\left({}^{u}C^{s}_{E}\,\mu - {}^{u}T^{s} \right) \qquad (2.56) $$

In this expression appears the summation vector Σ , and its time derivative (see Table (2.1)). Introducing this notation into equation (2.57):

$$ \dot{\Sigma} = \left(N\,\frac{\partial v}{\partial s} \right) \Sigma \qquad (2.57) $$

This is a linear homogeneous system of differential equations in Σ . At the initial conditions, the derivatives of the metabolite concentrations with respect to enzyme concentrations are zero, and as an immediate consequence, the unscaled control coefficients are zero. As the initial conditon for time is zero (see section (2.2)), the initial time coefficient is also zero. (If $t_0 \neq 0$, the definition of the time coefficient has to be extended, see section (2.4)). Then the summation vector at the initial conditions is equal to the vector of

zeros. The solution of equation (2.58) in these conditions is zero for all times:

$$\Sigma = 0 \qquad (2.59)$$

The last equation can be written in its equivalent notation:

$$^{u}C^{s}_{E}\,\mu = {}^{u}T^{s} \qquad (2.60)$$

Equation (2.60) is the summation relationship for unscaled metabolite concentration coefficients, written in vector form. It represents a number of scalar equations equal to the number of variable metabolites. The equalities between the components of these vectors are given in equation (2.32).

Postmultiplying equation (2.53) by **E** (enzyme concentration matrix) we obtain the relation between the unscaled flux contol coefficient matrix $^{u}C^{j}_{E}$, and concentration control coefficient matrix:

$$^{u}C^{j}_{E} = J + \frac{\partial v}{\partial s}\,{}^{u}C^{s}_{E} \qquad (2.61)$$

where , **J** is the flux matrix. The time derivative of the flux vector **j** is given by:

$$\frac{\partial j}{\partial t} = \frac{\partial v}{\partial s}\,\frac{\partial s}{\partial t} \qquad (2.62)$$

If we multiply both members by the variable time t , we obtain the relation between the unscaled flux and concentration time coefficient vectors:

$$^{u}T^{j} = \frac{\partial v}{\partial s}\,{}^{u}T^{s} \qquad (2.63)$$

Finally, we postmultiply equation (2.61) by the unity vector μ , and premultiply equation (2.60) by $\partial \mathbf{v} / \partial \mathbf{s}$. Combining the resulting equations and equation (2.63) we obtain:

$$ {}^{u}C^{J}_{E}\,\mu = J + {}^{u}T^{J} \qquad (2.64) $$

Equation (2.64) is the summation relationship for unscaled flux coefficients. The scalar relationships between the components are given in equation (2.34).

Now we assume that the metabolite concentration matrix **S** and the flux matrix **J** (both diagonal matrices) are invertible, which means that all metabolites and fluxes are different from zero. Then premultiplying equation (2.60) by $\mathbf{S}^{-1}$ (the inverse of **S**) we obtain the summation relationship for metabolite concentration coefficients (scaled):

$$ C^{S}_{E}\,\mu = T^{S} \qquad (2.65) $$

and premultiplying equation (2.64) by $\mathbf{J}^{-1}$ (the inverse of **J**) we obtain the summation relationship for flux coefficients (scaled) in matrix form:

$$ C^{J}_{E}\,\mu = \mu + T^{J} \qquad (2.66) $$

The relation between the flux and concentration control coefficients (scaled) is obtained by premultiplying both members of equation (2.61) by $\mathbf{J}^{-1}$, and premultiplying the concentration control coefficient matrix by $\mathbf{S}\,\mathbf{S}^{-1}$:

$$C^{J}_{E} = I_{vxv} + \varepsilon^{v}_{s} C^{s}_{E} \qquad (2.67)$$

2.8.3) Connectivity Relationships

If J is invertible, in equation (2.54), we can premultiply $\partial v / \partial s$ by $J J^{-1}$. Then postmultiplying by the unscaled elasticity coefficient matrix and rearranging the right member, we obtain:

$$u_{\cdot s} C^{s}_{E} \; u\varepsilon^{v}_{s} = N \frac{\partial v}{\partial s} \left(I_{sxs} + {}^{u}C^{s}_{E} \; u\varepsilon^{v}_{s} \right) \qquad (2.68)$$

Equation (2.68) is the connectivity relationship in matrix form. It represents s^2 number of scalar relationships, were s is the number of metabolites. Equation (2.49) is one of these scalar relationships. If we are dealing with an unbranched pathway, the number of steps (enzymes) is equal to the number of variable metabolites plus one . The number of metabolite control coefficients in this metabolic system is equal to the number of steps (s + 1) times the number of metabolites (s). Then equations (2.60) and (2.68) together constitute a system of differential equations, that has the same number of equations ($s + s^2$) as unkown metabolite control coefficients. Assuming that $\partial v / \partial s$, the time coefficients and the elasticity coefficients are known functions of time, we can integrate the linear system of differential equations. The solution shows us how the metabolite control coefficients (system variations) are related to the elasticity coefficients (local variations). Substituting this solution into equation (2.61) the relation between the flux control coefficients and the elasticitiy coefficients is obtained. If the pathway is not linear (e.g. branched) additional relationships are needed to complete the

analysis.

If the metabolic system is in a stable steady state, the left member of equation (2.68) equals to a (sxs) matrix of zeros (**0**). As in this metabolic situation the $\mathbf{N}\,\partial\,\mathbf{v}\,/\,\partial\,\mathbf{s}$ (the Jacobian matrix) is invertible, the expression betweeen parenthesis in the right member of equation (2.68) is equal to **0** . We assume that the metabolite concentration matrix **S** is invertible (the steady state concentrations of metabolites are all non-zero). Substracting the identity matrix from both members, premultiplying them by **S** , and postmultiplying them by $\mathbf{S}^{-1}$ we obtain:

$$\mathbf{C}^{\mathbf{s}}_{\mathbf{E}}\;\boldsymbol{\varepsilon}^{\mathbf{v}}_{\mathbf{s}} \;=\; -\,\mathbf{I}_{\mathbf{s}\mathbf{x}\mathbf{s}} \qquad (2.69)$$

Equation (2.69) is the metabolite concentration connectivity relationship in matrix form for steady state systems (Westerhoff & Chen, 1984).

We can conclude by noting equations (2.54), (2.60), (2.61), (2.64), (2.65), (2.66), (2.67) and (2.68) as representing the main results of our analysis, and they reveal the well known theorems of steady state control analysis, as the time evolution approaches a stable steady state.

2.9) Example

It is instructive to apply the general conclusions to a particular example. This will also reveal some of the biological relevance of the foregoing treatment. In what follows we shall point out what pattern of behaviour can be expected without commitment to specific mechanisms or to particular values of parameters.

We consider a metabolic pathway whose scheme is given in (2.70).

$$X_0 \xrightarrow{E_1} S \xrightarrow{E_2} X_1 \qquad (2.70)$$

The concentrations of metabolites X_0 and X_1 are held constant, S being the only metabolite whose concentration is free to move. For this scheme , with one variable metabolite concentration and two rates, the stoichiometry matrix is :

$$\mathbf{N} = \begin{bmatrix} 1 & -1 \end{bmatrix} \qquad (2.71)$$

The reactions, catalyzed by enzymes E_1 and E_2 respectively, are in general reversible, and their rate laws are subject only to the same restrictions as equation (2.2).

For this example, the components of equation (2.54) are:

$$\dot{C}^{u\,s}_{E_1} = J_1 + \frac{\partial (v_1 - v_2)}{\partial S} C^{u\,s}_{E_1}$$

$$\dot{C}^{u\,s}_{E_2} = -J_2 + \frac{\partial (v_1 - v_2)}{\partial S} C^{u\,s}_{E_2} \qquad (2.72)$$

Equations (2.72) constitute a system of differential equations that can be solved to obtain the unscaled concentration control coefficients. If we integrate, taking into account that at the initial condition of time (t = 0) the concentration control coefficients are zero, we

obtain:

$$ {}^{u}C^{s}_{E_1} = (J_1 - J_2) \int_0^t \frac{J_1}{J_1 - J_2} \, d\,t $$

$$ {}^{u}C^{s}_{E_2} = (J_1 - J_2) \int_0^t \frac{- J_2}{J_1 - J_2} \, d\,t \qquad (2.73) $$

In this particular example, the summation and connectivity relationships given by equations (2.60) and (2.68) respectively, take the form:

$$ {}^{u}C^{s}_{E_1} + {}^{u}C^{s}_{E_2} = {}^{u}T^{s} \qquad (2.74) $$

and

$$ {}^{u}\dot{C}^{s}_{E_1} \, {}^{u}\varepsilon^{v_1}_{s} + {}^{u}\dot{C}^{s}_{E_2} \, {}^{u}\varepsilon^{v_2}_{s} = \frac{\partial (v_1 - v_2)}{\partial S} \left(1 + {}^{u}C^{s}_{E_1} \, {}^{u}\varepsilon^{v_1}_{s} + {}^{u}C^{s}_{E_2} \, {}^{u}\varepsilon^{v_2}_{s} \right) $$

(2.75)

Equation (2.74), its time derivative, and equation (2.75) are functions of time, and constitute a system of equations that can be solved to obtain the unscaled control coefficients in terms of the unscaled elasticity coefficients. Integrating the system from time zero, we obtain:

$$ {}^{u}C^{s}_{E_1} = {}^{u}T^{s} \frac{\int_0^t \frac{{}^{u}\varepsilon_s^{(v_1-v_2)} - {}^{u}\varepsilon_s^{v_2}}{{}^{u}\varepsilon_s^{v_1} - {}^{u}\varepsilon_s^{v_2}} \, dt}{t} $$

and (2.76)

$$ {}^{u}C^{s}_{E_2} = {}^{u}T^{s} \frac{\int_0^t \frac{{}^{u}\varepsilon_s^{v_1} - {}^{u}\varepsilon_s^{(v_1-v_2)}}{{}^{u}\varepsilon_s^{v_1} - {}^{u}\varepsilon_s^{v_2}} \, dt}{t} $$

It will be noted that equations (2.73) and (2.76) are two forms of the same relationship, but the latter exhibits explicitly the concentration control coefficients in terms of the (time-dependent) elasticity and time coefficients.

The control coefficients (scaled) are obtained in terms of the scaled elasticity coefficients applying the appropriate transformations to equations (2.76).

$$ C^{s}_{E_1} = T^{s} \frac{\int_0^t \frac{\varepsilon_s^{(v_1-v_2)} - \varepsilon_s^{v_2}}{\varepsilon_s^{v_1} - \varepsilon_s^{v_2}} \, dt}{t} $$

and (2.77)

$$ C^{s}_{E_2} = T^{s} \frac{\int_0^t \frac{\varepsilon_s^{v_1} - \varepsilon_s^{(v_1-v_2)}}{\varepsilon_s^{v_1} - \varepsilon_s^{v_2}} \, dt}{t} $$

Once the unscaled concentration control coefficients are known, the unscaled flux control coefficients can be obtained using equation (2.61):

$$
\begin{aligned}
{}^{u}C^{J_1}_{E_1} &= J_1 + \frac{\partial v_1}{\partial S}\ {}^{u}C^{S}_{E_1} \\
{}^{u}C^{J_1}_{E_2} &= \frac{\partial v_1}{\partial S}\ {}^{u}C^{S}_{E_2} \\
{}^{u}C^{J_2}_{E_1} &= \frac{\partial v_2}{\partial S}\ {}^{u}C^{S}_{E_1} \\
{}^{u}C^{J_2}_{E_2} &= J_2 + \frac{\partial v_2}{\partial S}\ {}^{u}C^{S}_{E_2}
\end{aligned}
\qquad (2.78)
$$

The expressions that relate the scaled control coefficients are: (see equation (2.67)

$$
\begin{aligned}
C^{J_1}_{E_1} &= 1 + \varepsilon^{v_1}_{S}\ C^{S}_{E_1} \\
C^{J_1}_{E_2} &= \varepsilon^{v_1}_{S}\ C^{S}_{E_2} \\
C^{J_2}_{E_1} &= \varepsilon^{v_2}_{S}\ C^{S}_{E_1} \\
C^{J_2}_{E_2} &= 1 + \varepsilon^{v_2}_{S}\ C^{S}_{E_2}
\end{aligned}
\qquad (2.79)
$$

It is worth pointing out that, unlike the steady state solution (see below), we have four different (not two) flux control coefficients. This is because, at any time before the steady state, the two fluxes J_1 and J_2 are not necessarily equal to one another and, as shown here, respond differently to a particular enzyme variation. This is true, not only for the small system considered here (2.70), but for any system

of any complexity. The immediate biological relevance of this is that, in e.g. developing systems, different parts can show differential sensitivities even if at steady state no such differences are detectable.

Equations (2.76) to (2.79) show how the sensitivity of the metabolite concentration and fluxes, to changes in one enzyme concentration, are quantitatively affected by the kinetic properties of the individual enzymes, included in the elasticity coefficients. It is important to note that the elasticity coefficients appear within the time integral. This is so , because the effect of the local variations (given by the elasticity coefficients) on the system variations (given by the control coefficients) depend not only on the properties of the individual rates, and the topology of the network, but on the extent of time the system has, in fact, evolved. Furthermore, at the initial condition of time , even if the elasticity coefficients are non-zero, the metabolite concentration control coefficients are all zero, since the metabolite concentration has not changed. In these conditions the flux control coefficient (scaled) is one, if the flux considered corresponds to the step where the enzyme was changed, and zero if that flux is affected only by the movement of the metabolite concentration.

If the system is one that approaches a stable steady state, the limit of the functions given in equations (2.77), as time goes to infinity, is:

$$\left(C^{S}_{E_1} \right)_{ss} = \frac{1}{\left(\varepsilon^{v2}_{S} \right)_{ss} - \left(\varepsilon^{v1}_{S} \right)_{ss}}$$

and (2.80)

$$\left(C^{S}_{E_2} \right)_{ss} = \frac{-1}{\left(\varepsilon^{v2}_{S} \right)_{ss} - \left(\varepsilon^{v1}_{S} \right)_{ss}}$$

the subscript ss indicating steady state values. Substituting equations

(2.80) into equations (2.79) we obtain:

$$\left(C_{E_1}^{J_1} \right)_{ss} = \left(C_{E_1}^{J_2} \right)_{ss} = \frac{\left(\varepsilon_S^{v\,2} \right)_{ss}}{\left(\varepsilon_S^{v\,2} \right)_{ss} - \left(\varepsilon_S^{v\,1} \right)_{ss}}$$

$$\left(C_{E_2}^{J_1} \right)_{ss} = \left(C_{E_2}^{J_2} \right)_{ss} = \frac{-\left(\varepsilon_S^{v\,1} \right)_{ss}}{\left(\varepsilon_S^{v\,2} \right)_{ss} - \left(\varepsilon_S^{v\,1} \right)_{ss}} \qquad (2.81)$$

Equations (2.80) and (2.81) are the same expressions obtained for the control coefficients in terms of the elasticity coefficients, when the scheme (2.70) is studied using steady state control analysis (Kacser, 1983).

The equations derived in this section, and the general conclusions obtained hold whatever function the dependence of the rates on the concentration of S is assumed.

Let us now restrict our treatment to the case where the rate laws are monotonic functions in the concentration of S (decreasing for the first and increasing for the second step). With this assumption, the sign of the concentration control coefficients can be studied using equations (2.73). If the initial value of S (S_{ini}) is less than the steady state value (S_{ss}), then $J_1 > 0$ and $J_1 > J_2$ for any time. Considering these inequalities together with equation (2.73) we conclude that ${}^{u}C_{E1}^{S} > 0$ for any time. If, in addition, $S_{ini} > X_1 / K_2$ (K_2 is the equilibrium constant of the second step), then $J_2 > 0$ and ${}^{u}C_{E2}^{S} < 0$, for any time . But, if $S_{ini} < X_1 / K_2$, J_2 changes sign as time goes from zero to infinity. Then, in this last situation, for small time ${}^{u}C_{E2}^{S} > 0$ and for large time ${}^{u}C_{E2}^{S} < 0$. In the case where $S_{ini} > S_{ss}$, ${}^{u}C_{E2}^{S} < 0$. If, in addition, $S_{ini} < K_1 X_0$ (K_1 is the equilibrium

constant of the first step), ${}^{u}C^{S}_{E1} > 0$. But if $S_{ini} > K_1 X_o$, then ${}^{u}C^{S}_{E1}$ changes sign in the time course, being negative for small time and positive for large time. The sum of the concentration control coefficients, given by equation (2.74), is positive or negative depending on $S_{ini} < S_{ss}$ or $S_{ini} > S_{ss}$ respectively. It is obvious that the scaled concentration control coefficients have the same sign as the unscaled ones. We conclude that for monotonic rate laws, even though at steady state ${}^{u}C^{S}_{E1} > 0$ and ${}^{u}C^{S}_{E2} < 0$ and their sum is equal to zero (see equations (2.80)), the corresponding time dependent coefficients and their sum can be either positive or negative depending on the initial concentration of S and the time. The steady state flux control coefficients, given in equations (2.81), are each positive and less than one, their sum being equal to one. In time dependent situations, it may be shown that each of the four flux control coefficients (equations (2.79)) and the sum of those corresponding to the same flux, can be either positive or negative for different initial conditions and temporal points. These conclusions are independent of the enzyme mechanisms provided only that we could assume monotonicity. To illustrate one possible pattern of behaviour, in Figure (1), we show a diagram of the change in time of the two flux control coefficients (C^{J1}_{E1} and C^{J1}_{E2}) and their sum obtained by simulation of system (2.70) with particular mechanisms, parameters and initial conditions.

Using the assumptions of monotonicity of the rate laws we studied the signs of the time dependent control coefficients, comparing the results with those at steady state. Let as now impose additional restrictions to the rate laws, with the aim of making predictions about the magnitude of the control coefficients. We assume reversible Michaelis-Menten rate laws for both steps of scheme (2.70). The elasticity coefficients can be written as the sum of two terms, one that depends on the disequilibrium and other on the saturation (Westerhoff et al., 1984). In this case we consider two extreme

situations: a) the overall reaction (from X_0 to X_1) is near equilibrium, and b) both reactions are far from equilibrium and substancially saturated by the substrate at steady state. In situation a) the elasticity coefficients at steady state are large (Westerhoff, 1984) and, from equation (2.80), the concentration control coefficients are small. At the initial conditions the concentration control coefficients are zero. Between the initial conditions and the steady state we can find points where the interval of time elapsed from the initial conditions is such that the concentration of S can change significantly, but the elasticity coefficients are not large and the time coefficient is not small. At these points the concentration control coefficients can have values that are not small as they would be in the initial stages and at steady state (see equation (2.77)). Furthermore, when, in addition, unsaturated rate laws are considered, it can be shown (Acerenza, Sauro and Kacser, unpublished results) that the sum of the time dependent concentration control coefficients (scaled) can attain a maximun positive value of one, for cases where $S_{ini} < S_{ss}$ or considerably large negative values for cases where $S_{ini} > S_{ss}$. Now we consider the extreme situation b). If both reactions are far from equilibrium and saturated by substrate at steady state, the elasticity coefficients (at steady state) are small and the control coefficients are large (equations (2.80)). As was pointed above, the concentration control coefficients at the initial condition are zero. Then, in this situation, the concentration control coefficients, given by equations (2.77), take a wide range of values, as the system evolves from the initial condition to the steady state.

10) Discussion

In the definitions of control, time and elasticity coefficients (section (4)), and in the derivation of summation and connectivity

relationships several mathematical assumptions were made. The variables and their derivatives were considered to be continuous functions of the parameters and time. A large and simultaneous change in all enzyme concentrations by the same factor will not change the qualitative behaviour of the system, the only difference being a constant time scale transformation (see section (2.3)). Other changes, however, in some of the parameters could change, for example, a stable steady state, into sustained oscillations, if a bifurcation point is reached. Due to the discontinuities occurring in these points, bifurcation behaviour is excluded in our treatment. In equation (2.1), the temporal change of the metabolite concentrations is written as a linear combination of the kinetic laws of individual enzymes. This implies that, even if the concentrations of metabolites are changing in time, a quasi-stationary approximation for the different forms under which each enzyme exists, is plausible. If " hysteretic enzymes " (Neet & Ainslie, 1980) are present, however, these can present lags or bursts with relaxation times up to minutes, and in general they do not satisfy the last approximation. We also assumed that each enzyme affects only one step ("independence") and that the reaction rate is of first order with respect to the total concentration of this enzyme ("additivity"), (see equation (2.2)). There is some experimental evidence suggesting that enzymes can act non-independently, and that metabolites can be transfered by enzyme-enzyme interaction, but it is not established how frequent this phenomenon is in vivo (Srivastava & Bernhard, 1986; Srere, 1987). Enzymes that present association-dissociation mechanisms (Frieden, 1967) do not fulfill the additivity requisite, and are excluded from our treatment. Metabolic Control Analysis for steady state systems that are non-additive and non-independent has been recently developed (Kacser et al., 1990 and Kacser and Sauro, 1990) Furthermore, the present treatment excludes metabolic structures, such as conserved cycles, where the concentrations of metabolites are linked by

conservation equations (see equation (2.53)). All other systems that do not violate the mathematical assumptions made are, strictly speaking, included in our treatment, while systems which contain some of the above aspects may nevertheless be amenable to the present analysis if, quantitatively, the deviations are not significant.

The time response of (parts of) organisms can be an important factor in the proper functioning of the organism in its environment. Thus, transition from one steady state to another caused by some external stimulus must be under enzymatic (and eventually genetic) control. Similarly, certain periodic phenomena (from high frequency 'songs' to circadian rythms) are known to be genetically determined and may operate through some system of enzyme mediated steps. The distribution of control between the different steps of the network is therefore a critical aspect of the fitness of the organism. It was shown (and supported by one example) that in a system which had only positive steady state flux control coefficients, the control of the time course can display high negative coefficients. An enzyme (E_1) which have little control over the steady state flux, could be seen to exert a mayor (negative) control on the development of the system. The establishment of the relationship between the time-dependent control coefficients and the kinetic nature of the enzymes is a first step towards understanding the molecular basis of these phenomena.

It is known that in many cases (particularly in microoganisms) whole pathways are coordinately induced or repressed (see for example Stuart et al., 1986). This is very close to the summation transformation, where the control exerted by a simultaneous and equal change in all the enzyme concentrations of a pathway, by the same factor ('coordinate control') are considered (see section (2.5)). It should be noted that the coordinate control is directly related to the time scale transformation of the time course of the metabolite

concentrations (see section (2.3)).

The summation and connectivity relationships , as was stated in sections (2.8) and (2.9), can be used to write the control ceofficients in terms of the time and elasticity coefficients, when linear pathways are considered. These equations are not as simple as for steady state situations, because the elasticity coefficients appear inside the time integral (see equations (2.74) to (2.77)). In spite of this fact, the analysis of these relationships gives insight on the way the main features of the kinetics of each metabolic reaction can affect the control coefficients, even if a complete knowledge of the rate laws is not available (see section (2.9)). This is one important achievement of Metabolic Control Analysis. Unlike traditional approaches to the problems of 'control' which either are concerned with detailed mechanistic arguments or make use of *in vitro* values of parameters for simulations of putative *in vivo* models, Metabolic Control Analysis draws its principal conclusions without such specific assumptions.

The theory developed in the present paper is an extension of traditional Metabolic Control Analysis to time dependent systems. Each of the relationships derived has its counterpart in the known steady state theory. But even if many of the basic ideas here are not new, they are enlarged from the static vision of the stable steady state, to a wider domain that includes time evolution, oscillations and chaos.

Chapter 2 and 4 were transformed into two publications: i) Acerenza, L., Sauro, H. and Kacser, H. (1989) J. theor. Biol. **137**, 444 and ii) Acerenza, L., Kacser, H. (1990) Biochem. J. **269**, 697. In these papers I am a co-author with Henrik Kacser and Herbert Sauro. Their critical comments and permanent encouragement were an invaluable contribution to this work. Having started with this topic and being responsible for the main developments and results I hope not to be wrong to include these papers in the present thesis as my own work.

Table 2.1 : Definitions of vectors and matrices.

Symbol	Name (*)	Dimension (**)	Element ij (***)	Equation (****)
$\mathbf{N}$	Stoichiometry (M)	s x v	n_{ij}	Eq (2.1)
$\mathbf{s}$	Metabolite concentration (V)	s x 1	S_i	Eq (2.3)
$\dot{\mathbf{s}}$	Time derivative of $\mathbf{s}$ (V)		$\dot{S}_i$	------
$\mathbf{v}$	Rate (V)	v x 1	v_i	Eq (2.2)
$\mathbf{j}$	Flux (V)	v x 1	J_i	Eq (2.4)
$\mathbf{S}$	Metabolite concentration (M)	s x s	$S_{ii} = S_i$, $S_{ij} = 0$ $(i \neq j)$	------
$\mathbf{J}$	Flux (M)	v x v	$J_{ii} = J_i$, $J_{ij} = 0$ $(i \neq j)$	------
$\frac{\partial \mathbf{v}}{\partial \mathbf{s}}$	Metabolite concentration derivative of $\mathbf{v}$ (M)	v x s	$\frac{\partial v_i}{\partial s_j}$	Eq (2.17)
$\mathbf{e}$	Enzyme concentration (V)	v x 1	E_i	Eq (2.2)
$\mathbf{E}$	Enzyme concentration (M)	v x v	$E_{ii} = E_i$, $E_{ij} = 0$ $(i \neq j)$	------
${}^{u}C^{\mathbf{s}}_{\mathbf{E}}$	Unscaled concentration control coefficient (M)	s x v	${}^{u}C^{S_i}_{E_j}$	Eq (2.15)
${}^{u}\dot{C}^{\mathbf{s}}_{\mathbf{E}}$	Time derivative of ${}^{u}C^{\mathbf{s}}_{\mathbf{E}}$ (M)	s x v	${}^{u}\dot{C}^{S_i}_{E_j}$	Eq (2.50)
${}^{u}C^{\mathbf{J}}_{\mathbf{E}}$	Unscaled flux control coefficient (M)	v x v	${}^{u}C^{J_i}_{E_j}$	Eq (2.15)

$^{u}_{T}{}^{s}$	Unscaled concentration time coefficient (V)	s x 1	$^{u}_{T} S_i$	Eq (2.19)
$^{u}\dot{T}^{s}$	Time derivative of $^{u}_{T}{}^{s}$ (V)	s x 1	$\frac{\partial\, ^{u}_{T} S_i}{\partial\, t}$	------
$^{u}_{T} J$	Unscaled flux time coefficient (V)	v x 1	$^{u}_{T} J_i$	Eq (2.19)
$^{u}_{\varepsilon}{}^{v}_{s}$	Unscaled elasticity coefficient (M)	v x s	$^{u}_{\varepsilon}{}^{v_i}_{S_j}$	Eq (2.17)
$I_{s\,x\,s}$	Identity (M)	s x s	$d_{ii}=1$, $d_{ij}=0$ $(i\neq j)$	------
$I_{v\,x\,v}$	Identity (M)	v x v	$d_{ii}=1$, $d_{ij}=0$ $(i\neq j)$	------
μ	Unity (V)	v x 1	$\mu_i = 1$	------
Σ	Summation (V)	s x 1	$\Sigma_i = \sum_j {}^{u}_{E_j} C^{S_i} - {}^{u}_{T} S_i$	------
$\dot{\Sigma}$	Time derivative of Σ (V)	s x 1	$\frac{\partial\, \Sigma_i}{\partial\, t}$	------

(*) Name of the vector (V) or matrix (M).

(**) Dimension of the vector or matrix, where " s " is the number of metabolites and " v " the number of rates.

(***) Element of row " i " and column " j " of the vector or matrix.

(****) Equation where the elements, vector or matrix are introduced or defined.

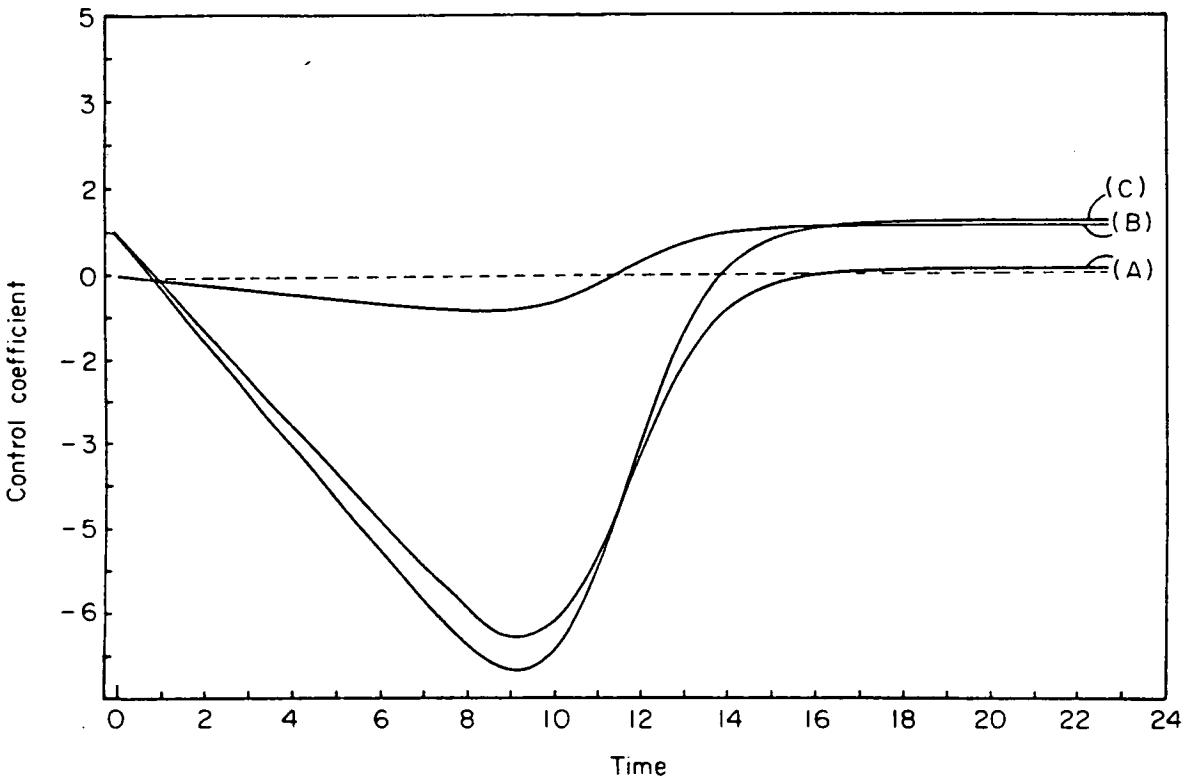

Fig. 2.1 : Time evolution of flux control coefficients in system (2.70).

The rate equations for both steps were represented by : $v_1 = V_1 (X_o - S / K_1)$ and $v_2 = V_2 (S - X_1 / K_2)$ $V_1 = 10$, $V_2 = 1$, $K_1 = 1$, $K_2 = 0.1$, $X_o = 1000.1$, $X_1 = 100$ and at $t_o = 0$, $S_o = S_{ss} / 100$. We show only changes in $C^{J_1}_{E_1}$ (curve A), $C^{J_1}_{E_2}$ (curve B), and their sum (curve C). The latter is also equal to $1 + T^{J_1}$. At large values of time they nearly reach their steady state values.

Chapter 3

Enzyme-enzyme interactions and parameter-unspecified control coefficients *

3.1) Introduction

Two assumptions were made in MCA regarding the functional relationship between the rate of an enzyme-catalized reaction and the concentration of the enzymes. The first is that the reaction rate of an isolated enzyme is proportional to the total concentration of this enzyme (i.e. additivity). The second assumption is that the catalytic properties of anyone enzyme are not affected by the presence of other enzymes (i.e. independence). When in a metabolic network one or more enzymes present non-additivity or non-independence the traditional theorems of MCA must be modified (Kacser et al., 1990 and Sauro and Kacser, 1990). The effect that changes in parameters (enzyme concentrations or others) that are non-proportional to the rates, have on the system's variables can be appropriately described by introducing parameter-unspecified control coefficients (C_V). Here a proof of the steady state summation and connectivity theorems in terms of C_V is given (see also Reder, 1988). The expressions for the concentration and flux control coefficients in terms of the elasticity coefficients are also derived. Matrix notation is used throughout. Finally, in section (3.3), I show an attempt to introduce a description in terms of parameter-unspecified control coefficients in time-dependent metabolic systems, which is successful only in a particular case.

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* see footnote on page 91

3.2) Parameter-unspecified control coefficients: the steady state.

In what follows, the parameters (concentrations of enzymes, external effectors, etc) are represented by the parameter vector **p**. The components of this column vector are the values of the parameters that the experimenter chooses to modulate. No assumption is made about the functional relationship between **p** and the rate vector **v** , thus, the rates are not necessarily proportional to the parameters. The definitions of vectors and matrices used in this section are given in Table (3.1) in pages 92-93.

In this section we only consider the steady state. The steady state concentrations are constant in time and therefore the steady state flux vector satisfy the following matrix equation (see eq(2.52)):

$$\mathbf{N}\ \mathbf{j} = \mathbf{0} \qquad (3.1)$$

where the stoichiometry matrix **N** is independent of the parameters. Differentiating eq(3.1) with respect to **p** we obtain:

$$\mathbf{N}\left(\frac{\partial \mathbf{v}}{\partial \mathbf{p}} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\ \frac{\partial \mathbf{s}}{\partial \mathbf{p}} \right) = \mathbf{0} \qquad (3.2)$$

The metabolite concentration (**S**) and the flux (**J**) matrices, and their inverses, can be used to scale the partial derivatives in the previous equations, i.e.

$$\mathbf{N}\left(\mathbf{J}\mathbf{J}^{-1}\frac{\partial \mathbf{v}}{\partial \mathbf{p}}\ \mathbf{p} + \mathbf{J}\mathbf{J}^{-1}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\ \mathbf{S}\mathbf{S}^{-1}\frac{\partial \mathbf{s}}{\partial \mathbf{p}}\ \mathbf{p} \right) = \mathbf{0} \qquad (3.3)$$

obtaining an expression in terms of the control and elasticity coefficients:

$$N\ J \left(\pi^{v}_{p} + \varepsilon^{v}_{s}\ C^{s}_{p} \right) = 0 \qquad (3.4)$$

The quantity between brackets in eq (3.4) is equal to the flux control coefficient matrix.

$$C^{J}_{p} = \pi^{v}_{p} + \varepsilon^{v}_{s}\ C^{s}_{p} \qquad (3.5)$$

Now we assume that the number of parameters is equal to the number of rates i.e. π^{v}_{p} is a square matrix. Moreover, we consider a set of parameters such that this matrix is invertible (i.e. the determinant is non-zero). The postmultiplication of eq (3.4) by its inverse, i.e. $(\pi^{v}_{p})^{-1}$, renders

$$N\ J \left(I_{v \times v} + \varepsilon^{v}_{s}\ C^{s}_{p}\ (\pi^{v}_{p})^{-1} \right) = 0 \qquad (3.6)$$

Now the parameter-unspecified control coefficient matrix (C_v) can be defined. The one corresponding to metabolite concentrations is

$$C^{s}_{v} \equiv C^{s}_{p}\ (\pi^{v}_{p})^{-1} \qquad (3.7)$$

The left hand member of this identity must only be interpreted as a symbol representing the product of matrices in the right hand side. It is not the partial derivative of the metabolite concentrations vector (s) with respect to the rate vector (v) (for an interpretation in terms of scalar modulation equations see Kacser et al., 1990). Some of its properties are analysed below. Substituting definition (3.7) into eq (3.6) we obtain:

$$N\ J\left(\ I_{v\times v}+\varepsilon^{v}_{s}\ C^{s}_{v}\ \right)=0 \qquad (3.8)$$

The matrix $N\ J\ \varepsilon^{v}_{s}$ is equal to $N\ (\partial v/\partial s)\ S$. $N\ (\partial v/\partial s)$ is the jacobian matrix and for a steady state system is invertible. **S** is a diagonal matrix whose elements are the metabolite concentrations. As any of these concentrations vanish, **S** is also invertible. Finally, as the product of two invertible matrices is an invertible matrix, we conclude that $N\ J\ \varepsilon^{v}_{s}$ is invertible. Based on this property, eq (3.9) can be easily solved for C^{s}_{v}.

$$C^{s}_{v}=-\left(\ N\ J\varepsilon^{v}_{s}\ \right)^{-1}N\ J \qquad (3.10)$$

The postmultiplication of eq (3.5) by $(\pi^{v}_{p})^{-1}$ renders:

$$C^{j}_{v}=I_{v\times v}+\varepsilon^{v}_{s}\ C^{s}_{v} \qquad (3.11)$$

where C^{s}_{v} was defined in eq(3.7) and C^{j}_{v}, the parameter-unspecified flux control coefficient matrix, is defined by

$$C^{j}_{v}\equiv C^{j}_{p}\,(\pi^{v}_{p})^{-1} \qquad (3.12)$$

Introducing eq(3.10) into (3.11) we obtain.

$$C^{j}_{v}=I_{v\times v}-\varepsilon^{v}_{s}\left(\ N\ J\varepsilon^{v}_{s}\ \right)^{-1}N\ J \qquad (3.13)$$

Eqs (3.10) and (3.13) show that C^{s}_{v} and C^{j}_{v} can be expressed in

terms of the fluxes and ε-elasticity coefficients (an example was given in eqs (1.18)). The parameters modulated do not appear explicitly in these expressions. Therefore, the $\mathbf{C}_{\mathbf{v}}$ do not depend on the parameters used to define them. If the parameters chosen are the enzyme concentrations, and the assumptions of additivity and independence are valid. $\pi^{\mathbf{v}}_{\mathbf{p}}$ is equal to the identity matrix. In this case $\mathbf{C}^{\mathbf{s}}_{\mathbf{v}}$ and $\mathbf{C}^{\mathbf{j}}_{\mathbf{v}}$ are equal to $\mathbf{C}^{\mathbf{s}}_{\mathbf{E}}$ and $\mathbf{C}^{\mathbf{j}}_{\mathbf{E}}$ respectively (eqs(3.7) and (3.12)). We can also conclude that under the assumption of proportionality between rate and enzyme concentration the following equations hold.

$$\mathbf{C}^{\mathbf{s}}_{\mathbf{E}} = \mathbf{C}^{\mathbf{s}}_{\mathbf{p}} (\pi^{\mathbf{v}}_{\mathbf{p}})^{-1} \qquad (3.14a)$$

$$\mathbf{C}^{\mathbf{j}}_{\mathbf{E}} = \mathbf{C}^{\mathbf{j}}_{\mathbf{p}} (\pi^{\mathbf{v}}_{\mathbf{p}})^{-1} \qquad (3.14b)$$

(because each member of these equations is equal to the corresponding $\mathbf{C}_{\mathbf{v}}$) or postmultiplying by $\pi^{\mathbf{v}}_{\mathbf{p}}$

$$\mathbf{C}^{\mathbf{s}}_{\mathbf{p}} = \mathbf{C}^{\mathbf{s}}_{\mathbf{E}} \pi^{\mathbf{v}}_{\mathbf{p}} \qquad (3.15a)$$

$$\mathbf{C}^{\mathbf{j}}_{\mathbf{p}} = \mathbf{C}^{\mathbf{j}}_{\mathbf{E}} \pi^{\mathbf{v}}_{\mathbf{p}} \qquad (3.15b)$$

Eqs(3.15a) and (3.15b) are the matrix version of the partitioned response theorem (Kacser and Burns, 1973).

The summation and connectivity theorems can be easily derived from the previous equations. The concentration summation theorem is obtained by postmultiplying eq(3.10) by μ (a unitary column vector). The product $\mathbf{J}\,\mu$ equals $\mathbf{j}$, and according to eq(3.1) $\mathbf{N}\,\mathbf{j} = \mathbf{0}$, therefore,

$$C^{s}_{v}\ \mu = 0 \qquad (3.16)$$

Eq(3.16) is the matrix form of the concentration summation theorem for parameter-unspecified control coefficients. Similarly, postmultiplying eq(3.13) by μ we obtain the matrix form of the flux summation theorem for parameter-unspecified control coefficients, i.e.

$$C^{j}_{v}\ \mu = \mu \qquad (3.17)$$

The concentration connectivity theorem is obtained by multiplying eq(3.8) by ε^{v}_{s} and premultiplying it by the inverse of $N\ J\ \varepsilon^{v}_{s}$ (this matrix is invertible as was discussed above). The result is:

$$I_{s\times s} + C^{s}_{v}\ \varepsilon^{v}_{s} = 0 \qquad (3.18)$$

Finally, by postmultiplication of eq(3.11) by ε^{v}_{s} and taking into account eq(3.18) the matrix version of the flux summation theorem for parameter-unspecified control coefficients is obtained:

$$C^{j}_{v}\ \varepsilon^{v}_{s} = 0 \qquad (3.19)$$

The theorems given in eqs(3.16) to (3.19) are identical to those satisfied by the enzyme control coefficients when all the rates are proportional to the corresponding enzyme concentration. Yet the theorems in terms of C_v apply irrespective of the functional relationship between rate and enzyme concentration.

The only way to produce a change in a variable is by modulation of a parameter. But the primary effect of changing a parameter is to change the rate to which the parameter belongs. Subsequently, the change in the rate spreads throughout the system affecting the values

of the variables. It is, therefore, natural to attempt to split the effect that a change in a parameter has on a variable (C^{Y}_{p}) as the product of two contributions i) the effect that the parameter has on the rate (π^{v}_{p}) and ii) the effect that the rate has on the variable (C^{Y}_{v}). This is symbolized by the equation

$$C^{Y}_{p} = C^{Y}_{v} \pi^{v}_{p} \qquad (3.20)$$

The usefulness of eq(3.20) relies on the fact that, even though the rate changes as a result of a parameter change, the values of C^{Y}_{v} do not depend on the particular set of parameters chosen. This is the case when the variable **Y** is the steady state metabolite concentration or flux vector, but is not necessarily true for other variables. As was shown above, MCA of steady state metabolite concentrations and fluxes may be described in terms of the C_{v} only , i.e. without explicit mention to parameters. Yet the effect of particular parameters can be asserted from eq(3.7) and (3.12) (which are particular cases of eq(3.20)).

3.3) Parameter-unspecified control coefficients: time-dependent systems

An extension of MCA to time-dependent systems was given in chapter 2. There the assumptions of additivity and independence were made. In this section, we shall briefly show some of the difficulties encountered when trying to extend time-dependent control analysis to parameters that are not proportional to the rate. Here (as in section 3.2 and in contrast to section 2.8.1), we make no assumptions about the functional relationship between the rate vector **v** and the parameter vector **p**. The definitions of vectors and matrices used below are given in Table 3.1. Following a similar procedure as in section (

2.8.1), we obtain an equation analogous to eq(2.54):

$$ {}^{u}\dot{C}^{s}_{p} = N \left(J \, \pi^{v}_{p} + \frac{\partial v}{\partial s} \, {}^{u}C^{s}_{p} \right) \qquad (3.21) $$

If the π-elasticity matrix π^{v}_{p} is invertible we can postmultiply eq(3.21) by its inverse obtaining:

$$ {}^{u}\dot{C}^{s}_{p} \, (\pi^{v}_{p})^{-1} = N \left(J + \frac{\partial v}{\partial s} \, {}^{u}C^{s}_{p} (\pi^{v}_{p})^{-1} \right) \qquad (3.22) $$

Using the rule for differentiation of a product it is easily shown that

$$ \overline{{}^{u}C^{s}_{p} (\pi^{v}_{p})^{-1}}^{\cdot} = {}^{u}\dot{C}^{s}_{p} (\pi^{v}_{p})^{-1} + {}^{u}C^{s}_{p} \overline{(\pi^{v}_{p})^{-1}}^{\cdot} \qquad (3.23) $$

and combining this equation with eq(3.22) we obtain

$$ \overline{{}^{u}C^{s}_{p} (\pi^{v}_{p})^{-1}}^{\cdot} = N \left(J + \frac{\partial v}{\partial s} \, {}^{u}C^{s}_{p} (\pi^{v}_{p})^{-1} \right) + {}^{u}C^{s}_{p} \overline{(\pi^{v}_{p})^{-1}}^{\cdot} \qquad (3.24) $$

Eq (3.24) is the counterpart to eq(3.6) when time-dependent metabolic systems are considered. At this point we could make an attempt to introduce parameter-unspecified control coefficients. Following this line of thought we define

$$ {}^{u}C^{s}_{v} \equiv {}^{u}C^{s}_{p} (\pi^{v}_{p})^{-1} \qquad (3.25) $$

Substituting eq(3.25) into (3.24) we finally obtain:

$$ {}^{u}\dot{C}^{s}_{v} = N \left(J + \frac{\partial v}{\partial s} {}^{u}C^{s}_{v} \right) + {}^{u}C^{s}_{p} \overline{\left(\dot{\pi}^{v}_{p} \right)^{-1}} \qquad (3.26) $$

The last term in the right hand side of eq(3.26) still depends explicitely on the π-elasticity matrix and on the parameter control coefficient matrix. It is therefore clear that by this simple procedure we have not succeeded to describe the control behaviour of the system by the parameter-unspecified coefficients only. However, in the case where π^{v}_{p} is constant in time the last term in eq(3.26) disappears. In this particular case eq(3.26) has the same form as eq(2.54), the only difference being that it is expressed in terms of $\mathbf{C_v}$. As a consequence, all the relationships following eq(2.54) in Chapter 2 (summation, connectivity, etc) are valid substituting $\mathbf{C_E}$ by $\mathbf{C_v}$. This is the only case where we can describe the control properties by parameter-unspecified coefficients. It is also important to note that if this is not the case the partition response theorem of the steady state (eq(3.15)) is not valid for time-dependent processes. I can only conclude that if a general description in terms of parameter-unspecified coefficients for time-dependent systems is to be developed, a different definition from eq(3.25) is required.

Chapter 3 is a sequel of the paper: Kacser, H., Sauro, H. and Acerenza, L. (1990) Eur. J. Biochem. **187**, 481. Most of the results appearing in this paper were developed by Kacser and Sauro before I joined the 'Edinburgh Group' and I only participated in the discussions in the last stages of this work. It would be difficult to assess what my particular contribution to this work was. Therefore, in chapter 3 I only include some aspects of the subject that were not considered in the paper.

Table 3.1 : Definitions of vectors and matrices.

Symbol	Name (*)	Dimension (**)	Element ij (***)	Equation(****)
$\mathbf{N}$	Stoichiometry (M)	s x v	n_{ij}	Eq (2.1)
$\mathbf{s}$	Metabolite concentration (V)	s x 1	S_i	Eq (2.3)
$\dot{\mathbf{s}}$	Time derivative of $\mathbf{s}$ (V)		$\dot{S}_i$	------
$\mathbf{v}$	Rate (V)	v x 1	v_i	Eq (2.2)
$\mathbf{j}$	Flux (V)	v x 1	J_i	Eq (2.4)
$\mathbf{S}$	Metabolite concentration (M)	s x s	$S_{ii} = S_i$, $S_{ij} = 0$ $(i \neq j)$	------
$\mathbf{J}$	Flux (M)	v x v	$J_{ii} = J_i$, $J_{ij} = 0$ $(i \neq j)$	------
$\frac{\partial \mathbf{v}}{\partial \mathbf{s}}$	Metabolite concentration derivative of $\mathbf{v}$ (M)	v x s	$\frac{\partial v_i}{\partial s_j}$	Eq (2.17)
$\mathbf{p}$	Parameter (V)	v x 1	p_j	------
$\frac{\partial \mathbf{v}}{\partial \mathbf{p}}$	Parameter derivative of $\mathbf{v}$ (M)	v x v	$\frac{\partial v_i}{\partial p_j}$	------
$\frac{\partial \mathbf{s}}{\partial \mathbf{p}}$	Parameter derivative of $\mathbf{s}$ (M)	s x v	$\frac{\partial S_i}{\partial p_j}$	------
$C_{\mathbf{p}}^{\mathbf{s}}$	Concentration control coefficient (M)	s x v	${}^{u}C_{p_j}^{S_i}$	------
$C_{\mathbf{p}}^{\mathbf{j}}$	Flux control coefficient (M)	v x v	${}^{u}C_{p_j}^{J_i}$	------

C^{s}_{v}	p-unspecified concentration control coefficient (M)	s x v	$C^{S_i}_{v_j}$	Eq (3.7)
C^{j}_{v}	p-unspecified flux control coefficient (M)	v x v	$C^{J_i}_{v_j}$	Eq (3.12)
${}^{u}C^{s}_{p}$	Unscaled concentration control coefficient (M)	s x v	${}^{u}C^{S_i}_{p_j}$	------
${}^{u}\dot{C}^{s}_{p}$	Time derivative of ${}^{u}C^{s}_{p}$ (M)	s x v	${}^{u}\dot{C}^{S_i}_{p}$	------
${}^{u}C^{s}_{v}$	Unscaled p-unspecified concentration control coefficient (M)	s x v	${}^{u}C^{S_i}_{v_j}$	Eq (3.25)
${}^{u}\dot{C}^{s}_{v}$	Time derivative of ${}^{u}C^{s}_{v}$ (M)	s x v	${}^{u}\dot{C}^{S_i}$	Eq (3.26))
ε^{v}_{s}	ε-elasticity coefficient (M)	v x s	$\varepsilon^{v_i}_{S_j}$	Eq (2.18)
π^{v}_{p}	π-elasticity coefficient (M)	v x v	$\pi^{v_i}_{p_j}$	Eq (1.3)
$I_{s\,x\,s}$	Identity (M)	s x s	$d_{ii}=1$, $d_{ij}=0$ $(i\neq j)$	------
$I_{v\,x\,v}$	Identity (M)	v x v	$d_{ii}=1$, $d_{ij}=0$ $(i\neq j)$	------

(*) Name of the vector (V) or matrix (M).

(**) Dimension of the vector or matrix, where " s " is the number of metabolites and " v " the number of rates.

(***) Element of row " i " and column " j " of the vector or matrix.

(****) Equation where the elements, vector or matrix are introduced or defined.

Chapter 4

On the consequences of large changes: the Co-ordinate Control Operation *

4.1) Introduction

In the previous chapters we study the sensitivity of metabolic variables to small (infinitesimal) changes in the values of individual parameters. In what follows, a different method from modulating individual parameters (Co-ordinate Control Operation, CCO) will be described. It applies to time-dependent systems and has the advantage of not being restricted to small (infinitesimal) changes. First, we assume, for each enzyme measured in isolation, the validity of the steady state approximation and the proportionality between reaction rate and enzyme concentration. Under these assumptions the time-dependent and time-invariant variables are classified according to the relationships they fulfil when the CCO is applied. A method is given to test these relationships in experimental systems and to quantify deviations from the predicted behaviour, when the assumptions are not fulfilled.

4.2) Parameters and variables

A metabolic system is, basically, a network constituted of molecules, x_i , 'connected' by chemical reactions. The rates of interconversion between each pair of molecules are given by the rate

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* see footnote on page 79

laws v_k . These may be functions of the concentrations, x_i, involved (free metabolites, free enzymes, enzyme-metabolite complexes, enzyme-enzyme complexes, etc), temperature, pressure, pH, ionic strength, etc.

The 'parameters' of the system are the quantities that can be manipulated independently of each other. Once their values are fixed at the initial point of time, they remain constant during the whole interval of time that the system is studied. In what follows, we consider as parameters the total concentration of each enzyme (free plus complex forms) and physicochemical quantities such as temperature and pressure. The fluxes or free concentrations that act as inputs of the system (e.g. sources and sinks of matter and external effectors) are either held constant or changed in time in some pre-determined way (an example of the later situation is given in Markus et al., 1984).

The 'variables' of the system are the quantities whose values depend on the values of the parameters. We may define two types of variables : time-dependent and time-invariant, whether their magnitudes do or do not change with time respectively. The instantaneous concentrations x_i , mentioned above, and other quantities that are functions of these concentrations (e.g. fluxes), are time-dependent variables. The succesive values that they take in time depend on the values to which the parameters and initial concentrations are set at the initial time. Examples of time-invariant variables are: the stable steady state concentrations and fluxes, the transition time of a metabolite (Heinrich and Rapoport, 1975) or a pathway (Easterby, 1981, 1986) to a stable steady state, the period and amplitude of variables that exhibit sustained oscillations (Hofmann et al., 1985; Goldbeter & Moran, 1987 ; Mizraji et al., 1988) and the maximun Lyapunov exponent that characterizes a chaotic regime (Hess & Markus, 1987).

First, we centre the attention on the time-dependent variables

x_i. The change of each x_i with time, dx_i/dt, may be written as the balance of all the rates, v_k, that affect its concentration directly:

$$\frac{dx_i}{dt} = \sum_{k=1}^{p} n_{ik} v_k \qquad i = 1, \ldots, q \qquad (4.1)$$

Here n_{ik} is the stoichiometric coefficient of the molecule x_i in the reaction k. It is positive, negative or zero if x_i is produced, consumed or not altered directly by the rate v_k respectively. For a given set of values of the parameters and initial concentrations, the solutions of eqs (4.1) constitute the time courses of the concentrations: $x_i(t)$, $i=1,\ldots,q$. Introducing these $x_i(t)$ into the rate laws v_k , we obtain the time courses of the fluxes: $J_k(t)$, $k=1,\ldots,p$.

The values of the time-invariant variables may be obtained from the time courses of the time-dependent variables. For example, when a system settles to a stable steady state, an estimate of the steady state values of metabolite concentrations and fluxes may be obtained from the time courses, waiting a 'long enough' period of time. In a system that exhibits sustained oscillations in time, the period of oscillation (the interval of time between two consecutive maxima in the time course) is such a time-invariant variable.

4.3) The Co-ordinate Control Operation

Changes in the values of the parameters affect, to varying degrees, the values of the variables (control of variables by parameters). For a time-dependent variable, one may define a 'reference time course' generated by a chosen set of values of the parameters, the 'reference parameter values'. If one or more of the reference parameter values are altered at the initial time, the resulting time course may be significantly different from the reference one. In

what follows, we shall study the control of the variables by a particular group of parameters: the total enzyme concentrations, E_j. We assume that all the m enzyme concentrations are simultaneously changed by the same arbitrary factor α (not necessarily ≈ 1). If $E_{j,r}$ (j=1,...,m) are the values of the enzyme concentrations that generate the reference time course, then the new time course is obtained using enzyme concentrations $E_{j,\alpha}$ (j=1,...,m) given by:

$$E_{j,\alpha} = \alpha E_{j,r} \qquad (4.2)$$

We call this equal and simultaneous change in all the enzyme concentrations: the coordinate-control operation (abbreviated: CCO) (briefly introduced in Acerenza et al, 1989; Acerenza, 1989). The subscripts r and α will be used to indicate the value of a parameter or variable before (reference) and after the CCO is applied respectively. We shall use this operation throughout the following treatment. This approach will reveal certain simple properties of time-dependent metabolic systems, when some assumptions are made (see below). Furthermore we suggest how the resulting relationships, and hence the assumptions made, may be experimentaly tested in reconstituted systems or biological extracts. We shall discuss the practical problems associated with attempting to apply a CCO in a later section.

4.4) Assumptions

We now make some assumptions concerning the properties of the metabolic system. These shall be used to derive some theoretical consequences of the CCO in sections (4.5) and (4.6). The analysis of cases where there is a breakdown of the assumptions is considered in sections (4.9) to (4.11).

In the general case (see section (4.2)), the q concentrations

x_i that appear in eqs (4.1) may be classified in two groups: n free metabolite concentrations, S_i, and q-n enzyme concentrations in their different forms (free or combined with metabolites), C_i. If the steady state approximation for the concentrations C_i is plausible , then $d C_i / d t = 0$ for each C_i (Segel, 1988). Applying these conditions to eqs (4.1), the reduced resulting system of differential equations,

$$\frac{d S_i}{d t} = \sum_{j=1}^{m} n_{ij} \; v_j \; , \qquad i = 1 , \ldots , n \; , \qquad (4.3)$$

involves only the free metabolite concentrations as variables. In addition, we assume that the rates v_j are proportional to the corresponding total enzyme concentrations E_j,

$$v_j = E_j \; f_j \; , \qquad j = 1 , \ldots , m \; , \qquad (4.4)$$

where f_j are functions of the concentrations S_i and parameters, and are independent of enzyme concentrations and time. The Michaelis-Menten rate equation, for example, fulfills eq (4.4).

Applying the CCO to a metabolic system whose rates are given by equation (4.4), the resulting rates, $v_{j,\alpha}$, are related to the reference rates, $v_{j,r}$ (see eq (4.2)), as follows,

$$v_{j,\alpha} = \alpha \; v_{j,r} \; , \qquad j = 1 , \ldots , m \; . \qquad (4.5)$$

Then, under the assumptions described by eqs (4.3) and (4.4), the first important consequence of the CCO is to multiply each term of the right hand member of eqs (4.3) by the same factor α. It is important to note that, if matter is introduced into the system via one or more constant input fluxes, these should also be modified

according to eq (4.5) when applying the CCO. However, any constant (input) concentrations, if present, should not be modified when the rate that transforms them is given by eq (4.4). The discussion of the case where the inputs are changed in time, by the experimentalist, is postponed to section (4.5).

4.5) Co-ordinate Control of time-dependent variables

In this section, we outline some consequences of the coordinate-control operation, related to the control of time-dependent variables. Some mathematical details of this treatment are given in chapter 2.

Combining eqs (4.3) and (4.5) we obtain the relationship between the derivative of S_i with respect to time after and before (reference) the CCO:

$$\left(\frac{d S_i}{d t} \right)_{\alpha} = \alpha \left(\frac{d S_i}{d t} \right)_{r} \qquad (4.6)$$

The only effect of a simultaneous change in all the enzyme concentration by a factor α, is to make the metabolite concentrations change at a rate that is α times the original one. Then, the CCO is equivalent to a change in the time scale of the time courses of the metabolite concentrations. For each time t_r of the reference time courses (the reference time courses are the functions of time, $S_i(t)$, obtained with the reference parameter values) there exists one time t_α, in the new time courses, that has the same value for all the metabolite concentrations. The value of t_α is given by:

$$t_\alpha = \frac{t_r}{\alpha} \qquad (4.7)$$

and, hence,

$$S_{i,\alpha}(t_r/\alpha) = S_{i,r}(t_r) \qquad (4.8)$$

From eqs (4.5) and (4.8) we obtain the analogous relationship for the fluxes:

$$J_{j,\alpha}(t_r/\alpha) = \alpha\ J_{j,r}(t_r) \qquad (4.9)$$

Eqs (4.8) and (4.9) tell us that when applying the CCO to a time-dependent metabolic system, which satisfies the assumptions made in eqs (4.3) and (4.4), the instantaneous values of the metabolite concentrations are 'shifted' from the time t to t/α, while, the instantaneous values of the fluxes are multiplied by the factor α and 'shifted' from t to t/α. It is important to note that, if matter is introduced in the system via concentrations or fluxes that change in time, these inputs should be altered in the same way as shown by eqs (4.8) and (4.9) respectively, when the CCO is applied.

An immediate consequence of eq (4.8) is that if one plots the metabolite concentrations after the CCO against α multiplied by time, the resulting curve should coincide with the reference time course (see Figs (4.1a) and (4.1b)). This result will be used in section (4.8). (A similar procedure is used as a test for inactivation of a single enzyme during assay, Selwyn, 1965, see also Cornish-Bowden, 1979).

Scheme (4.1): $X_0 \xrightarrow{v_1} S \xrightarrow{v_2} X_1$

4.6) Co-ordinate Control of time-invariant variables:

4.6.1) with dimension of time .

Time-invariant variables with dimension of time, that characterize some temporal aspect of the time course of the metabolite concentrations satisfy eq (4.7). Examples of these variables are transition times, period of oscillation and the reciprocal of the maximum Lyapunov exponent (mentioned in section (4.2)). Then if T_r is the value of such a time-invariant variable with dimension of time, obtained from the reference time-course, after the CCO the new value of the variable, T_α , is given by:

$$T_\alpha = \frac{T_r}{\alpha} \qquad (4.10)$$

That is, a simultaneous increase (decrease) in all enzyme concentrations by a factor α, causes a decrease (increase), in the value of T, by the same factor.

4.6.2) of a transition to a stable steady state .

If the metabolic system is one that approaches a stable steady state, after a long enough period of time the variables exhibit approximately constant values independent of time. Therefore, eqs (4.8) and (4.9) take the form,

$$S_{i,\alpha}^{ss} = S_{i,r}^{ss} \qquad (4.11)$$

$$J_{j,\alpha}^{ss} = \alpha \; J_{j,r}^{ss} \qquad (4.12)$$

where the superscript ss indicates steady state values. Eqs (4.11) and (4.12) show the effect that the CCO has on the steady state

metabolite concentrations and fluxes.

To characterize the transition between the initial conditions and the steady state one may use the transition time as defined by Easterby (1981). This is, of course, a time-invariant variable that behaves according to eq (4.10) when the CCO is applied.

4.6.3) of sustained oscillations .

Here we consider the situation where the time-dependent variables (metabolite concentrations and fluxes) exhibit stable oscillations in time. In this type of behaviour, the values of the time-dependent variables repeat at constant intervals of time, T (period of oscillation). Two time-invariant variables are frequently used to characterize oscillatory phenomena: the period and amplitude. The period is a time-invariant variable with dimension of time, and when the CCO is applied, it behaves as shown in eq (4.10). The amplitude of oscillation (A_{S_i}) for a metabolite concentration S_i may be defined as half the difference between the maximum (S_i^{max}) and minimum (S_i^{min}) values: $A_{S_i} = (S_i^{max} - S_i^{min}) / 2$. The maximum and minimum values, and hence the amplitude, depend on the reference parameter values. If we apply the CCO, and wait until the system settles to a new stable oscillation, even though the value of the period is modified as described by eq (4.10), the maximum and minimum values of the metabolite concentrations are not altered (see eq (4.8)):

$$\begin{aligned} S_{i,\alpha}^{max} &= S_{i,r}^{max} \\ S_{i,\alpha}^{min} &= S_{i,r}^{min} \end{aligned} \qquad (4.13)$$

Introducing eqs (4.13) into the definition of amplitude we immediatly

obtain:

$$A_{s_i,\alpha} = A_{s_i,r} \qquad (4.14)$$

i.e. the value of the amplitude is unaffected by the CCO. The fluxes J_j may be calculated by introducing the metabolite concentrations into the rate equations given in eq (4.4). If the metabolite concentrations are periodic functions of time, with period T, the corresponding fluxes are periodic functions of time with the same period. Therefore, when applying the CCO, the period of these fluxes is also modified according to eq (4.10). The metabolite concentrations corresponding to the maximum and minimum fluxes are not modified and, therefore, the same applies to the values of f_j, at these points, introduced in eq (4.4). However, as the rates are proportional to enzyme concentration, even if the values of f_j are unaltered, the new maximum and minimum values of the flux are α times those of the reference oscillation:

$$\begin{aligned} J_{j,\alpha}^{max} &= \alpha \; J_{j,r}^{max} \\ J_{j,\alpha}^{min} &= \alpha \; J_{j,r}^{min} \end{aligned} \qquad (4.15)$$

The amplitude of oscillation (A_{J_j}) for a flux J_j may be defined as: $A_{J_j} = (J_j^{max} - J_j^{min}) / 2$. Combining eqs (4.15) with this definition, the relationship between the flux amplitude before and after the CCO is obtained :

$$A_{J_j,\alpha} = \alpha \; A_{J_j,r} \qquad (4.16)$$

Besides the period and amplitude, another quantity that may be used to characterize an oscillatory regime is the mean value in a cycle. The mean value of a time-dependent variable Y in an interval of time T is

defined by the expression:

$$\bar{Y} = \frac{\int_0^T Y \, dt}{T} \qquad (4.17)$$

It is important to note that we evaluate the mean value in an interval of time equal to the period of oscillation. When applying the CCO, the resulting mean value can be written as follows:

$$\bar{Y}_\alpha = \frac{\int_0^{T\alpha} Y_\alpha \, dt}{T_\alpha} \qquad (4.18)$$

In the following, we discuss the relationship between $\bar{Y}_r$ and $\bar{Y}_\alpha$, when the variable is a metabolite concentration or flux that shows stable oscillations. As was mentioned before, the period of oscillation, T, satisfies eq (4.10). If Y is a metabolite concentration, eq (4.8) is fulfilled and can be written in an equivalent way: $S_{i,\alpha}(t) = S_{i,r}(\alpha t)$. Using this equation together with eqs (4.10), (4.17) and (4.18), and properties of integrals, we finally obtain:

$$\overline{S_{i,\alpha}} = \overline{S_{i,r}} \qquad (4.19)$$

In the case where Y is a flux, eqs (4.9), (4.10), (4.17) and (4.18) are used to obtain:

$$\overline{J_{j,\alpha}} = \alpha \, \overline{J_{j,r}} \qquad (4.20)$$

In other words, the simultaneous change of all enzyme concentrations by a factor α, do not affect the mean values of the metabolite concentrations, but alters all the mean fluxes by the same factor (being the mean values evaluated in a period of oscillation). Eqs (4.19) and (4.20) may be seen as equivalent to the steady state conditions

(4.11) and (4.12) when stable oscillations are considered..

4.7) Classification of the variables

In sections (4.5) and (4.6) we considered the effect that the CCO has on time-dependent and time-invariant variables respectively. In each one of these groups we may distinguish variables that behave like a metabolite concentration (S-type) or like a flux (J-type). Such a classification may serve as a summary of the relationships established and constitutes the basis of experimental tests.

4.7.1) Time-invariant variables .

All the time-invariant variables, Y , considered in section (4.6), may be classified in two groups, S-type and J-type, according to the expected response when the system is subject to the CCO . We define as S-type time-invariant variables those that remain unaltered after the CCO:

$$S_\alpha = S_r \qquad (4.21)$$

Examples of this type of variable are: S_i^{ss} (eq (4.11)), S_i^{max} and S_i^{min} (eq (4.13)), A_{S_i} (eq (4.14)) and $\overline{S_i}$ (eq (4.19)). J-type time-invariant variables appear multiplied by the factor α when the CCO is applied:

$$J_\alpha = \alpha\ J_r \qquad (4.22)$$

and examples of this type are: J_j^{ss} (eq (4.12)), J_j^{max} and J_j^{min} (eq (4.15)), A_{J_j} (eq (4.16)) and $\overline{J_j}$ (eq (4.20)). The reciprocal of T (1 / T) also belongs to this type (see eq (4.10)).

It should be noted that, if Y is a J-type time-invariant variable, then Y/α behaves like a S-type time-invariant variable (see eqs (4.21) and (4.22)).

4.7.2) Time-dependent variables.

The time-dependent variables may also be classified as S-type or J-type depending on the predicted behaviour when the CCO is applied (see section (4.5)). The effect of the CCO on a S-type time-dependent variable is simply expressible as an alteration in the time scale of its time course :

$$S_\alpha (t/\alpha) = S_r (t) \qquad (4.23)$$

The time-dependent metabolite concentrations are S-type variables (eq (4.8)). In the case of J-type variables, the CCO scales simultaneously the time and the variable according to the following relationship:

$$J_\alpha (t/\alpha) = \alpha \; J_r (t) \qquad (4.24)$$

Examples of J-type variables are the time-dependent fluxes (eq (4.9)).

We therefore see that the values of a S-type (J-type) time-dependent variable, corresponding to different α and identical αt, behave like the values of a S-type (J-type) time-invariant variable. Similarly to the case of time-invariant variables, if Y is a J-type time-dependent variable, Y/α behaves like a S-type time-dependent variable .

4.8) Test of the general relationships

The data obtained from a coordinate-control operation experiment may be used to test the general relationships summarized in section (4.7) (eqs (4.21) to (4.24)). Here we propose simple plots to test these relationships. Depending on whether the variables analysed are time-dependent or time-invariant, the procedures are slightly different.

4.8.1) Time-invariant variables .

To test the behaviour of a time-invariant variable, Y, the basic experimental information needed is a table with the values of the variable corresponding to different α values (Y_α vs α).

The 'direct coordinate-control plot' (D-plot) is simply the plot: Y_α / Y_r against α. Y_r is the value of the variable when $\alpha = 1$ (reference point). The expected result of a D-plot for a S-type variable is a straight line where the quotients, Y_α / Y_r , are equal to one for all α (see eq (4.21) and Fig (4.2)). J-type variables should give a straight line, with tangent one (45 degrees), that, extrapolated to α equal to zero, passes through the origin (see eq (4.22) and Fig 4(.2)). If the D-plot is not as expected, the variable shows departures from the theoretical relationship (eq (4.21) for S-type and eq (4.22) for J-type variables). Provided no systematic errors in applying the CCO have been introduced (see section (4.12)), this result should be interpreted as a breakdown of the assumptions introduced in section (4.4). On the other hand, if the D-plot is as expected, the deviations from the assumptions, if they exist, do not contribute significantly to the behaviour of the variable when the enzymes are changed.

4.8.2) Time-dependent variables.

To test eqs (4.23) and (4.24) the experimental information needed is the time courses for different values of α, namely $Y_\alpha(t)$. Based on the properties of time-dependent variables, discussed in section (4.7), we suggest to plot the data in a 'rescaling coordinate-control plot' (R-plot). The R-plot for S-type variables is: $S_\alpha(t)$ against αt. If the variable behaves according to eq (4.23), the plots for different α should appear superimposed on the reference curve ($\alpha = 1$) (see Fig (4.1)). Similarly, in the case of J-type variables, the R-plot is: $J_\alpha(t)/\alpha$ against αt. As a consequence of eq (4.24), the curves corresponding to the different time courses $J_\alpha(t)$, should appear superimposed on the reference time course $J_r(t)$ (where $\alpha = 1$) in the R-plot (not shown). If such an R-plot does not give coincident curves, this would be an indication that the assumptions made in section (4.4) are not fulfilled, and that these deviations contribute significantly to the behaviour of the system variable when the enzymes are modified.

As was mentioned in section (4.7) the values of a S-type (J-type) time-dependent variable, corresponding to different α and identical αt, behave like the values of a S-type (J-type) time-invariant variable. Therefore, a D-plot may be constructed with these values.

What we have called the R-plot , for S-type time-dependent variables, is similar to a test used to detect inactivation of an enzyme during assay (Selwyn, 1965).

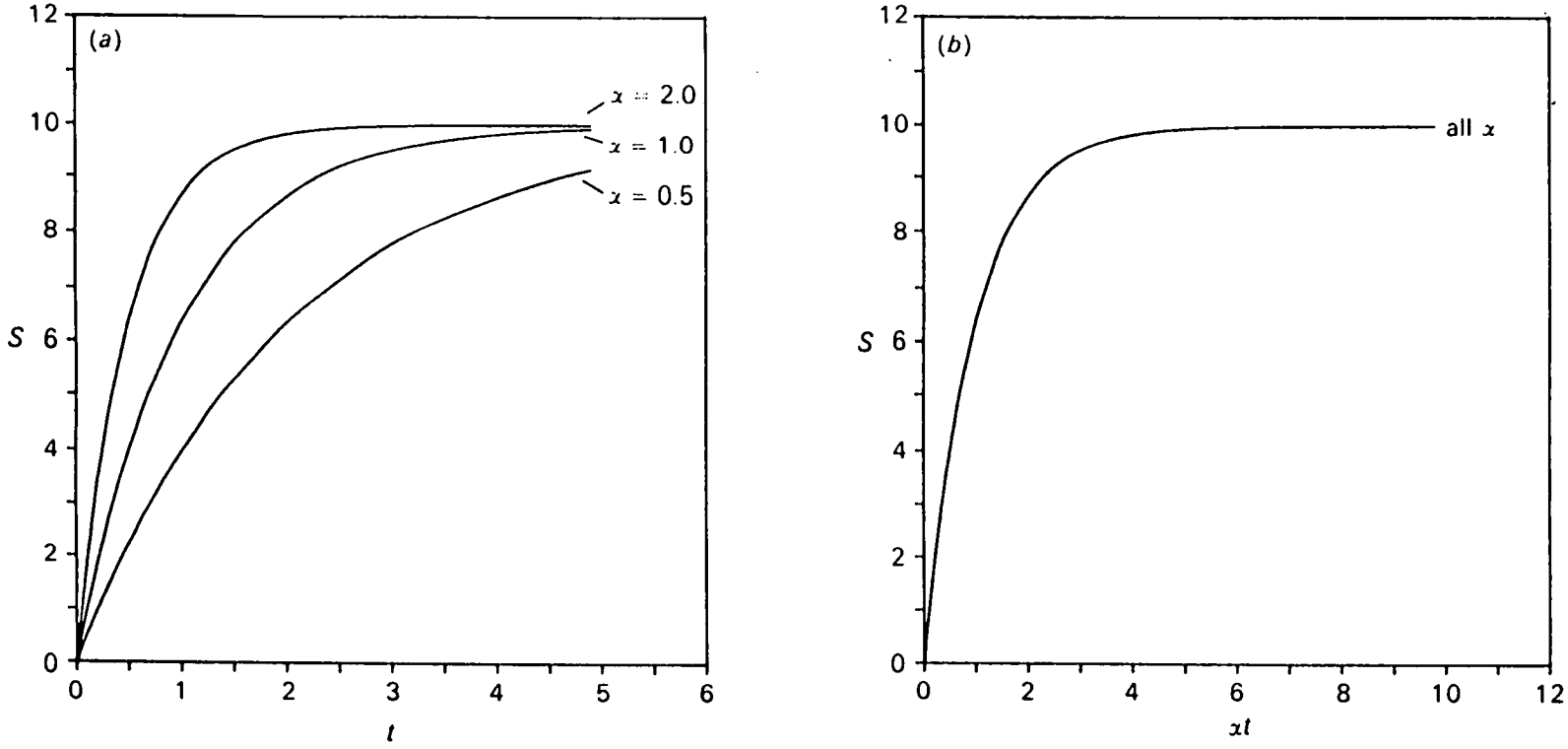


Fig (4.1). Example where assumptions (eqs (4.3) and (4.4)) are valid . a) Concentration of S vs time, and b) R-plot.

In a), we show time courses of the concentration of S (scheme (4.1)) corresponding to different α values. The concentrations of X_0 and X_1 are constant . The rate laws v_1 and v_2 are proportional to the corresponding enzyme concentrations. In b), we plot the same ordinate values as in a), but against α multiplied by time. In this rescaling plot the three curves coincide. It shoud be noted that, due to the rescaling procedure, the curves, in a), corresponding to $\alpha = 0.5$, 1.0 and 2.0 end in b) at $\alpha t = 2.5$, 5.0 and 10.0 respectively. (In Figs (4.3a) and (4.3b) we show the same types of plots in a situation were assumption eq (4.4)) is not valid).

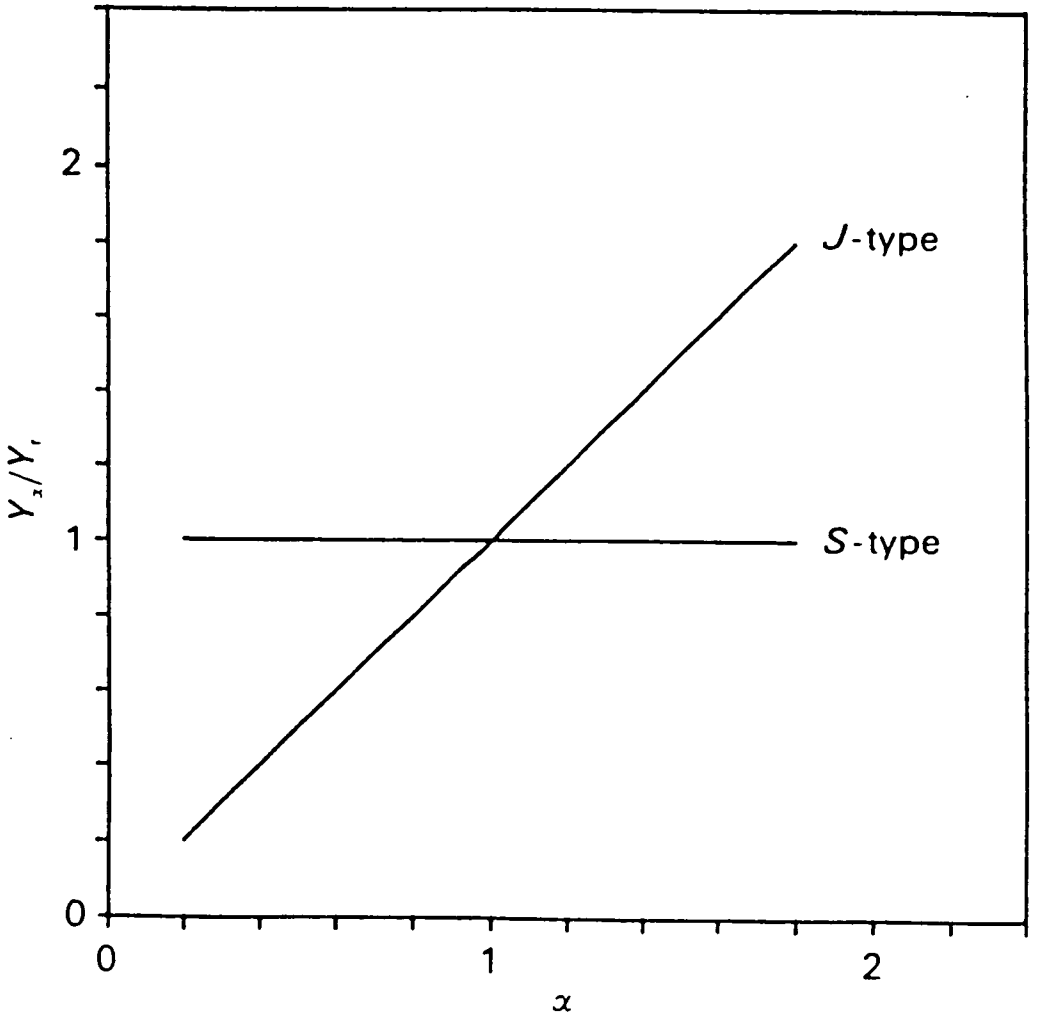


Fig (4.2). D-plots. Y_α / Y_r against α . when eqs (4.3) and (4.4) apply.

The horizontal straight line is the D-plot of a S-type variable while the straight line of 45 degrees corresponds to a J-type variable.

4.9) Breakdown of the assumptions

The relationships derived in sections (4.5) and (4.6), and summarized in section (4.7), are based on the assumptions introduced in eqs (4.3) and (4.4). If the steady state approximation for the different forms of the enzyme concentrations is not valid or the rates are not proportional to the corresponding enzyme concentrations, the system variables may exhibit significant deviations from the predicted behaviour (eqs (4.21) to (4.24)) when applying the CCO. Here we enumerate some enzyme mechanisms that are known to violate those assumptions and, when embedded in a metabolic network, are potential generators of deviations.

Many proteins described in the literature have a quaternary structure. Depending on the experimental conditions, more than one polymeric form may coexist in significant amount. If a protein with catalytic function shows these structural features, it constitutes a candidate to generate rate laws that do not behave as eq (4.4). (see e.g. Kurganov, 1978). The simplest example of association-dissociation between homologous subunits is the monomer-dimer equilibrium. In section (4.11) we shall analyse some effects of this type of mechanism on transients to a stable steady state. The existence of associations between different enzymes (heterologous associations) may generate rate laws that depend on more than one enzyme concentration, showing departures from eq (4.4). Some consequences of homologous and heterologous associations on the control properties of steady state metabolic concentrations and fluxes have recently been addressed (Kacser et al., 1989; Sauro & Kacser, 1989).

The time courses of some enzymatic reactions show 'lags' or 'bursts' under normal assay conditions (see e.g. Neet & Ainslie, 1980). This phenomenon is associated with the existence of slow conformational transitions in the enzyme mechanism. In these cases

the steady state approximation is not valid and, therefore, it is not possible to express the behaviour in time by eqs (4.3). The existence of slow conformational changes may have mayor effects on the control of the time course of a variable, while having no effect on the control properties of the steady state of the system.

Some concentrations of metabolites within a system appear to be linked by conservation constraints (e.g. NAD + NADH = constant). If the total concentrations of enzymes are negligible with respect to the concentrations of conserved metabolites to whom they bind, the steady state approximation is valid. In this frequently considered situation, as there is no significant sequestration of the conserved metabolites by the enzymes, when the CCO is applied, eq (4.4) is also valid.. Even if the total concentrations of the enzymes are of the same order as the conserved metabolite concentrations, the steady state assumption may still be satisfied, provided that those concentrations are much smaller than the Michaelis constant (see: Segel, 1988). Due to the low 'affinity' (large Michaelis constant) between enzyme and metabolite, the fraction of the metabolite under complex form is still small. If, however, the total concentrations of the enzymes and the metabolites are of the same order, but greater than the Michaelis constant , the validity of the steady state assumption is no longer ensured. Furthermore, in this conditions, there is considerable sequestration of the conserved metabolites, and we may expect significant deviations in the system variables when the CCO is applied.

The enzyme mechanisms mentioned above may be responsible for the appearence of departures from the quantitative relationships derived in sections (4.5) and (4.6). Furthermore, they may be the cause of a qualitative change in the dynamics of the system, if a 'bifurcation point' is reached when applying the CCO. Such situation, e.g., may transform a sustained oscillation into a stable steady state , or vice versa .

4.10) Quantification of the deviations

The D-plots and R-plots may be used to test the existence of deviations from the predicted relationships (eqs (4.21) to (4.24)). Such a case is illustrated in Figs (4.3a) and (4.3b). Here we introduce additional plots to assess the quantitative importance of the deviations. These plots constitute a phenomenological description of the deviations. Furthermore, as we shall see, they may be useful in the search of the origin of the deviations.

In section (4.7), we discussed two properties of metabolic variables: a) if Y is a J-type variable (time-dependent or time-invariant), then the values of the variable divided by α, Y/α, behave like a S-type variable (time-dependent or time-invariant respectively), and b) if $Y(t)$ is a time-dependent variable (S-type or J-type), then the values of the variable for the same αt and different α, $Y_\alpha(t)$, behave like a time-invariant variable (of the same type). This properties allow us to transform the values of any of the variables, described in section (4.7), into the values of a S-type time-invariant variable. If we want, for example, to compare the deviations of a S-type variable with those of a J-type variable, or to compare the deviations of a time-dependent variable corresponding to different time points, such transformtions should be applied. The plots, that we shall introduce in this section, are defined for S-type time-invariant variables. However, they may also be used in the analysis of other types of variables, applying the appropriate transformations described above.

To quantify deviations, the data from a CCO experiment may be plotted in, at least, two different ways. In what follows we define and discuss two plots, that we call: 'reference point sensitivity

coordinate-control plot' (RPS-plot) and 'point to point sensitivity coordinate-control plot' (PPS-plot).

4.10.1) Reference point sensitivity coordinate-control plot (RPS-plot)

The RPS-plot is established to characterize the changes of a variable with respect to a unique reference point (point corresponding to $\alpha = 1$). We define a deviation function d_r (for a S-type time-invariant variable) as: $d_r = [(S_\alpha - S_r) / S_r] / (\alpha - 1)$. A plot of d_r against α represents the relative change in the value of the variable, with respect to the reference value (S_r), per relative change in the enzyme concentrations, $\alpha - 1$ (see eq (4.2)), when the CCO is applied. The ordinate values in this plot would constitute a measure of the quantitative importance of the deviations, for different α changes. The sign of the ordinate values is positive or negative if the change in the variable is in the same or opposite direction to the change in the enzyme concentrations respectively. In the absence of deviations, the ordinate values d_r are equal to zero, for all α.

4.10.2) Point to point sensitivity coordinate-control plot (PPS-plot)

In the previous (RPS) plot we used a unique reference point. Alternatively, it is possible to establish a plot where each set of enzyme concentrations serve succesively as the reference point.

Let E_{n-1}, E_n and E_{n+1} ($E_{n-1} < E_n < E_{n+1}$) be three consecutive values of the concentration of anyone enzyme, and S_{n-1} , S_n and S_{n+1} the corresponding values of a S-type time-invariant variable, resulting from the application of the CCO. The point E_n is momentarily considered as the reference point. The relative change in

the variable per relative change in the enzyme concentration, from E_n to E_{n+1} , is: $d_{+1}=[(S_{n+1} - S_n)/S_n]/[(E_{n+1}-E_n)/E_n)]$. Similarly, the relative change in the variable per relative change in the enzyme concentration, from E_n to E_{n-1} , is: $d_{-1}=[(S_{n-1} - S_n)/S_n]/[(E_{n-1}-E_n)/E_n)]$. If the increase and decrease of the enzyme concentration from the reference point are equal (i.e. $E_{n+1}-E_n = E_n-E_{n-1}$), then the relative change in the variable per relative change in the enzyme concentration at the reference point may be estimated by the simple arithmetic mean: $d_n=(d_{+1}+d_{-1})/2$. From p experimental points, p-2 values of d_n may be calculated (d_2 to d_{p-1}).

We define the PPS-plot as: d_n against E_n. The ordinates in this plot may be considered as an estimate of the deviation in the variable corresponding to each E_n when the CCO is applied. In the absence of deviations the ordinates are equal to zero. The signs of the ordinates are positive or negative if the change in the variable is in the same or opposite direction to the change in the enzyme concentrations respectively.

It should be noted that if the experimental data are given as S_α against α, the relative changes in the enzyme concentrations may be calculated directly from the values of α: $(E_{n+1}-E_n)/E_n = (\alpha_{n+1}-\alpha_n)/\alpha_n$ and $(E_{n-1}-E_n)/E_n = (\alpha_{n-1}-\alpha_n)/\alpha_n$ (see eq (2)). In addition, the value of d_n may be plotted against α_n.

The arithmetic mean used above to calculate d_n may not be a good estimation when $E_{n+1}-E_n \neq E_n-E_{n-1}$. In this case we propose to use $d_n = [(E_{n+1}-E_n)\, d_{-1} + (E_n-E_{n-1})\, d_{+1}]/(E_{n+1}-E_{n-1})$. This weighted mean is equivalent to obtain the value of d_n by linear interpolation between d_{+1} and d_{-1} . Here the enzyme concentrations may also be substituted by the corresponding α values without changing the results.

There is a link between the values of the ordinates in a PPS-plot and the summation relationships of control analysis. This is given in the appendix. The construction of these plots is illustrated in section (4.11).

4.11) Example

Here we show, by way of simulation, how the proposed plots can be used to test and quantify deviations. Although the example chosen is of a monomer-dimer equilibrium (eq (4.4) is violated), the same treatment can be applied to any of the deviations discussed in the previous section.

We consider one metabolic pathway, whose structure is represented in scheme (4.1). The first step is catalyzed by an enzyme that presents a monomer-dimer equilibrium. X_0 and X_1 are the constant source and sink concentrations respectively. S is the only metabolite whose concentration is free to move. The rate for the first step is:

$$v_1 = a_m M + 2 a_d D \qquad (4.25)$$

where a_m and a_d are the specific activities of the monomer and dimer subunits, respectively. The total concentrations of monomer and dimer, M and D, appearing in eq (4.25), are given by: $M = [-1 + (1 + 8\,\mathrm{Kapp}\,E_1)^{1/2}] / (4\,\mathrm{Kapp})$ and $D = \mathrm{Kapp}\,M^2$. E_1 is the total enzyme concentration expressed in monomer units ($E_1 = M + 2D$). Kapp (the apparent equilibrium constant), a_m and a_d depend on the concentration of the substrate X_0, and are independent of E_1. It should be noted that, if $a_m = a_d$, then v_1 is proportional to E_1, and eq (4.4) is fulfilled. Here we consider situations where this is not

the case.

The second step, in scheme (4.1), is catalyzed by an irreversibe Michaelis-Menten enzyme,

$$v_2 = \text{kcat}\ E_2\ \frac{S}{K_M + S} \qquad (4.26)$$

E_2 is the total enzyme concentration and kcat and K_M are constants.

The time course of the metabolite concentration is obtained solving the differential equation:

$$\frac{dS}{dt} = v_1 - v_2 \qquad (4.27)$$

where v_1 and v_2 are given in eqs (4.25) and (4.26) respectively. Fig (4.3a) shows the reference time course, $\alpha = 1$, obtained for a particular set of reference parameter values (given in the legend of Fig (4.3)), and the time courses after applying the CCO using values of α different from 1. The corresponding curves (not shown) for the flux carried by the second step (flux 2) are obtained substituting the instantaneous values of the metabolite concentration into eq (4.26).

The R-plots corresponding to Fig (4.3a) are shown in Fig (4.3b). The five curves in each R-plot do not coincide, and this fact is a positive test for the existence of deviations from the predicted relationship (eqs (4.23)). Similar results are obtained in the R-plot for flux 2 (not shown) which reveals significant deviations from eq (4.24). We are going to characterize and quantify these deviations at two different points of time: $\alpha t = 1$ and the steady state . The values $S_\alpha^{(1)}$, appearing in Table (1), are the ordinates corresponding to the abscissa $\alpha t = 1$ in the plot of Fig (4.3b). The values $J_\alpha^{(1)}$ are calculated from the ordinates ($J_\alpha^{(1)}$) / α) corresponding to the abscissa $\alpha t = 1$ of the R-plot corresponding to flux 2 (not shown).

The steady state values (S_{α}^{ss} and J_{α}^{ss}) are the constant values attained after a 'long enough time'. From the steady state values, another time-invariant variable, namely, the transition time of the system can be calculated (Easterby, 1981, 1986): $\tau = S^{ss} / J^{ss}$. Table (1) shows how this value changes with α.

The PPS-plot, calculated from the data of Table (4.1), appears in Fig (4.4). Here the deviations are different, for the different variables (concentration of S, flux2 and τ) and αt ($\alpha t = 1$ and steady state, for time-dependent variables). Due to the values chosen for the parameters, the deviations are positive. In the case of the metabolite concentrations (S-type variables) positive deviations mean that, when applying the CCO, the variable moves in the same direction as the enzyme concentrations. For the fluxes and the reciprocal of τ (J-type variables) positive deviations indicate that the change in the variable is greater than the proportional increase expected when the CCO is applied with α greater than unity. It is important to note that the deviation for S^{ss} increases with α, while the deviations for the other metabolite concentration and fluxes decrease. These properties of the PPS-plot constitute a quantitative phenomenological description of the deviations.

In what follows, we analyse how the properties of the component rates (v_1 and v_2) affect the resulting behaviour of the variables when the CCO is applied. This analysis is based on infinitessimal changes ($\alpha \approx 1$) as used in control analysis. It will provide us with an interpretation of the deviations appearing in Fig (4.4). It may be shown that, in the simple example under consideration, the ordinates of the PPS-plot for the metabolite

concentration may be estimated by:

$$D_S(t) = \left(\pi_{E_1}^{v_1} - 1 \right) C_{v_1}^{S} \qquad (4.28)$$

Here, $\pi_{E_1}^{v_1} = (E_1/v_1)(\partial v_1/\partial E_1)$. This π elasticity is equal to one when the rate v_1 is proportional to the enzyme concentration E_1 . $C_{v_1}^{S}$ $=(v_1/S)/(\partial S/\partial v_1)$ is the control coefficient. In general, this control coefficient is time-dependent, though in the limit, it represents the usual steady state value [see appendix and Kacser et al., 1989].The analogous equation for flux 2 is:

$$D_{J_2}(t) = \left(\pi_{E_1}^{v_1} - 1 \right) C_{v_1}^{J_2} \qquad (4.29)$$

It is important to note that eqs (4.28) and (4.29) are valid in this particular example, because $\pi_{E_1}^{v_1}$ is independent of time (In a more general case they must be substituted by expressions that involve integrals, what will be the subject of a forthcoming publication) . Therefore, for this particular example, the signs and magnitudes of the deviations in a PPS-plot depend on the product of two factors: a) the sign and magnitude of the deviation of the rate ($\pi_{E_1}^{v_1} - 1$) and b) the sign and magnitude of the effect that a change in the rate has on the variable ($C_{v_1}^{S}$ or $C_{v_1}^{J_2}$) . The values of these quantities are given in Table (4.2). In the case studied both factors (a) and b)) are positive, which results in a positive deviation in the PPS-plot .The deviation of the rate ($\pi_{E_1}^{v_1} - 1$) decreases with α. $C_{v_1}^{J_2}$ ($\alpha t = 1$) shows the same tendency, while $C_{v_1}^{J_2}$ (steady state) is constant and equal to one (first step is irreversible) and $C_{v_1}^{S}$ (αt = 1) increases slightly with α. This dependence with α explains the decreasing curves exhibited by the three variables under consideration (Fig(4.4)). On the other hand. $C_{v_1}^{S}$ (steady state) increases with

α in such a way that the product $C^{S}_{v_1}$ ($\pi^{v_1}_{E_1}$ - 1) increases too, being the cause of the increase in the deviation with α in the PPS-plot. It should be pointed out that the increase in $C^{S}_{v_1}$ is due to an increase in the saturation of the second enzyme with α. However, as S is built up from zero, the saturation effect is not important at the early stages of the time course ($\alpha t = 1$).

It is important to note that, even though the values of α used to construct the PPS-plot are relatively large, the deviations calculated with eqs (4.28) and (4.29), which are based on infinitessimal changes, are in reasonable agreement (see Table (4.2)) with the values of the ordinates, d_n, in Fig (4.4).

Eqs (4.28) and (4.29) illustrate that the existence of strong deviations from proportionality between rate and enzyme concentration (e.g. $\pi^{v_1}_{E_1} >> 1$) may be irrelevant to the behaviour of a metabolic variable if the magnitude of the control coefficient ($C^{Y}_{v_1}$) is small. However, in other cases, the deviation from proportionality in the rate equation may be greatly amplified, if the variable shows a high value of the control coefficient (e.g. S^{ss} in the situation shown above).

4.12) Discussion

The ideal CCO consists in the change of all the enzyme concentrations by the same factor, without any alteration in the other parameters of the experiment (Exceptions are time-invariant input fluxes and time-dependent input metabolite concentrations and fluxes, see sections (4.4) and (4.5)). It now remains to discuss how far this operation can be applied to experimental systems. As always, there are special problems which will be encountered in particular applications.

The CCO may be applied to reconstituted systems. These systems are built up by the use of component molecules that had been previously purified. They are of course much more simple than the biological systems they intend to mimic. However, they may give insight concerning, e.g., the main components and conditions needed to obtain a certain behaviour (see e.g. Eschrich et al., 1983, oscillations; Torres et al., 1989, transition time; Salerno et al., 1982, transition time). The composition of a reconstituted system is known and under the control of the experimentalist. Therefore, the CCO may, in principle, be applied to reconstituted systems in a simple way. These seem to be the most immediate experimental applications.

In the case of a biological extract, many aspects of the composition are probably not known, which makes it more difficult to apply the CCO. One way to approach this goal might be to take a fraction of the extract and make a complete enzyme inactivation (e.g. denaturation by temperature or proteolitic enzymes). Mixing the original extract and the one subjected to inactivation, in different proportions, we might obtain dilutions of the active enzyme concentrations, without altering the concentrations of the other components of the system. In the ideal conditions, the inactivator and the products of inactivation must not react with non-enzymatic components of the system. It is evident that, the agent used to inactivate the enzymes should be totally removed before the mixing is done. If there is considerable enzyme inactivation during the experiments, (spontaneous or induced by unremoved inactivator) the total enzyme concentrations may not be treated as parameters and will constitue a source of deviations. An alternative method consists of successive dilutions of the extract which would reduce all enzyme concentrations by the same factor. It is however necessary to supplement with all the metabolites which are not generated in the

system to maintain the original concentrations. Such an attempt was made by Das & Busse (1985) in studying glycolitic oscillations in yeast extracts. Although the (NAD + NADH) and (ATP + ADP + AMP) were maintained constant, other co-factors may have been altered by the dilutions. The PPS-plot for the period, which can be constructed with the data obtained from the above publication, shows both positive and negative deviations. This suggests a change of sign of the control coefficient but, in view of the experimental difficulties referred above, this interpretation may be questionable. If it is desired to extrapolate from experiments on biological extracts to the *in vivo* situation, it is important to note that in the preparation of the extract a dilution takes place. In so doing the quantitative importance of the deviations may be modified.

The application of the CCO to an *in vivo* system appears to be difficult. One may think that the use of, for example, haploid, diploid and tetraploid yeast can be a way to achieve this goal. However, in these series, the volume increases proportional to the gene ploidy, leaving most of the enzyme concentrations approximatly unchanged (Mortimer, 1958; Ciferri et al., 1969). On the other hand, some enzyme concentrations (e.g. enzymes bound to membranes) may suffer significant changes (Hilger, 1972). This situation is therefore far from what we define as CCO.

A method where enzyme concentrations can be manipulated *in vivo* consists in using conditions when co-ordinate repressions/inductions of pathways occur. These are well known in both fungal and bacterial micro-organisms. By definition the CCO requires all enzymes in the system to be simultaneously altered and this is certainly not the case in the above systems. Nevertheless, such studies may approach the requirements of a CCO if the system outside the pathway does not interact significantly with it when such

repressions/inductions are effected. It is an almost universal observation that single null mutants in one pathway do not impose double (or multiple) requirements on other pathways. This argues against important interactions between pathways. The absence of such interactions, however, will have to be established rigorously or the system will have to be manipulated to eliminate them.

The non-existence of deviations, as a result of a CCO experiment, is informative by itself. This fact indicates that either the assumptions (eqs (4.3) and (4.4)) are fulfilled or their violation is unimportant concerning the behaviour of the variables. On the other hand, the discovery of deviations strongly suggests that the properties of one or more components of the network do not coincide with the assumptions made . Furthermore, a positive test for deviations shows that these features of the components have a significant effect on the behaviour of the variables when the enzyme concentrations are changed. The experimental design to perform the CCO does not necessarily rely on a detailed knowledge of the structure of the metabolic system. However, if we want to have an interpretation to the deviations, the existing profuse amount of information concerning the structure of metabolic systems and the kinetic properties of its component reactions may be useful. This information (e.g. non-proportionality between a rate, v_i, and an enzyme concentration, E_i) may suggest candidates to be the 'cause' of the deviations in a variable of the system (Y). To test the candidate, the values of the control and π coefficients (e.g. $C^{Y}_{E_i}$ and $\pi^{v_i}_{E_i}$) should be experimentally obtained, in the same conditions used when the CCO was applied (see section (4.11)). We conclude that the coordinate-control operation and coordinate-control plots may be used as a first approach to study the control properties of time-dependent metabolic systems. They constitute a possible way to obtain relevant information and may guide the design of later experiments, leading to a deeper understanding of how metabolic networks work.

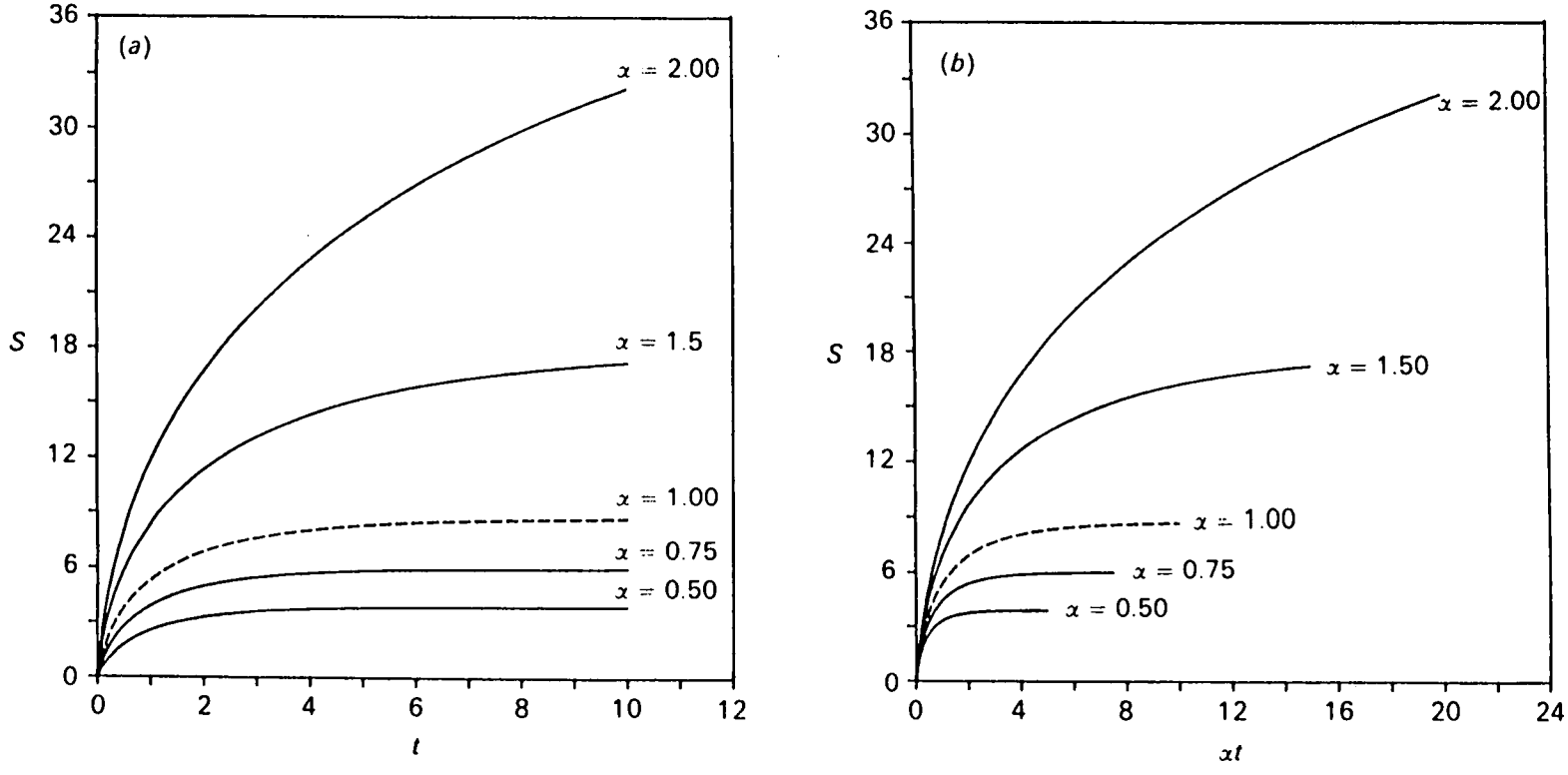


Fig (4.3). Example where assumption eq. (4.4) is not valid: monomer-dimer equilibrium. a) Concentration of S vs time, and b) R-plot.

In the example of section (4.11), the reference parameter values used to generate the reference time course ($\alpha = 1$, dashed line) are: $a_m = 1$, $a_d = 10$, Kapp = 0.1, $K_A = 3$, $E_1 = 4$ and $V_A = 22$ and the concentration of S at the initial time zero (S^{in}) is zero. The same values for a_m, a_d, Kapp, K_A and S^{in} are used to calculate the curves for $\alpha = 0.5$, 0.75, 1.5 and 2.0, while the values of E_1 and V_A are multiplied by the corresponding α (see eq (4.2)). The time courses for different α are given in a). In b) the same concentrations of S are plotted against α multiplied by time.

Table (4.1). Concentration of S and flux, (J) vs α.

The concentrations appearing in this table may be obtained from Fig (4.3a) and (4.3b). The plots for the fluxes are not shown. The values given correspond to the steady state , ss, and $\alpha t = 1$, (1). τ_α is equal to $S_\alpha^{ss} / J_\alpha^{ss}$.

α	S_α^{ss}	J_α^{ss}	$S_\alpha^{(1)}$	$J_\alpha^{(1)}$	τ_α
0.50	3.90	6.22	3.31	5.77	0.63
0.75	6.02	11.01	4.37	9.78	0.55
1.00	8.76	16.39	5.29	14.04	0.53
1.50	18.12	28.31	6.79	22.88	0.64
2.00	45.77	41.29	7.94	31.94	1.11

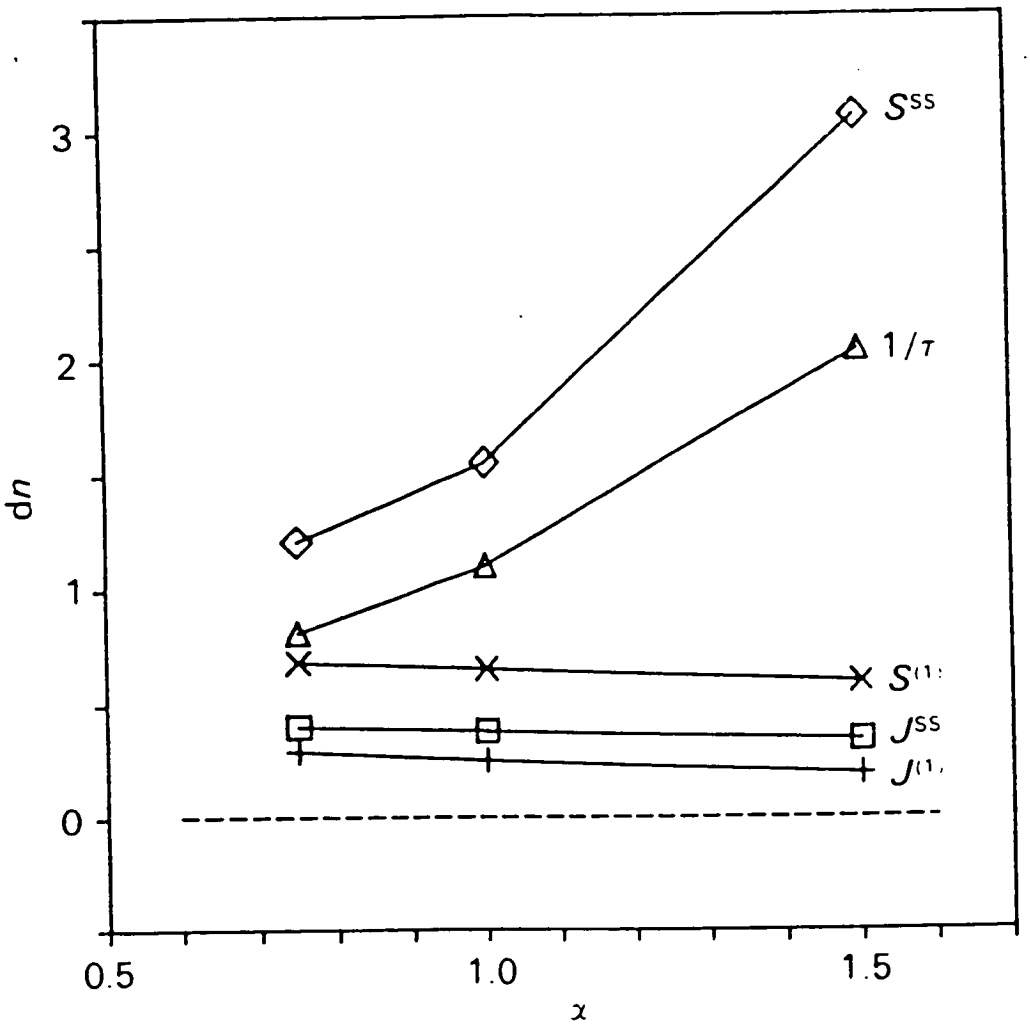


Fig. (4.4). PPS-plot, d_n against α, in a case where assumption eq (4.4) is not valid. Behaviour of time-invariant and time-dependent variables.

These plots are constructed using the data from Table (4.1). The ordinates corresponding to d_n, S_α^{ss} (◇), $S_\alpha^{(1)}$ (×), J_α^{ss} (□), $J_\alpha^{(1)}$ (+) and $1 / \tau_\alpha$ (△), are calculated as described in section (4.10). The dashed line indicates the plot of all variables expected in the absence of deviations.

Table (4.2). Control coefficients and deviations.

The values of the parameters used to generate the coefficients appearing in this table are given in Fig (4.3). Deviations D_S and D_J were obtained using eqs (4.28) and (4.29). They are evaluated at two different points: $\alpha t = 1$ and ss (steady state). The values of d_n were obtained from the PPS-plot of Fig (4.4). The numerical simulations were carried out using the program SCAMP (Sauro, 1986).

	α	$C^{S}_{v_1}$	$C^{J}_{v_1}$	$\pi^{v_1}_{E_1} - 1$	D_S	d^{S}_{n}	D_J	d^{J}_{n}
ss	0.75	3.01	1.00	0.39	1.19	1.21	0.39	0.40
	1.00	3.92	1.00	0.37	1.45	1.55	0.37	0.38
	1.50	7.04	1.00	0.33	2.30	3.06	0.33	0.34
$\alpha t = 1$	0.75	1.73	0.70	0.39	0.68	0.68	0.28	0.29
	1.00	1.75	0.63	0.37	0.65	0.65	0.23	0.25
	1.50	1.76	0.54	0.33	0.58	0.59	0.18	0.19

4.13) Appendix : Relationship of the CCO to control analysis : summation relationships.

4.13.1) The assumptions of eqs (4.3) and (4.4) are valid.

Let us consider a time-invariant variable Y . A small relative change in Y, dY/Y, originated by simultaneous small relative changes in all the enzyme concentrations, dE_j/E_j ($= \alpha - 1$), can be written as the sum of the contributions of each individual enzyme:

$$\frac{dY}{Y} = \sum_{j=1}^{m} C_{E_j}^{Y} \frac{dE_j}{E_j} \qquad (A4.1)$$

where $C_{E_j}^{Y}$ is the control coefficient of the variable Y, by the enzyme concentration E_j, and it is defined as follows:

$$C_{E_j}^{Y} \equiv \frac{E_j}{Y} \frac{\partial Y}{\partial E_j} \qquad (A4.2)$$

When the changes in E_j correspond to a CCO (see eq (4.2)) $dE_j/E_j = = \alpha - 1$ (for j=1,...,m). If the assumptions introduced in eqs (4.3) and (4.4) are valid, the relative change in the variable, dY/Y, is zero in the case of a S-type time-invariant variable , S , and $\alpha - 1$ for a J-type time-invariant variable, J (see eqs (4.21) and (4.22)) . Introducing these results into eq (A4.1), we obtain:

$$\sum_{k} C_{E_k}^{S} = 0 \qquad (A4.3)$$

$$\sum_{k} C_{E_k}^{J} - 1 = 0 \qquad (A4.4)$$

Equations (A4.3) and (A4.4) are the summation relationships for S-type and J-type time-invariant variables respectively. Examples of these variables are given in section (4.7). Particular cases of eqs (A4.3) and (A4.4) are the summation relationships for the control coefficients of the steady state metabolite concentrations and fluxes (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). The reciprocal of a time-invariant variable with dimension of time, $1/T$, fulfils eq (A4.4). Note that $C^{1/T}_{E_k} = - C^{T}_{E_k}$ and therefore, for these variables ,

$$\sum_k C^{T}_{E_k} = - 1$$

This general relationship was previously obtained for particular definitions of transition time (see Heinrich & Rapoport, 1975; Torres et al. 1989; Melendez-Hevia et al. 1989), but is general for any variable which obeys the transformation eq (4.1 0).

The summation relationships for the control coefficients of S-type and J-type time-dependent variables can be written as follows:

$$\sum_k C^{S}_{E_k} - T^{S} = 0 \qquad \text{(A4.5)}$$

$$\sum_k C^{J}_{E_k} - 1 - T^{J} = 0 \qquad \text{(A4.6)}$$

The 'time coefficients', T^S and T^J are defined by: $T^Y = (t/Y)/(\partial Y/\partial t)$, where Y stands for S or J (see Acerenza et al., 1989).

4.13.2) Deviations from the assumptions of eqs (4.3) and (4.4).

Eqs (A4.3) to (A4.6) are derived using the assumptions introduced in eqs (4.3) and (4.4). If these assumptions are not fulfiled , the left hand members of eqs (A4.3) to (A4.6) are not equal to zero. It may be shown that, when the CCO is applied with small changes ($\alpha \approx 1$), those left hand members are not equal to zero but approximatly equal to a deviation term (D) given by eqs (A4.7) to (A4.10) respectively.

$$D_S = \frac{\frac{S_\alpha - S_r}{S_r}}{\alpha - 1} \qquad (A4.7)$$

$$D_J = \frac{\frac{(J_\alpha / \alpha) - J_r}{J_r}}{\alpha - 1} \qquad (A4.8)$$

$$D_S(t) = \frac{\frac{S_\alpha(\alpha t_\alpha = t) - S_r(t)}{S_r(t)}}{\alpha - 1} \qquad (A4.9)$$

$$D_J(t) = \frac{\frac{(J_\alpha(\alpha t_\alpha = t)/\alpha) - J_r(t)}{J_r(t)}}{\alpha - 1} \qquad (A4.10)$$

It is important to note that, these D values are the better approximations to the left hand members of eqs (A4.3) to (A4.6) the closer α tends to 1 .

The deviations given in eqs (A4.7) to (A4.10) are those plotted against α in a PPS-plot (see section (4.10)). The only difference is that, in the PPS-plot, we use the mean between positive and negative $\alpha - 1$ values to compensate (partially) the error introduced by the use of relatively large changes. It is immediate to show that all D values are zero when the assumptions eqs (4.3) and (4.4) apply.

Chapter 5

A minimal oscillator with negative feedback

5.1) Introduction

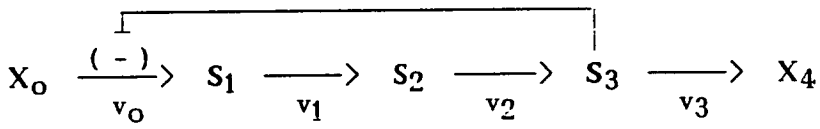
The existence of feedback loops is a common feature of metabolic networks. The mechanism by which an 'end product' of a pathway inhibits an enzyme upstream is called a negative feedback. In this case. the accumulation of the 'end product' tends to diminish the flux that produces it. As a consequence this type of mechanism makes the metabolite concentrations and fluxes within the pathway less sensitive to external perturbations. The same interactions that tend to maintain the steady state values of the variables at certain physiological levels. introduce simultaneously the possibility of existence of unstable steady states. The stability of some models including negative feedback loops has been studied (Hunding, 1974; Rapp. 1975a and b). The usual assumptions made in these models were that the first enzyme was cooperatively inhibited by the end product and that the other enzymes were operationg with first order kinetics. Under these assumptions it was shown that the smaller the number of first order reactions in the linear chain the grater the cooperativity required to obtain oscillations. Moreover. cooperativity was a necessary condition for oscillations (Viniegra-Gonzalez and Martinez. 1969). Mizraji and co-workers have studied the stability of negative feedback loops using discrete and distributed time delays (Mizraji et al.. 1988).

They have shown that the fact that oscillations could only arrise in the presence of cooperativity was a sequel of the linear kinetics hypothesis.

Here, I show that the model in scheme (5.1) may exhibit sustained oscillations even when the first enzyme is non-cooperative. This simple metabolic oscillator with negative feedback is also used to analyse the control properties of the period of oscillation.

5.2) The oscillator

We consider a metabolic model, whose structure is represented in scheme (5.1) (Acerenza, 1990).



(5.1)

S_j (j = 1, 2, 3) are variable metabolite concentrations while X_0 and X_4 are maintained constant. The first step is catalized by a MWC allosteric enzyme. Its activity is modulated by a negative effector, S_3, whose concentration is internally adjusted by the system. For the sake of simplicity we also assume exclusive binding and non-cooperativity. The rate equation for the first step is given by:

$$v_0 = k_0 E_0 \frac{X_0 / K_0}{1 + X_0 / K_0 + L (1 + S_3 / K_I)} \qquad (5.2)$$

where k_0, K_0, L and K_I are constants and E_0 is the enzyme

concentration. The other steps in scheme (5.1) are catalyzed by irreversible Michaelis-Menten enzymes, their rate laws being:

$$v_j = k_j E_j \frac{S_j}{K_j + S_j} \qquad (j = 1, 2, 3) \qquad (5.3)$$

where k_j and K_j are constants and E_j are the enzyme concentrations. The behaviour in time of the metabolite concentrations is described by the system of three differential equations:

$$\frac{dS_j}{dt} = v_{j-1} - v_j \qquad (j = 1, 2, 3) \qquad (5.4)$$

where the rates v_j are given in eqs(5.2) and (5.3). Due to the non-linearities appearing in these equations the time courses of the metabolite concentrations must be obtained by numerical simulation. However, the steady state metabolite concentrations may be algebraically solved in terms of the parameter values (see Appendix 5.5).

The stability of the steady state is studied using the Hurwitz Criterion (see e.g. Stucki, 1978), the condition of unstable steady state being:

$$(d_1 + d_2 + d_3)(d_1 d_2 + d_1 d_3 + d_2 d_3) - d_1 d_2 (d_3 - d_o) < 0 \qquad (5.5)$$

where

$$d_o = \left(\frac{\partial v_o}{\partial S_3} \right)_{ss} = \frac{- k_o E_o \ (X_o / K_o) \ (L / K_I)}{(1 + X_o / K_o + L (1 + S_3^{ss} / K_I))^2} \qquad (5.6)$$

and

$$d_j = \left(\frac{\partial v_j}{\partial S_j} \right)_{ss} = \frac{k_j E_j K_j}{(K_j + S_j)^2} \qquad (j = 1, 2, 3) \qquad (5.7)$$

In the particular case where $k_1 E_1 = k_2 E_2 = k_3 E_3$ and $K_1 = K_2 = K_3$ the steady state concentrations are equal and $d_1 = d_2 = d_3 \equiv d_h$. Therefore inequality (5.5) reduces to:

$$8 \ d_h < -d_o \qquad (5.8)$$

If we use parameter values for which condition (5.5) is fulfilled, the metabolite concentrations exhibit sustained oscillations in time. Stability analysis of the homologous models to the one represented in scheme (5.1) with two or three steps, i.e. one or two Michaelis-Menten enzymes, do not show unstable steady states. Consequently, the model described above constitutes a minimal oscillator with negative feedback.

5.3) The control of period

The period of oscillation depends on the values at which all the

parameters are fixed at the initial time. In what follows we will be interested in a particular group of parameters, namely the enzyme concentrations.

In the previous chapter we analyse the consequences that a simultaneous change in all the enzyme concentrations by the same factor α (Co-ordinate Control Operation, CCO) has on the values of different metabolic variables. There, we assumed that the steady state approximation and the proportionality between rate and enzyme concentration were valid for each enzyme mechanism. Under these assumptions some relationships were derived. In particular, we showed (section 4.6.1) that in an oscillating system the period of oscillation before (T) and after (T_α) the CCO is applied fulfil the following relationship:

$$T_\alpha = \frac{T}{\alpha} \qquad (5.9)$$

An immediate consequence of eq(5.9) is the summation relationship for the period control coefficients

$$\sum_i C^T_{E_i} = -1 \qquad (5.10)$$

where

$$C^T_{E_i} = \frac{E_i}{T} \frac{\partial T}{\partial E_i} \qquad (5.11)$$

are the control coefficients of the period with respect to the enzyme concentrations. The model given in section (5.2) satisfies the

assumptions made to derive eqs(5.9) and (5.10), and therefore these equations apply to that model. According to eq(5.9) if all the enzyme concentrations are increased by a factor α, the period decreases by the same factor (see Fig(5.1)). This is an intuitive result, because when all the enzymes are increased by a factor α the evolution of the system is α times faster and hence the period is α times smaller. By analogy one may be mislead to conclude that the increase in anyone enzyme concentration will decrease the period. However, eqs(5.9) and (5.10) do not describe what is the effect that an increase in one enzyme has on the period when all the other enzyme concentrations are not altered. This was studied by numerical simulation of eq(5.4). The parameter values used satisfy eq(5.5). In Fig(5.2) we show one example of the results obtained for the period of oscillation as a function of the enzyme concentrations. For all the numerical situations studied, an increase in E_1 and E_2 decreases the period while an increase in E_0 and E_3 increases the period. As a consequence, C^T_{E1} and C^T_{E2} are negative while C^T_{E0} and C^T_{E3} are positive. The sum of the negative coefficients must be, in absolute terms, greater than the sum of positive coefficients in such a way that the sum of the four coefficients is equal to -1 (see eq(5.10)).

5.4) Discussion

The stability analysis of models with one or two variable metabolite concentrations, where the last metabolite is a negative effector of the first enzyme, does not show unstable steady states (this analysis is not shown). The model described in section (5.2) is therefore the shortest of this type to exhibit sustained oscillations and constitutes a minimal oscillator. It must be pointed out, however, that a model with only two variable metabolite concentrations may

show sustained oscillations if a positive feedback is present (see e.g. Heinrich et al., 1977 .).

When all enzyme concentrations are increased by the same factor (CCO) the period of oscillation decreases by that factor. This general result, that applies to the model analysed in this chapter, can be easily understood in terms of a change in the time scale of the time course of the metabolite concentrations (see sections 4.5 and 4.6.1). It is however less easy to understand why the period control coefficients of the first and last enzymes in the model (5.1) are positive while the ones corresponding to the second and third enzymes are negative. The transition time τ introduced in eq(1.30) can be calculated using the unstable steady state values of the metabolite concentrations and fluxes which, in turn, can be calculated with the equations given in Appendix (5.5). The signs of the transition time control coefficients with respect to the four enzymes in the model are equal to the corresponding ones of the period control coefficients described in section (5.4). This result suggests that the effect that an enzyme concentration has on the period, strongly depends on how the transition time is affected by that enzyme concentration change.

Many hormones (e.g. growth hormone) must be delivered in a periodic manner to exert their physiological effect. Similarly, in the slime mold *Dictyostelium discoideum* periodic pulses of cyclic AMP control aggregation and differentiation after starvation. In these cases periodic signaling represents an optimal mode of intercellular communication that allows for maximum responsiveness where constant stimuli bring desensitization (Martiel and Goldbeter, 1987). There is not enough experimental evidence, however, to decide whether metabolic oscillations (e.g. glycolitic oscillations) are physiologically or pathologically relevant.

5.5) Appendix: The steady state metabolite concentrations.

The steady state concentrations (S_1^{ss}, S_2^{ss} and S_3^{ss}) are derived solving the non-linear algebraic system of equations obtained by seting eqs(5.4) equal to zero. The result is:

$$S_3^{ss} = K_3 \left(Y-Z + \left(\left(Y-Z \right)^2 + 4\, Y \right)^{1/2} \right) / 2$$

where

$$Y = \frac{K_I\, X_o\, V_o}{K_3\, L\, K_o\, V_3} \quad \text{and} \quad Z = \frac{K_I \left(1 + L + X_o / K_o \right)}{K_3\, L},$$

$$S_2^{ss} = \frac{K_2\, v_3}{V_2 - v_3}$$

where

$$v_3 = \frac{V_3\, S_3}{K_3 + S_3^{ss}}$$

and

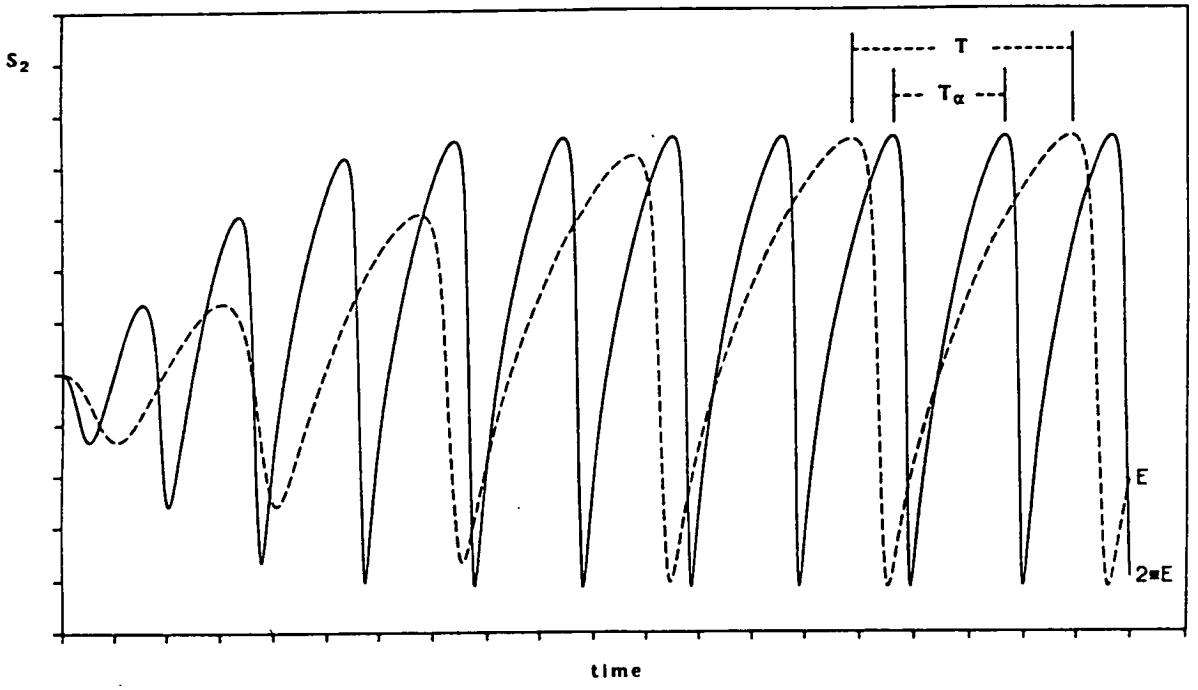
$$S_1^{ss} = \frac{K_1\, v_2}{V_1 - v_2}$$

where

$$v_2 = \frac{V_2\, S_2^{ss}}{K_2 + S_2^{ss}} .$$

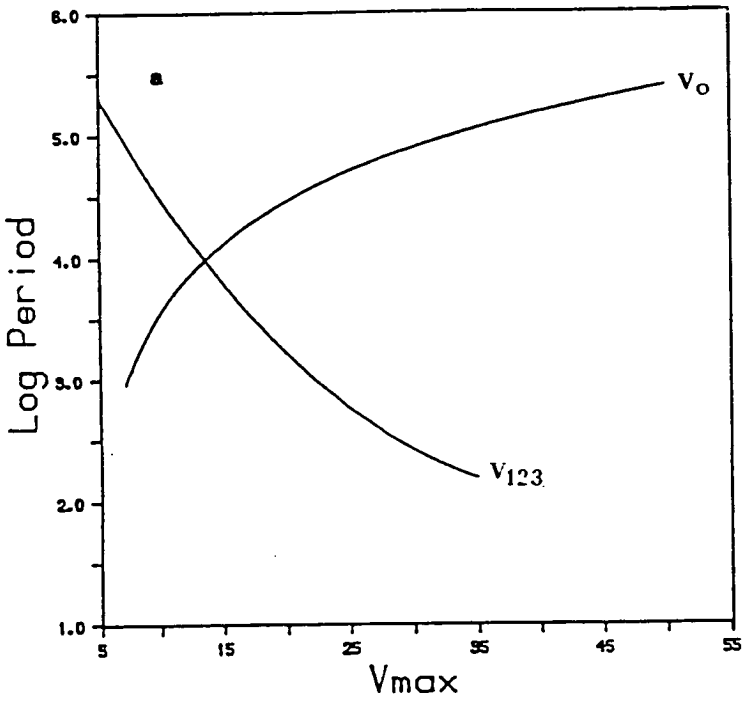
A sufficient condition for the existence of the steady state is:

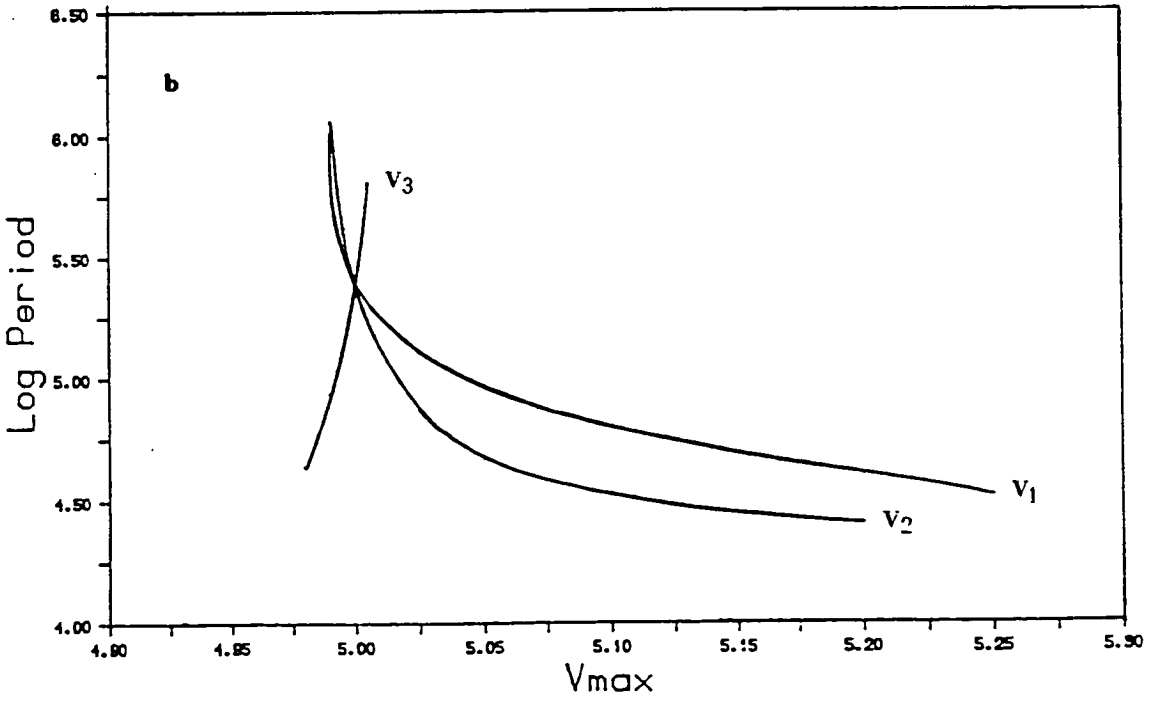
$$V_1 \geq V_2 \geq V_3$$



Fig(5.1) Effect of the CCO on the period of oscillation.

The model used is shown in scheme (5.1). The system of equations (5.4) was solved with $X_o = 5$, $K_o = 0.02$, $K_I = 0.2$, $L = 10$, $K = 5$. The reference values (dashed curve) of the maximum rates are $V_o = 50$ and $V_j = 5$. After the CCO is applied (continuous line) we use $V_o = 100$ and $V_j = 10$ (i.e. $\alpha = 2$). One may see from the graphs that doubling all the maximum rates decreases the period T to half its reference value, $T_\alpha = T / \alpha$. Numerical simulations were carried out using the program SCAMP (Sauro, 1986).





Fig(5.2) Log(period of oscillation) vs Vmax .

The values of the parameters used appear in the legend to Fig(5.1). The curves labelled V_o. V_1, V_2, and V_3 correspond to independent changes in these maximum rates, while the one labelled V_{123} corresponds to an equal and simultaneous change in V_1, V_2 and V_3.

Chapter 6

Final Remarks

This final chapter is a summary of some aspects of metabolic behaviour and control which I think would require further investigation.

1) In this thesis we were mainly concerned with the control of time-dependent metabolic processes by parameters which are proportional to the rates. The attempt in chapter 3 to extend this treatment to any parameter, irrespective of their functional relationship to the rates, was only successful in a particular case. The question still remains whether there is a general and useful description of the control of time-dependent metabolic systems in terms of parameter-unspecified control coefficients.

2) Most of the definitions and relationships of MCA apply only to infinitesimal changes. It is, of course, possible to design experiments where the response of metabolic variables to small changes in the parameters is obtained. The outcome of these experiments may be analysed using MCA. It is, however, a standard procedure today to change the dose of a certain gene by genetic manipulation. The result of adding an extra copy of the gene will usually have the effect of doubling the corresponding enzyme concentration (or if multi-copy vectors are used the increase is by a larger factor). This type of modulation can not be considered a small change, and we still do not have a framework to analyse what effect

on the variables is to be expected. On the other hand, there are physiological situations, such as the release of a hormone, where the concentrations, in the target cells, increase by a large factor. These changes of what could be considered an external effector are also not amenable to the analysis of small changes. In chapter 4 we studied the effect of simultaneous changes in all the enzyme concentrations by the same, not necesarilly small, factor. The effects caused by large changes in individual parameters, however, remains to be investigated.

3) Enzyme concentrations are usually considered as parameters. This assumption is valid when e.g. we measure the response of extracted or reconstituted systems under conditions where enzyme denaturation is negligible. In living organisms we know that enzyme concentrations may be considered constant during periods of time in which the rates of synthesis and degradation do not change. Enivronmental changes, however, may trigger enzymatic induction-repression mechanisms. In addition, in time scales corresponding to developmental processes the enzyme concentrations are internally modified by the system and must be considered as variables. Further study is needed to deal with this type of situations.

4) The present thesis was concerned with systems that are homogeneous in space. Some aspects of the control of temporal patterns (i.e. metabolic oscillations) were studied. The control analysis of spatial patterns, relevant to the phenomenon of morphogenesis, is still to be developed.

5) Metabolic systems are constituted of a large number of elements which appear to be operating in a coordinated way. It has been suggested that some kind of hierarchical metabolic structure could be present such as . for example, a time hierarchy (Reich and

Sel'kov, 1975) or a 'reaction level' hierarchy (Westerhoff et al., 1990). Further studies are needed to give light into these matters.

6) The understanding of metabolic processes together with the development of new methods and techniques will have a future impact on many branches of applied science and biotechnology. It is of biotechnological interest to increase the fluxes to some desired products such as the fluxes to antibiotics in micro-organisms. Techniques are available to add additional copies of cloned genes which will result in a large increase in the corresponding enzyme concentration. What enzyme or enzymes have to be up-modulated in order to achieve the desired increase in flux is a problem that requires further investigation. Many drugs are known where the pharmacological effect starts by the inhibition of a specific enzyme. The final effect, however, depends not only on the efficiency of the drug to inhibit the protein but also on the effect that the inactivation of the protein has on the metabolic state. Thus, a deeper understanding of quantitative aspects of metabolism may be an essential guide in the design of medical strategies.

References

ACERENZA, L. (1990) in Control of Metabolic Proccesses (Cornish-Bowden, A. & Cardenas, M. L., eds.), Chapter 25, p 297 Plenum Press .

ACERENZA, L. & KACSER, H. (1990) Biochem. J. **269**, 697.

ACERENZA, L., SAURO, H.M. & KACSER, H. (1989). J. theor. Biol, **137**, 423.

ALAMGIR, M. & EPSTEIN, I.R. (1983) J. Am. Chem. Soc., **105**, 2500.

BEADLE, G.W. & TATUM, E.L. (1941) Genetics **27**, 499.

BLACKMAN, F.F. (1905) Ann. Bot. **14**, 281.

BOITEUX, A., HESS, B. & SEL'KOV, E.E. (1980). Curr. Topics Cell. Regul. **17**, 171.

BURNS, J.A., CORNISH-BOWDEN, A., GROEN, A.K., HEINRICH, R., KACSER, H., PORTEOUS, J.W., RAPOPORT, S.M., RAPOPORT, T.A., STUCKI, J.W., TAGER, J.M., WANDERS, R.J.A. & WESTERHOFF, H.V. (1985). Trends Biochem. Sci. **10**, 16.

BURTON, A.C. (1937) J. Cell. Comp. Physiol. **9**, 1.

BURTON, A.C. (1939) J. Cell. Comp. Physiol. **14**, 327.

CASCANTE, M., FRANCO, R. & CANELA, E.I. (1989a) Math. Biosci. **94**, 271.

CASCANTE, M., FRANCO, R. & CANELA, E.I. (1989b) Math. Biosci. **94**, 289.

CHANCE, B., ESTABROOK, R.W. and GHOSH, A. (1964). Proc. Natl. Acad. Sci. **51**, 1244.

CHANCE, B., GHOSH , A., HIGGINS, J. & MAITRA, P.K. (1964). Ann. N.Y. Acad. Sci. **115**, 1010.

CIFERRI, O., SORA, S. & TIBONI, O. (1968). Genetics, **61** , 567.

COHEN, P. (1976) in Outline Series in Biology. Chapman and Hall, London.

CORNISH-BOWDEN, A. (1976). Principles of Enzyme Kinetics. Butterworths.

CORNISH-BOWDEN, A. (1979). Fundamentals of Enzyme Kinetics. Butterworths.

CORNISH-BOWDEN, A. (1989) J. theor. Biol. **136**, 365.

CORNISH-BOWDEN, A. (1990) in Control of Metabolic Proccesses (Cornish-Bowden, A. & Cardenas, M. L., eds.), Chapter 1, p 31, Plenum Press.

CORNISH-BOWDEN, A. (1991) Eur. J. Biochem. **195**, 103.

CRABTREE, B. & NEWSHOLME, E.A. (1985) Curr. Topics in Cell.

Regul. **25**, 21.

DAS, J. & BUSSE, H.-G.(1985). J. Biochem. **97**, 719.

DECROLY, O. & GOLDBETER, A. (1982). Proc. Natl. Acad. Sci. USA **79**, 6917.

DEGN, H. (1968) Nature **217**, 1047.

DENTON, R.M. & POGSON C.I. (1976) in Outline Series in Biology. Chapman and Hall, London.

DERR, R.F. (1985) Biochem. Arch. **1**, 239.

DONACHIE, W.D. (1962) Ph.D Thesis, University of Edinburgh.

EASTERBY, J.S. (1981). Biochem. J. **199**, 155.

EASTERBY, J.S. (1984). Biochem. J. **219**, 843.

EASTERBY, J.S. (1986). Biochem. J. **233**, 871.

ESCHRICH, K., SCHELLENBERGER, W. & HOFMANN, E. (1980) Arch. Biochem. Biophys. **205**, 114.

EVANS, M.G. & POLANYI, M. (1935) Trans. Faraday Soc. **31**, 875.

EYRING, H. (1935) J. Chem. Phys. **3**, 107.

FELL, D.A. & SAURO, H.M. (1985). Eur. J. Biochem. **148**, 555.

FRIEDEN, C. (1967). J. Biol. Chem. **242**, 4045.

GARFINKEL, D. & HESS, B. (1964) J. Biol. Chem. **239**, 971.

GAVISH, B. (1986) in The Fluctuating Enzyme (G. Rickey Welch Ed.) John Wiley & Sons Inc.

GIERSCH, C. (1988) J. theor. Biol. **134**, 451.

GLEIK, J. (1989) Chaos. Making a new science. Sphere Books Ltd p 275.

GLANSDORFF & PRIGOGINE (1971) Thermodynamic theory of structure stability and fluctuations, Wiley-Interscience.

GOLDBETER, A. & KOSHLAND D.E. Jr. (1982) Quart. Rev. Biophy., **15**, 555.

GOLDBETER, A. & MORAN, F. (1987). in The Organization of Cell Metabolism (Welch, G.R. & Clegg , J.S., eds.), p 291, Plenum.

HATLEE, M.D. & KOZAK, J.J. (1980) J. Chem. Phys. **72**, 4358.

HEINRICH, R. & RAPOPORT, T.A. (1974). Eur. J. Biochem. **42**, 89.

HEINRICH, R. & RAPOPORT, T.A. (1975). Biosystems **7**, 130.

HEINRICH, R., RAPOPORT, S.M. & RAPOPORT, T.A. (1977) Progr. Biophys. Mol. Biol. **32**, 1

HESS, B. & MARKUS, M. (1987) TIBS **12**, 45.

HIGGINS, J. (1965) in Control of Energy Metabolism (B. Chance, R.W. Estabrook and J.R. Williamson Eds.) Academic Press, p 13.

HIGGINS, J., FRENKEL, R., HULME, E., LUCAS, A. & RANGAZAS, G. (1973). In Biological and Biochemical Oscillators (Chance, B., Pye, E.K., Ghosh, A.M. and Hess, B., eds), Academic Press, New York, p. 127.

HILGER, F. (1973) J. Gen. Microbiol. **75**, 23.

HOFMANN, E., ESCHRICH, K. & SCHELLENBERGER, W. (1985) in Advances in Enzyme Regulation (Weber, G., ed.), Vol 23, p 331, Pergamon Press, Oxford and New York.

HOFMEYR, J.-H.S (1989) Eur. J. Biochem. **186**, 343.

HOFMEYR, J.-H.S., KACSER, H. & VAN DER MERWE, K.J. (1986). Eur. J. Biochem. **155**, 631.

HOROWITZ, N.H. (1945) Proc. Natnl. Acad. Sci. **31**, 151.

HUNDING, A. (1974). Biophys. Struct. Mechanism **1** , 47.

KACSER, H. (1957) in The Strategy of the Genes, George Allen & Unwin Ltd. p 191.

KACSER, H. (1983). Biochem. Soc. Trans. **11** , 35.

KACSER, H. (1987). The Biochemistry of Plants **11** , 39.

KACSER, H. (1987). in The Organization of Cell Metabolism (Welch, G.R. & Clegg , J.S., eds.), p 327, Plenum.

KACSER, H. & BURNS, J.A. (1968) Quantitative Biology and Metabolism (A. Locher Ed.) Springer-Verlag, p 11.

KACSER, H. & BURNS, J.A. (1973). Symp. Soc. Exp. Biol. **27**, 65.

KACSER, H. & BURNS, J.A. (1979). Biochem. Soc. Trans. **7**, 1149.

KACSER, H. & BURNS, J.A. (1981) Genetics **97**, 639.

KACSER, H. & PORTEOUS, J.W. (1987). Trends Biochem. Sci. **12**, 5.

KACSER. H, SAURO, H.M. & ACERENZA, L. (1990) Eur. J. Biochem. **187**, 481.

KOHN, M.C., WHITLEY, L.M. & GARFINKEL, D. (1979). J. theor. Biol. **76**, 437.

KOHN, M.C. & CHIANG, E. (1982). J. theor. Biol. **98**, 109.

KRAMERS, H.A. (1940) Physika **7**, 284.

KURGANOV, B.I. (1978). Allosteric Enzymes: Kinetic Behaviour. John Wiley & Son.

LAMBA, P. & HUDSON, J.L. (1985) Chem. Eng. Commun. **32**, 369.

LEINHARD, G.E. (1973) Science **180**, 149.

LEWIS, W.C. McC. (1918) J. Chem. Soc. **113**, 471.

LWOFF, A. & ULLMANN, A. (eds.) (1978) Selected Papers in Molecular Biology by Jacques Monod. Academic Press.

MANGEL, M. (1978) J. Chem. Phys. **69**, 3697.

MARKUS, M., & HESS, B. (1990) in Control of Metabolic Proccesses (Cornish-Bowden, A. & Cardenas, M. L., eds.), Chapter 26, p 303, Plenum Press .

MARKUS, M., KUSCHMITZ, D. & HESS, B. (1984). FEBS Lett. **172**, 235.

MARTIEL, J.-L & GOLDBETER, A. (1987) Biophys. J. **52**, 807.

MELENDEZ-HEVIA, E., TORRES, N.V., SICILIA, J. & KACSER, H. (1990). Biochem. J. **265**, 195.

MENDEL, G. (1866) Verhandlungen des naturforschenden Vereines in Brunn **4**, 3.

MIZRAJI, E., ACERENZA, L. & HERNANDEZ, J. (1988). BioSystems **22**, 11.

MIZRAJI, E., ACERENZA, L. & LIN, J. (1987) J. theor. Biol. **129**, 163.

MONOD, J. (1942) Recherches sur la croissance des cultures bacteriennes, Hermann & Cie.

MORAN, F. & GOLDBETER, A. (1984) Biophys. Chem. **20**, 149.

MORTIMER, R. (1958) Radiation Research **9**, 312.

NEET, K.E. & AINSLIE, G.R., Jr. (1980). In Methods in Enzymology (Purich, D.L., ed) Academic Press, New York, **64**, 192.

NICOLIS, G. & PRIGOGINE, I. (1977). Self-Organization in Non-Equilibrium Systems, Wiley, New York.

ONSAGER, L. (1931) Phys. Rev. **37**, 405.

OVADI, J., OSMAN, M.I. & BATKE, J. (1983) Eur. J. Biochem. **133**, 433.

PORTEOUS, J.W. (1985) Circulation, Respiration and Metabolism (Gilles, R. ed.), Springer Verlag, Berlin.

PROCACCIA, I. (1988). Nature **333**, 618.

RABITZ, H., KRAMER, M. & DACOL, D. (1983) Ann. Rev. Phys. Chem. **34**, 419.

RAPP, P.E. (1975a) Math. Biosci. **23**, 289.

RAPP, P.E. (1975b) Math. Biosci. **25**, 165.

RAPP, P.E. (1979). J. Exp. Biol. **81**, 281.

REDER, C (1988). J. theor. Biol., **135**, 175.

REICH, J.G. & SEL'KOV, E.E. (1975) Biosystems **7**, 39.

SALERNO, C., OVADI, J., KELETI, T. & FASELLA, P. (1982) Eur. J. Biochem. **121** , 511.

SAURO, H.M. (1986). PhD Thesis, Oxford Polytechnic.

SAURO, H.M. & KACSER, H. (1990) Eur. J. Biochem. **187**, 493

SAURO, H.M. SMALL, J.R. & FELL, D.A. (1987). Eur. J. Biochem. **165**, 215.

SAVAGEAU, M. (1971) Arch. Biochem. Biophys. **145**, 612.

SEGEL, L.A. (1988). Bull. Math. Biol. **50**, 579.

SELWYN, M.J. (1965). Biochim. Biophys. Acta, **105**, 193.

SCHUSTER, H.G. (1984) Deterministic Chaos, Physik-Verlag, Weinheim.

SMALL, J.R. (1988) Ph.D Thesis, Oxford Polytechnic.

SMALL, J.R. & FELL, D. (1989) J. theor. Biol. **136**, 181.

SMALL, J.R. & FELL, D. (1990) Eur. J. Biochem. **191**, 413.

SORRIBAS, A. & BARTRONS, R. (1986). Eur. J. Biochem. **158**, 107.

SRERE, P.A. (1987). Ann. Rev. Biochem. **56**, 89.

SRISASTAVA, D.K. & BERNHARD, S.A. (1986). Curr. Topics Cell. Regul. **28**, 1.

STUART, F., PORTEOUS, D.J., FLINT, H.J. & KACSER, H. (1986). J. Gen. . Microbiol. **132**, 1159.

STUCKI, J.W. (1978) Prog. Biophys. Molec. Biol. **33**, 99.

TOMOVIC, R. & VUKOBRATOVIC, M. (1972) General Sensitivity Theory. New York: American Elsevier.

TORRES, N.V., SOUTO, R. & MELENDEZ-HEVIA, E. (1989). Biochem. J. **260**, 763.

TRAUTZ, M. (1916) Z. Anorg. Allgem. Chem. **96**, 1.

UMBARGER, H.E. (1956) Science **123**, 848.

VINIEGRA-GONZALEZ, G. & MARTINEZ, H. (1969) Proc. Biophys. Soc. Abstracts, **13**, A210.

WESTERHOFF, H.V., KOSTER, J.G., VAN WORKUM, M. & RUDD, K.E. (1990) in Control of Metabolic Proccesses (Cornish-Bowden, A. & Cardenas, M. L., eds.), Chapter 35, p 399 Plenum Press .

WESTERHOFF, H.V. & CHEN, Y. (1984). Eur. J. Biochem. **142**, 425.

WESTERHOFF, H.V., GROEN, A.K. & WANDERS, R.J.A. (1984). Bioscience Reports **4**, 1.

WRIGHT, B.E. & ALBE, K.R. (1990) in Control of Metabolic Proccesses (Cornish-Bowden, A. & Cardenas, M. L., eds.), Chapter 27, p 317, Plenum Press .

WYMAN, J. (1975). Proc. Natl. Acad. Sci. USA **72**, 3983.

YATES, R.A. & PARDEE, A.B. (1956) J. Biol. Chem. **221**, 757.

J. theor. Biol. (1989) **137**, 423-444

Control Analysis of Time-dependent Metabolic Systems†

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Metabolic Control Analysis is extended to time dependent systems. It is assumed that the time derivative of the metabolite concentrations can be written as a linear combination of rate laws, each one of first order with respect to the corresponding enzyme concentration. The definitions of the control and elasticity coefficients are extended, and a new type of coefficient ("time coefficient", "T") is defined. First, we prove that simultaneous changes in all enzyme concentrations by the same arbitrary factor, is equivalent to a change in the time scale. When infinitesimal changes are considered, these arguments lead to the derivation of general summation theorems that link control and time coefficients. The comparison of two systems with identical rates, that only differ in one metabolite concentration, leads to a method for the construction of general connectivity theorems, that relate control and elasticity coefficients. A mathematical proof in matrix form, of the summation and connectivity relationships, for time dependent systems is given. Those relationships allow one to express the control coefficients in terms of the elasticity and time coefficients for the case of unbranched pathway.

1. Introduction

Metabolic Control Analysis, as developed by Kacser & Burns (1973) and Heinrich & Rapoport (1974), is concerned with the effect of changes in enzyme concentration or activity on the steady state metabolite concentrations and fluxes of metabolic systems (metabolic variables). The basic relationships are the summation and connectivity theorems. They allow one to express the behaviour of the system variables in terms of the kinetic properties of the isolated enzymatic reactions that build up the metabolic network. The theory was subject to later developments, and theorems were obtained for branched pathways, substrate cycles and moiety conserved cycles (Kacser, 1983; Westerhoff & Chen, 1984; Fell & Sauro, 1985; Hofmeyr *et al.*, 1986; Sorribas & Bartrons, 1986). A matrix method was derived (Fell & Sauro, 1985; Sauro *et al.*, 1987) that allows the determination of the flux and concentration control coefficients of enzymes from their kinetic properties represented by the elasticity coefficients. All these developments were applicable to metabolic systems with asymptotically stable steady states (Wyman, 1975; Nicols & Prigogine, 1977). For recent reviews in this area see Kacser & Porteous (1987) and Kacser (1987).

Many biological systems exhibit a quasi-steady state behaviour during considerable intervals of time, for which the above treatment is a good approximation. On

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the other hand, fluctuations of the environment, both temporary and permanent, will affect the internal composition, giving rise to transient changes in the values of the variables. Furthermore, as is well known, the widely distributed allosteric mechanisms in metabolism are potential generators of temporal and spatial patterns (Goodwin, 1965; Hunding, 1974; Rapp, 1979; Boiteux *et al.*, 1980; Mizraji *et al.*, 1988). Even a simple model with two positive feedback loops (Decroly & Golbeter, 1982) can generate a wide range of different behaviours, such as birhythmicity, oscillations and chaos (Procaccia, 1988). Although abundant theoretical and experimental evidence is available concerning unstable steady states and transients, metabolic control analysis for these situations has not been developed. A few contributions in this field have been made (Higgins *et al.*, 1973; Kohn *et al.*, 1979; Kohn & Chiang, 1982), but no complete theoretical treatment is available.

The main goal of this paper is to extend Metabolic Control Analysis to systems whose variables are functions of time. First, in Section 2, we give a description of the metabolic system. In Section 3 we study the consequences of changing the concentrations of all enzymes by the same factor. In Section 4 we extend the definitions of control and elasticity coefficients to time dependent systems. A new type of coefficients ("time coefficient") is introduced. Next, summation relationships between the control coefficients are derived. In Section 6, we compare two situations where all the rates are identical, differing only in the concentration of one metabolite. The conclusions obtained are used in Section 7 to show how to construct connectivity relationships. A mathematical proof for the summation and connectivity relationships are given in Section 8. In Section 9, we illustrate, by an example, how the control coefficients can be expressed in terms of the elasticity coefficients.

In this paper we lay the conceptual and mathematical foundations for the control treatment of metabolic systems that exhibit time-dependent phenomena of biological relevance. While the usual approach to such questions involves the simulation of particular systems with assumed parameters (which are rarely known *in vivo*), our treatment enables us to make some general statements which are independent of detailed mechanistic considerations.

2. The System

We consider a metabolic system ($\mathscr{S}$) whose dynamics is described by a system of differential equations:

$$\frac{\mathbf{ds}}{\mathbf{d}t} = \mathbf{Nv} \tag{1}$$

where $\mathbf{s}$ is the column vector of concentrations of metabolites S_i, t the independent variable time, $\mathbf{v}$ the column vector of the rates v_j, and $\mathbf{N}$ the stoichiometry matrix. The element n_{ij} of this matrix is the stoichiometry coefficient of the metabolite S_i in the reaction j, and is positive, negative or zero if S_i is product, substrate or is not transformed in the reaction respectively. In $\mathbf{v}$ we assume that each individual rate law is of the form:

$$v_j = E_j \mathbf{f}_j(\mathbf{s}, k_j). \tag{2}$$

Here E_j is the total enzyme concentration of step j, and the function f_j depends on the concentrations of some intermediates of the pathway and of the parameters k_j. f_j is independent of enzyme concentrations and time. k_j includes kinetic constants, external effectors or other parameters related to the step j. We also assume that the stoichiometry matrix is constant.

The solution of eqn (1) is

$$\mathbf{s} = \mathbf{s}(\mathbf{k}, \mathbf{s}_0, t_0, t) \tag{3}$$

$(\mathbf{s}_0, t_0)$ is the vector of initial conditions of metabolite concentrations and time, and $\mathbf{k}$ is the vector of parameters of the system. Without loss of generality we assume $t_0 = 0$. Combining eqns (2) and (3) we obtain the flux for each step, J_j, as a function of time:

$$J_j = E_j f_j[\mathbf{s}(\mathbf{k}, \mathbf{s}_0, 0, t), k_j]. \tag{4}$$

We use the symbol v_j in eqn (2) and J_j in eqn (4). Although both quantities have the same value when evaluated in the system, in v_j we consider the metabolite concentrations as parameters (isolated reaction), while in J_j they are affected by change of enzyme concentrations and all the other parameters. A further distinction will be made in Section 4.

3. The Time Scale

We compare the system described previously ($\mathcal{S}$) with another metabolic system ($\mathcal{S}_\alpha$). The only difference between them is that all the enzyme concentrations of $\mathcal{S}_\alpha$ ($E_{j,\alpha}$) are obtained by multiplying those of $\mathcal{S}$ by the same arbitrary (not necessarily small) constant α.

$$E_{j,\alpha} = \alpha E_j. \tag{5}$$

The dynamics of the new metabolic situation is described by a new system of differential equations:

$$\frac{d\mathbf{s}_\alpha}{dt_\alpha} = \mathbf{N}\mathbf{v}_\alpha \tag{6}$$

$\mathbf{s}_\alpha$ and $\mathbf{v}_\alpha$ are the vectors of metabolite concentrations and rates respectively. Even though t_α is measured by the same time scale as t, it is useful to mark the time in the system $\mathcal{S}_\alpha$ by a different symbol. The solution of eqn (6) can be written with the same notation of eqn (3) as follows:

$$\mathbf{s}_\alpha = \mathbf{s}_\alpha(\mathbf{k}_\alpha, \mathbf{s}_0, 0, t_\alpha) \tag{7}$$

where we take the same initial condition as in $\mathcal{S}$ $(\mathbf{s}_0, t_0 = 0)$.

From eqns (2) and (5) we immediately obtain: $\mathbf{v}_\alpha = \alpha\mathbf{v}$. Substituting this relation into eqn (6), the equation takes the form:

$$\frac{d\mathbf{s}_\alpha}{d(\alpha t_\alpha)} = \mathbf{N}\mathbf{v} \tag{8}$$

where αt_α is a new time scale (for a similar concept see Selwyn, 1965). The right members of eqns (1) and (8) are identical. Then, the only effect of changing all enzyme concentrations by a factor α, is to affect the time scale, without qualitative modification of the dynamics of the metabolic system. Taking into account these considerations we can write the solution $\mathbf{s}_\alpha$ in terms of the solution s given by eqns (7) and (3), respectively.

$$\mathbf{s}_\alpha = \mathbf{s}(\mathbf{k}, \mathbf{s}_0, 0, \alpha t_\alpha). \tag{9}$$

Finally, comparing eqns (3) and (9) we obtain the relation between t_α and t so that the solutions of eqns (1) and (6) have the same value. That is if

$$t_\alpha = t/\alpha \tag{10}$$

then

$$\mathbf{s}_\alpha(t/\alpha) = \mathbf{s}(t). \tag{11}$$

In other words, if for a time t, the system $\mathcal{S}$ has a particular set of values of metabolite concentrations, then the system $\mathcal{S}_\alpha$ exhibit the same values of concentrations at the time t/α. This statement is true for any point of the temporal evolution of the system.

The flux through reaction j for $\mathcal{S}_\alpha$ is

$$J_{j,\alpha} = E_{j,\alpha} f_j[\mathbf{s}_\alpha(\mathbf{k}_\alpha, \mathbf{s}_0, 0, t_\alpha), k_j]. \tag{12}$$

Combining eqns (5), (10), (11) and (12) we obtain:

$$J_{j,\alpha}(t/\alpha) = \alpha J_j(t). \tag{13}$$

Equation (13) tell us that, if for any time t, the fluxes in $\mathcal{S}$ present certain values, then $\mathcal{S}_\alpha$ has values of fluxes that are α times those of $\mathcal{S}$ at a time t/α (where all metabolite concentrations have the same values as in $\mathcal{S}$).

If α is greater (smaller) than one, the system $\mathcal{S}_\alpha$ evolves α times faster (slower), that is:

$$\left(\frac{\mathrm{d}\mathbf{s}}{\mathrm{d}t}\right)_\alpha = \alpha \frac{\mathrm{d}\mathbf{s}}{\mathrm{d}t}. \tag{14}$$

Then, as is stated by eqn (11), to obtain the same values of metabolite concentrations in both systems we have to look in $\mathcal{S}_\alpha$ at a smaller (greater) time t/α. But, at that smaller (greater) time, as $\mathcal{S}_\alpha$ evolves α times faster (slower), the fluxes are α times greater (smaller) than in the reference system $\mathcal{S}$, as appears in eqn (13).

If $\mathcal{S}$ and $\mathcal{S}_\alpha$ are at a stable steady state, the variables (metabolite concentrations, fluxes, etc.) have constant values in time. In this case eqns (11) and (13) can be written as $\mathbf{s}_\alpha = \mathbf{s}$ and $J_{j,\alpha} = \alpha J_j$ respectively. In other words, in a stable steady state situation, for any time, both systems have the same values of metabolite concentrations, and all the fluxes in $\mathcal{S}_\alpha$ are α times the corresponding ones in $\mathcal{S}$.

These results are related to the conclusions derived from the Summation relationships (Section 5).

4. Control, Elasticity and Time Coefficients

In the last section we considered the consequences of a simultaneous change in all enzyme concentrations by a factor α. This factor can be, in principle, any real number and the enzyme concentrations in both metabolic situations ($\mathcal{S}$ and $\mathcal{S}_\alpha$) can differ by a large finite amount. From now on, however, we are going to deal with infinitesimal differences in enzyme concentrations, as is usual in control analysis. The first question we are going to pose is: how does an infinitesimal relative change in a particular enzyme concentration (at the initial conditions) affect the value of one variable Y (metabolite concentration or flux) at any time? The quantitative answer to this question is given by the value of the control coefficient of the variable, at that time, with respect to the enzyme concentration. We define two types of control coefficient for time dependent metabolic systems. The "unscaled control coefficients" (${}^uC^Y_{E_k}$) tell us what is the absolute change in the value of the variable per relative change in one enzyme concentration. And what we simply call "control coefficients" ($C^Y_{E_k}$) account for the relative change in the variable per relative change in enzyme concentration. For the variable Y the unscaled control coefficient with respect to enzyme concentration E_k can be mathematically defined ($\equiv$) as follows:

$$ {}^uC^Y_{E_k} \equiv E_k \left(\frac{\partial Y}{\partial E_k} \right)_{E_j, t} . \tag{15} $$

The subscripts outside the parenthesis indicate that while changing E_k, all the other enzyme concentrations (E_j), parameters in general and time are held constant. The control coefficients (scaled) can be obtained from the unscaled control coefficients dividing by the variable:

$$ C^Y_{E_k} \equiv \frac{E_k}{Y} \left(\frac{\partial Y}{\partial E_k} \right)_{E_j, t} . \tag{16} $$

These, apart from their time dependence, are the ones used in classical control analysis at steady state (Burns *et al.*, 1985). It is important to note that the coefficients defined by eqns (15) and (16) depend on the properties of the whole metabolic system.

Next, we introduce another type of coefficient: the elasticity coefficients. These tell us what the relative change of a local (isolated) rate is, given by eqn (2), when the concentration of one of the metabolites which explicitly appears in that rate equation changes by an infinitesimal amount, holding all the other variables constant. In this case we define unscaled and scaled elasticity coefficients with respect to the metabolite concentration. The "unscaled elasticity coefficients" are defined in mathematical terms as follows:

$$ {}^u\varepsilon^{v_k}_{s_i} \equiv \frac{1}{v_k} \left(\frac{\partial v_k}{\partial s_i} \right)_{s_j} . \tag{17} $$

The scaled elasticity coefficients, which we simply call "elasticity coefficients" a obtained from the unscaled ones by multiplying by the corresponding metaboli concentration:

$$\varepsilon_{S_i}^{v_k} \equiv \frac{S_i}{v_k}\left(\frac{\partial v_k}{\partial S_i}\right)_{S_j}. \qquad (1$$

These, apart from their time dependence, will be recognized as identical to th elasticity coefficients used in steady state analysis. From eqns (17) and (18) it clear that the elasticity coefficients can be defined only if the rates are non-zer The mathematical function that gives the elasticity coefficients in terms of th metabolite concentrations can be obtained by making the partial derivative of [given by eqn (2)] with respect to S_i and then scaling with the appropriate facto The elasticity coefficients are defined for isolated rates, and in that sense the represent local variations in the metabolic system. However, the actual values the elasticity coefficients depend on the values of the metabolite concentrations th are functions of time. Through these concentrations, the elasticity coefficients a affected by the properties of the whole system.

The definitions of control and elasticity coefficients given in eqns (16) and (18 respectively, constitute an extension of the steady state coefficients (Burns *et a* 1985). In general, the time-dependent control and elasticity coefficients have differe values for different times; but if the metabolic system is one that approaches a stab steady state, then in the limit, they are identical to the coefficients defined for steac state.

Finally, we define a new type of coefficient: the "time coefficients". They tell how a relative infinitesimal change in the time of observation of the metabol system affects the value of the variable (metabolite concentration or flux) und consideration, when all the parameters (enzyme concentrations, etc.) are he constant. As before, we define unscaled and scaled time coefficients with respect the variable. Their mathematical definition is given, respectively, by:

$${}^{u}T^{Y} \equiv t\left(\frac{\partial Y}{\partial t}\right)_{E_j} \qquad (1$$

and

$$T^{Y} \equiv \frac{t}{Y}\left(\frac{\partial Y}{\partial t}\right)_{E_j}. \qquad (2$$

These coefficients are directly related to the time evolution properties of the variabl It is clear from eqns (19) and (20) that if the system is in a stable steady state, wh all the variables have constant values, their time coefficients are zero. This is t reason why they do not appear explicitly in control analysis of steady state syste It has to be pointed out that if we do not choose the initial time equal to zero (s Section 2), the definitions given in eqns (19) and (20) have to be modified, substituti the factor t by $(t - t_0)$.

We have introduced the unscaled coefficients, which are not used in steady state nalysis. These are required when the connectivity relationships are considered. urthermore, unscaled control and time coefficients, can be used when, in the time volution of the system, the variable at a particular time is zero.

5. Summation Relationships

Here, using some results of Section 3, we derive summation relationships for me-dependent metabolic systems. We symbolize by Y any variable of the system . This variable depends, in general, on the parameters, initial conditions and time. n this section we consider Y as a function of all enzyme concentrations and time nly.

$$Y = Y(E_1, \ldots, E_n, t). \tag{21}$$

Ve compare the system $\mathcal{S}$ with another metabolic system $\mathcal{S}_\beta$. They differ in the alues of all enzymes concentrations by infinitesimal amounts. The relative difference etween the enzyme concentrations is a constant β (the same for all enzymes). That :

$$E_{k,\beta} = E_k + dE_k \qquad (k = 1, \ldots, n) \tag{22}$$

here

$$\mathrm{d}E_k = \beta E_k \qquad (k = 1, \ldots, n). \tag{23}$$

n comparing the systems $\mathcal{S}$ and $\mathcal{S}_\beta$ we will take a different time in each one, t and , respectively, their inverse being different by an infinitesimal amount $\mathrm{d}(1/t)$.

$$1/t_\beta = 1/t + \mathrm{d}(1/t). \tag{24}$$

Ve choose the relative differences between the inverses of the times of observation, and t_β, for each time, such that they are a constant equal to β: $\mathrm{d}(1/t) = \beta(1/t)$. aking into account that $\mathrm{d}(1/t) = -(\mathrm{d}t)/t^2$, this leads to:

$$\mathrm{d}t = -\beta t. \tag{25}$$

rom eqns (22) and (23) it follows that $E_{k,\beta} = (1+\beta)E_k$. Similarly, from eqns (24) nd (25) we obtain $t_\beta = t/(1+\beta)$. Then eqns (23) and (25) are equivalent conditions o eqns (5) and (10), respectively, when infinitesimal differences are considered with $\alpha = 1+\beta$). From eqn (11), we know that any metabolite concentration S_i has ne same value in $\mathcal{S}$ and $\mathcal{S}_\beta$. Similarly if we consider eqn (13), all the fluxes J_j differ n the same relative amount β. Then if eqns (23) and (25) are fulfilled, the differences etween the metabolite concentrations $\mathrm{d}S_i$, and the differences between fluxes $\mathrm{d}J_j$, re:

$$\mathrm{d}S_i = 0 \tag{26}$$

nd

$$\mathrm{d}J_j = \beta J_j. \tag{27}$$

The infinitesimal difference in any variable Y [see eqn (21)], between $\mathscr{S}$ and $\mathscr{S}$ is given in terms of the differences in enzyme concentrations and time by t following equation:

$$dY = \sum_k \frac{\partial Y}{\partial E_k} dE_k + \frac{\partial Y}{\partial t} dt. \quad (2$$

We can introduce eqns (23) and (25) into eqn (28) obtaining:

$$dY = \beta \left(\sum_k E_k \frac{\partial Y}{\partial E_k} - t \frac{\partial Y}{\partial t} \right). \quad (2$$

In the right member of eqn (29) appear the unscaled control and time coefficier defined by eqns (15) and (19), respectively, and therefore eqn (29) can be writt as follows:

$$dY = \beta \left(\sum_k {}^u C^Y_{E_k} - {}^u T^Y \right). \quad (3$$

This equation can be written in terms of the scaled control and time coefficier defined in eqns (16) and (20), dividing both members by Y:

$$dY/Y = \beta \left(\sum_k C^Y_{E_k} - T^Y \right). \quad (3$$

If Y is the metabolite concentration S_i, we combine eqn (26) with eqns (30) a (31), and as β is a non-zero constant, we obtain:

$$\sum_k {}^u C^{S_i}_{E_k} = {}^u T^{S_i} \quad (3$$

and

$$\sum_k C^{S_i}_{E_k} = T^{S_i}. \quad (3$$

We call eqn (32) the "unscaled summation relationship" for metabolite concentrati coefficients. The "summation relationship" (scaled), obtained from the unscal one, dividing by the metabolite concentration, appears in eqn (33).

Now we consider the case when Y is the flux through reaction j, J_j. Combini eqn (27) with eqns (30) and (31) we obtain, respectively,

$$\sum_k {}^u C^{J_j}_{E_k} = J_j + {}^u T^{J_j} \quad (3$$

and

$$\sum_k C^{J_j}_{E_k} = 1 + T^{J_j}. \quad (3$$

Equations (34) and (35) are the unscaled and scaled summation relationships f flux coefficients.

All the summation relationships, obtained in this section, are independent of t value of the infinitesimal constant β. They are links between the control and tir coefficients, and impose constraints on the values these coefficients can attain.

If $\mathcal{S}$ and $\mathcal{S}_\beta$ represent stable steady state situations, as the metabolite concentra-
ons and fluxes are constant in time, all the time coefficients are zero. In this case
qns (33) and (35) take the form of the well known summation theorems of steady
ate control analysis (Kacser & Burns, 1973, 1979; Heinrich & Rapoport, 1974).

6. The Invariance of Rates

In this section, we compare the original system $\mathcal{S}$, with another metabolic system
$_\gamma$. They differ in the concentration of one metabolite S_i, and in the values of some
arameters and time. S_i appears explicitly in some of the rate laws v_k ($k = 1, \ldots, l$; $l \leq$
) introduced in eqn (2). We assume that for a particular time and values of the
arameters of $\mathcal{S}$, the values of the quantities of $\mathcal{S}_\gamma$, given below with subscript γ,
re related to those of $\mathcal{S}$ as follows:

$$S_{i,\gamma} = S_i + \mathrm{d}S_i \tag{36}$$

$$E_{k,\gamma} = E_k + \mathrm{d}E_k \qquad (k = 1, \ldots, l) \tag{37}$$

$$t_\gamma = t + \mathrm{d}t. \tag{38}$$

$_k$ are the enzyme concentrations corresponding to rates v_k mentioned above, and
is time. The other parameters are identical in both systems.

For a particular infinitesimal value $\mathrm{d}S_i$, we adjust $\mathrm{d}E_k$ ($k = 1, \ldots, l$), so that the
ates v_k ($k = 1, \ldots, l$) of $\mathcal{S}_\gamma$ are equal to those of $\mathcal{S}$, and therefore their relative
ifferences are zero.

$$\frac{\mathrm{d}v_k}{v_k} = {}^{u}\varepsilon_{s_i}^{v_k}\,\mathrm{d}S_i + \frac{\mathrm{d}E_k}{E_k} = 0 \qquad (k = 1, \ldots, l). \tag{40}$$

he unscaled elasticity coefficients that appear in these equations were defined in
qn (17). Here the relative difference in the enzyme concentrations is multiplied by
factor equal to one, because the rates are proportional to total enzyme concentration
see eqn (2)]. Simultaneously, for the values of $\mathrm{d}E_k$ calculated from eqns (40) we
an adjust $\mathrm{d}t$ introduced in eqn (38), such that the derivative of S_i, with respect to
$(\dot{S}_i)$, has the same value in both systems, and the difference $\mathrm{d}\dot{S}_i$, is zero. This
ifference can be given in terms of the differences in enzyme concentrations and
me as follows:

$$\mathrm{d}\dot{S}_i = \sum_{k=1}^{l} \frac{\partial \dot{S}_i}{\partial E_k}\,\mathrm{d}E_k + \frac{\partial \dot{S}_i}{\partial t}\,\mathrm{d}t = 0. \tag{41}$$

hen, for a particular value of $\mathrm{d}S_i$, if we choose appropriate values of $\mathrm{d}E_k$ ($k =$
, ..., l) and $\mathrm{d}t$, all the rates immediately related to S_i (v_k, $k = 1, \ldots, l$ and $\dot{S}_i$) are
ie same in $\mathcal{S}$ and $\mathcal{S}_\gamma$. It has to be noted that, for a given $\mathrm{d}S_i$, the values of $\mathrm{d}E_k$
$k = 1, \ldots, l$) and $\mathrm{d}t$ are different for each time.

To obtain the invariance of rates immediately related to S_i, we only adjust the
ifferences in enzymes concentrations corresponding to those rates, maintaining all
ie other enzyme concentrations and parameters constant. For this reason, all the
ther rates in $\mathcal{S}$ and $\mathcal{S}_\gamma$ are also the same. In this situation we expect that all the

fluxes, metabolite concentrations and their time derivatives are the same in b systems, S_i being the only variable with different value in both systems.

The quantitative conclusions obtained above are used in the next section construct connectivity relationships for time-dependent metabolic systems. E though these conclusions depend on the validity of the considerations made, t consequences (connectivity relationships) are verified by a separate mathemati procedure in Section 8.

7. Connectivity Relationships

The quantitative conclusions obtained from the comparison of systems $\mathcal{S}$ and described above, are now used to construct relationships that link the control a elasticity coefficients. S_i is the metabolite whose concentration is different in systems we are considering, the difference being:

$$dS_i = \sum_{k=1}^{l} \frac{\partial S_i}{\partial E_k} dE_k + \frac{\partial S_i}{\partial t} dt. \quad (4$$

As all other concentrations are equal in both systems, we can write for S_j ($j \neq$

$$dS_j = \sum_{k=1}^{l} \frac{\partial S_j}{\partial E_k} dE_k + \frac{\partial S_j}{\partial t} dt = 0. \quad (4$$

Using eqns (40) we can eliminate dE_k ($k = 1, \ldots, l$) from eqns (41–43), obtain eqns (44–46), respectively.

$$dS_i \left[\sum_{k=1}^{l} \left(E_k \frac{\partial \dot{S}_i}{\partial E_k} \right) {}^{u}\varepsilon_{s_i}^{v_k} \right] = \frac{\partial \dot{S}_i}{\partial t} dt \quad (4$$

$$dS_i \left[1 + \sum_{k=1}^{l} \left(E_k \frac{\partial S_i}{\partial E_k} \right) {}^{u}\varepsilon_{s_i}^{v_k} \right] = \frac{\partial S_i}{\partial t} dt \quad (4$$

$$dS_i \left[\sum_{k=1}^{l} \left(E_k \frac{\partial S_j}{\partial E_k} \right) {}^{u}\varepsilon_{s_i}^{v_k} \right] = \frac{\partial S_j}{\partial t} dt \qquad (j \neq i). \quad (4$$

The time derivatives that appear in the right members of eqns (44–46) are link by the following equation:

$$\frac{\partial \dot{S}_i}{\partial t} = \sum_{h=1}^{m} \left(\frac{\partial \dot{S}_i}{\partial S_h} \right) \frac{\partial S_h}{\partial t} \quad (4$$

where the sum is carried over all the metabolite concentrations including S_i, t appear explicitly in the differential equation corresponding to $\dot{S}_i$ [eqn (1)].

Equations (44–47) have to be satisfied simultaneously, so we combine them eliminating the time derivatives, obtaining:

$$\sum_{k=1}^{l} \left(E_k \frac{\partial \dot{S}_i}{\partial E_k} \right) {}^{u}\varepsilon_{s_i}^{v_k} = \frac{\partial \dot{S}_i}{\partial S_i} + \sum_{h=1}^{m} \left[\frac{\partial \dot{S}_i}{\partial S_h} \sum_{k=1}^{l} \left(E_k \frac{\partial S_h}{\partial E_k} \right) {}^{u}\varepsilon_{s_i}^{v_k} \right]. \quad (4$$

e quantities between curved parenthesis, in the right member of the last equation, e the unscaled concentration control coefficients. In the left member appear the alogous coefficients for $\dot{S}_i$. For a particular set of the values of the parameters, n (48) is a function of time only. Then if we interchange the derivatives with spect to time and enzyme, in the metabolite concentration control coefficients of we obtain a differential equation in the metabolite concentration control efficients. Taking into account these considerations, eqn (48) can be written as llows:

$$\sum_{k=1}^{l} {}^{u}\dot{C}_{E_k}^{S_i} \, {}^{u}\varepsilon_{s_i}^{v_k} = \frac{\partial \dot{S}_i}{\partial S_i} + \sum_{h=1}^{m} \left[\frac{\partial \dot{S}_i}{\partial S_h} \sum_{k=1}^{l} {}^{u}C_{E_k}^{S_h} \, {}^{u}\varepsilon_{s_i}^{v_k} \right] \tag{49}$$

here

$${}^{u}\dot{C}_{E_k}^{S_i} = \frac{\partial}{\partial t} ({}^{u}C_{E_k}^{S_i}). \tag{50}$$

quation (49) is one connectivity relationship for a time dependent metabolic system. relates the metabolite concentration control coefficients and elasticity coefficients. the procedure used, a number of connectivity relationships equal to the number metabolite concentrations to the square can be constructed. A mathematical rivation of all the connectivity relationships, in a matrix form, is given in ction 8.

If we consider a stable steady state situation, the right members of eqns (45) and 6) vanish. As dS_i is non-zero, the quantities between square brackets in both uations must be zero. After appropriate scaling, and using eqns (16) and (18), ns (45) and (46) for steady state conditions become

$$\sum_{k} C_{E_k}^{S_j} \varepsilon_{s_i}^{v_k} = -\delta_{ij} \tag{51}$$

here δ_{ij} is the Kronecker δ [$\delta_{ii} = 1$ and $\delta_{ij} = 0$ $(i \neq j)$]. These expressions are the nnectivity relationships for steady state concentration control coefficients Vesterhoff & Chen, 1984).

8. Summation and Connectivity Relationships (Matrix Equations)

The definitions of the vectors and matrices used in this section are given in able 1.

(A) PRELIMINARY EQUATIONS

First, we obtain general equations that are used in the derivation of summation nd connectivity relationships. The solution of eqn (1) is given in eqn (3), and the sulting fluxes in eqn (4). Substituting the solution in both members of eqn (1), is equation takes the form:

$$\dot{\mathbf{s}} = \mathbf{N}\mathbf{j} \tag{52}$$

TABLE 1

Definitions of vectors and matrices

Symbol	Name†	Dimension‡	Element *ij*‖	Equation§
$\mathbf{N}$	Stoichiometry (M)	$s \times v$	n_{ij}	eqn (1)
$\mathbf{s}$	Metabolite concentration (V)	$s \times 1$	S_i	eqn (3)
$\dot{\mathbf{s}}$	Time derivative of s (V)	$s \times 1$	$\dot{S}_i$	—
$\mathbf{v}$	Rate (V)	$v \times 1$	v_i	eqn (2)
$\mathbf{j}$	Flux (V)	$v \times 1$	J_i	eqn (4)
$\mathbf{S}$	Metabolite concentration (M)	$s \times s$	$S_{ii} = S_i$, $S_{ij} = 0\ (i \neq j)$	—
$\mathbf{J}$	Flux (M)	$v \times v$	$J_{ii} = J_i$, $J_{ij} = 0\ (i \neq j)$	—
$\partial\mathbf{v}/\partial\mathbf{s}$	Metabolite concentration derivative of v (M)	$v \times s$	$\partial v_i/\partial s_j$	eqn (17)
$\mathbf{e}$	Enzyme concentration (V)	$v \times 1$	E_i	eqn (2)
$\mathbf{E}$	Enzyme concentration (M)	$v \times v$	$E_{ii} = E_i$, $E_{ij} = 0\ (i \neq j)$	—
${}^{u}\mathbf{C}^{s}_{E}$	Unscaled concentration control coefficient (M)	$s \times v$	${}^{u}C^{S_i}_{E_j}$	eqn (15)
${}^{u}\dot{\mathbf{C}}^{s}_{E}$	Time derivative of ${}^{u}\mathbf{C}^{s}_{E}$ (M)	$s \times v$	${}^{u}\dot{C}^{S_i}_{E_j}$	eqn (50)
${}^{u}\mathbf{C}^{j}_{E}$	Unscaled flux control coefficient (M)	$v \times v$	${}^{u}C^{J_i}_{E_j}$	eqn (15)
${}^{u}\mathbf{T}^{s}$	Unscaled concentration time coefficient (V)	$s \times 1$	${}^{u}T^{S_i}$	eqn (19)
${}^{u}\dot{\mathbf{T}}^{s}$	Time derivative of ${}^{u}\mathbf{T}^{s}$ (V)	$s \times 1$	$\partial {}^{u}T^{S_i}/\partial t$	—
${}^{u}\mathbf{T}^{j}$	Unscaled flux time coefficient (V)	$v \times 1$	${}^{u}T^{J_i}$	eqn (19)
${}^{u}\boldsymbol{\varepsilon}^{v}_{s}$	Unscaled elasticity coefficient (M)	$v \times s$	${}^{u}\varepsilon^{v_i}_{S_j}$	eqn (17)
$\mathbf{I}_{s\times s}$	Identity (M)	$s \times s$	$d_{ii} = 1$, $d_{ij} = 0\ (i \neq j)$	—
$\boldsymbol{\mu}$	Unity (V)	$v \times 1$	$\mu_i = 1$	—
$\boldsymbol{\Sigma}$	Summation (V)	$s \times 1$	$\sum_i = \sum_j {}^{u}C^{S_i}_{E_j} - {}^{u}T^{S_i}$	—
$\dot{\boldsymbol{\Sigma}}$	Time derivative of $\boldsymbol{\Sigma}$ (V)	$s \times 1$	$\partial \sum_i/\partial t$	—

† Name of the vector (V) or matrix (M).

‡ Dimension of the vector or matrix, where "s" is the number of metabolites and "v" the numbe rates.

‖ Element of row "i" and column "j" of the vector or matrix.

§ Equation where the element, vector or matrix is introduced or defined.

where **j** is the flux vector, and we symbolize by **ṡ** the partial derivative of the soluti with respect to time. The derivative of the flux vector with respect to **e** (enzy concentration vector) is:

$$\frac{\partial \mathbf{j}}{\partial \mathbf{e}} = \frac{\partial \mathbf{v}}{\partial \mathbf{e}} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \mathbf{e}}. \qquad (5$$

Here we assume that the derivative of **j** with respect to **e** at fixed **s**, equals t derivative of **v** with respect to **e**$[(\partial \mathbf{j}/\partial \mathbf{e})\mathbf{s} = \partial \mathbf{v}/\partial \mathbf{e}]$, and the derivative of **j** with resp to **s** at fixed **e**, equals the derivative of **v** with respect to **s**$[(\partial \mathbf{j}/\partial \mathbf{s})\mathbf{e} = \partial \mathbf{v}/\partial \mathbf{s})$. This true only if the concentrations of metabolites are not linked by conservati equations (see also Reder, 1988). We differentiate eqn (52) with respect to **e** a postmultiply both members by **E** (the enzyme concentration matrix), rememberi that the stoichiometry matrix **N** is constant. In the left member appears the successi partial derivatives of **s** with respect to time (first) and enzyme concentration (secon Assuming that the concentrations of metabolites and their derivatives are continuo

unctions of time and enzyme concentration, we can change the order of partial ifferentiation. After these considerations we obtain:

$$^{u}\dot{\mathbf{C}}^{s}_{E} = \mathbf{N}\left(\mathbf{J} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,{}^{u}\mathbf{C}^{s}_{E}\right). \tag{54}$$

or a particular set of the values of the parameters, eqn (54) is a function of time nly. If we assume that the fluxes (elements of **J**) and their derivatives with respect o metabolite concentrations ($\partial \mathbf{v}/\partial \mathbf{s}$) are known functions of time, this equation epresents a system of linear differential equations in the metabolite concentration ontrol coefficients ($^{u}\mathbf{C}^{s}_{E}$). The solution tells us how the control coefficients depend n the fluxes, and their derivatives with respect to metabolite concentration (these an be seen as totally unscaled elasticity coefficients). Equation (54) can be used, ven when one or more rates are zero, at some point of the interval of time considered and elasticity coefficients given in eqns (17) and (18) are not defined].

Taking the derivative of eqn (52) with respect to time, it is easy to derive the quation that relates the vector of time coefficients ($^{u}\mathbf{T}^{s}$) with its time derivative ($^{u}\dot{\mathbf{T}}^{s}$):

$$^{u}\dot{\mathbf{T}}^{s} = \dot{\mathbf{s}} + \mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,{}^{u}\mathbf{T}^{s}. \tag{55}$$

(B) SUMMATION RELATIONSHIPS

To prove the summation relationships, we begin by post multiplying eqn (54) by unit column vector, $\boldsymbol{\mu}$, obtaining:

$$^{u}\dot{\mathbf{C}}^{s}_{E}\boldsymbol{\mu} = \dot{\mathbf{s}} + \mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,{}^{u}\mathbf{C}^{s}_{E}\boldsymbol{\mu}. \tag{56}$$

hen we subtract, member by member, eqn (55) from eqn (56), to eliminate $\dot{\mathbf{s}}$.

$$^{u}\dot{\mathbf{C}}^{s}_{E}\boldsymbol{\mu} - {}^{u}\dot{\mathbf{T}}^{s} = \mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\left({}^{u}\mathbf{C}^{s}_{E}\,\boldsymbol{\mu} + {}^{u}\mathbf{T}^{s}\right). \tag{57}$$

n this expression the summation vector $\boldsymbol{\Sigma}$, and its time derivative appear (see Table). Introducing this notation into eqn (57):

$$\dot{\boldsymbol{\Sigma}} = \left(\mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\right)\boldsymbol{\Sigma}. \tag{58}$$

his is a linear homogeneous system of differential equations in $\boldsymbol{\Sigma}$. At the initial onditions, the derivatives of the metabolite concentrations with respect to enzyme oncentrations are zero, and as an immediate consequence, the unscaled control oefficients are zero. As the initial condition for time is zero (see Section 2), the nitial time coefficient is also zero. (If $t_0 \neq 0$, the definition of the time coefficient as to be extended, see Section 4). Then the summation vector at the initial conditions s equal to the vector of zeros. The solution of eqn (58) in these conditions is zero or all times:

$$\boldsymbol{\Sigma} = 0. \tag{59}$$

The last equation can be written in its equivalent notation:

$$^{u}\mathbf{C}^{s}_{E}\,\boldsymbol{\mu} = {}^{u}\mathbf{T}^{s}. \tag{6}$$

Equation (60) is the summation relationship for unscaled metabolite concentrati coefficients, written in vector form. It represents a number of scalar equations equ to the number of variable metabolites. The equalities between the components these vectors are given in eqn (32).

Postmultiplying eqn (53) by E (enzyme concentration matrix) we obtain t relation between the unscaled flux control coefficient matrix $^{u}\mathbf{C}^{j}_{E}$, and concentrati control coefficient matrix:

$$^{u}\mathbf{C}^{j}_{E} = \mathbf{J} + \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,{}^{u}\mathbf{C}^{s}_{E}, \tag{6}$$

where J is the flux matrix. The time derivative of the flux vector j is given by:

$$\frac{\partial \mathbf{j}}{\partial \mathbf{t}} = \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,\frac{\partial \mathbf{s}}{\partial \mathbf{t}}. \tag{6}$$

If we multiply both members by the variable time t, we obtain the relation betwe the unscaled flux and concentration time coefficient vectors:

$$^{u}\mathbf{T}^{j} = \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\,{}^{u}\mathbf{T}^{s}. \tag{6}$$

Finally, we postmultiply eqn (61) by the unity vector $\boldsymbol{\mu}$, and premultiply eqn (6 by $\partial \mathbf{v}/\partial \mathbf{s}$. Combining the resulting equations and eqn (63) we obtain:

$$^{u}\mathbf{C}^{j}_{E}\boldsymbol{\mu} = \mathbf{j} + {}^{u}\mathbf{T}^{j}. \tag{6}$$

Equation (64) is the summation relationship for unscaled flux coefficients. The scal relationships between the components are given in eqn (34).

Now we assume that the metabolite concentration matrix S and the flux matr J (both diagonal matrices) are invertible, which means that all metabolites ar fluxes are different from zero. Then, by premultiplying eqn (60) by $\mathbf{S}^{-1}$ (the inver of S) we obtain the summation relationship for metabolite concentration coefficien (scaled):

$$\mathbf{C}^{s}_{E}\boldsymbol{\mu} = \mathbf{T}^{s} \tag{6}$$

and premultiplying eqn (64) by $\mathbf{J}^{-1}$ (the inverse of J) we obtain the summatic relationship for flux coefficients (scaled) in matrix form:

$$\mathbf{C}^{j}_{E}\boldsymbol{\mu} = \boldsymbol{\mu} + \mathbf{T}^{j}. \tag{6}$$

The relation between the flux and concentration control coefficients (scaled) obtained by premultiplying both members of eqn (61) by $\mathbf{J}^{-1}$, and premultiplyir the concentration control coefficient matrix by $\mathbf{SS}^{-1}$:

$$\mathbf{C}^{j}_{E} = \mathbf{I}_{v\times v} + \boldsymbol{\varepsilon}^{v}_{s}\mathbf{C}^{s}_{E}. \tag{6}$$

(C) CONNECTIVITY RELATIONSHIPS

If **J** is invertible, in eqn (54), we can premultiply $\partial \mathbf{v}/\partial \mathbf{s}$ by $\mathbf{J}\mathbf{J}^{-1}$. Then postmultiplying by the unscaled elasticity coefficient matrix and rearranging the right member, we obtain:

$$ {}^{u}\dot{\mathbf{C}}_{\mathbf{E}}^{\mathbf{s}}\,{}^{u}\boldsymbol{\varepsilon}_{\mathbf{s}}^{\mathbf{v}} = \mathbf{N}\frac{\partial \mathbf{v}}{\partial \mathbf{s}}(\mathbf{I}_{s\times s} + {}^{u}\mathbf{C}_{\mathbf{E}}^{\mathbf{s}}\,{}^{u}\boldsymbol{\varepsilon}_{\mathbf{s}}^{\mathbf{v}}). \tag{68} $$

Equation (68) is the connectivity relationship in matrix form. It represents s^2 number of scalar relationships, were s is the number of metabolites. Equation (49) is one of these scalar relationships. If we are dealing with an unbranched pathway, the number of steps (enzymes) is equal to the number of variable metabolites plus one. The number of metabolite control coefficients in this metabolic system is equal to the number of steps $(s+1)$ times the number of metabolites (s). Then eqns (60) and (68) together constitute a system of differential equations that has the same number of equations $(s+s^2)$ as unknown metabolite control coefficients. Assuming that $\partial \mathbf{v}/\partial \mathbf{s}$, the time coefficients and the elasticity coefficients are known functions of time, we can integrate the linear system of differential equations. The solution shows us how the metabolite control coefficients (system variations) are related to the elasticity coefficients (local variations). Substituting this solution into eqn (61) the relation between the flux control coefficients and the elasticity coefficients is obtained. If the pathway is not linear (e.g. branched) additonal relationships are needed to complete the analysis.

If the metabolic system is in a stable steady state, the left member of eqn (68) equals to a $(s \times s)$ matrix of zeros (**0**). As in this metabolic situation $\mathbf{N}\partial \mathbf{v}/\partial \mathbf{s}$ (the Jacobian matrix) is invertible, the expression between parenthesis in the right member of eqn (68) is equal to **0**. We assume that the metabolite concentration matrix **S** is invertible (the steady state concentrations of metabolites are all non-zero). Subtracting the identity matrix from both members, premultiplying them by $\mathbf{S}^{-1}$, and postmultiplying them by **S** we obtain:

$$ \mathbf{C}_{\mathbf{E}}^{\mathbf{s}}\boldsymbol{\varepsilon}_{\mathbf{s}}^{\mathbf{v}} = -\mathbf{I}_{s\times s}. \tag{69} $$

Equation (69) is the metabolite concentration connectivity relationship in matrix form for steady state systems (Westerhoff & Chen, 1984).

We can conclude by noting eqns (54), (60), (61), (64), (65), (66), (67) and (68) as representing the main results of our analysis. They approach the well known theorems of steady state control analysis, as the time evolution tends to a stable steady state.

9. Example

It is instructive to apply the general conclusions to a particular example. This will also reveal some of the biological relevance of the foregoing treatment. In what follows we shall point out what pattern of behaviour can be expected without commitment to specific mechanisms or to particular values of parameters.

We consider a metabolic pathway whose scheme is given in eqn (70).

$$X_0 \xrightarrow{E_1} S \xrightarrow{E_2} X_1. \tag{70}$$

The concentrations of metabolites X_0 and X_1 are held constant, S being the onl metabolite whose concentration is free to move. For this scheme, with one variabl metabolite concentration and two rates, the stoichiometry matrix is:

$$\mathbb{N} = [1 \quad -1]. \tag{71}$$

The reactions, catalyzed by enzymes E_1 and E_2 respectively, are in general reversible and their rate laws are subject only to the same restrictions as eqn (2).

For this example, the components of eqn (54) are:

$$\begin{aligned} {}^u\dot{C}^s_{E_1} &= J_1 + \frac{\partial(v_1 - v_2)}{\partial S}\, {}^uC^s_{E_1} \\ {}^u\dot{C}^s_{E_2} &= -J_2 + \frac{\partial(v_1 - v_2)}{\partial S}\, {}^uC^s_{E_2}. \end{aligned} \tag{72}$$

Equations (72) constitute a system of differential equations that can be solved t obtain the unscaled concentration control coefficients. If we integrate, taking int account that at the initial condition of time ($t = 0$) the concentration contro coefficients are zero, we obtain:

$$\begin{aligned} {}^uC^s_{E_1} &= (J_1 - J_2) \int_0^t \frac{J_1}{J_1 - J_2}\, \mathrm{d}t \\ {}^uC^s_{E_2} &= (J_1 - J_2) \int_0^t \frac{-J_2}{J_1 - J_2}\, \mathrm{d}t. \end{aligned} \tag{73}$$

In this particular example, the summation and connectivity relationships give by eqns (60) and (68), respectively, take the form:

$${}^uC^s_{E_1} + {}^uC^s_{E_2} = {}^uT^s \tag{74}$$

and

$${}^u\dot{C}^s_{E_1}\, {}^u\varepsilon^{v_1}_s + {}^u\dot{C}^s_{E_2}\, {}^u\varepsilon^{v_2}_s = \frac{\partial(v_1 - v_2)}{\partial S}\,(1 + {}^uC^s_{E_1}\, {}^u\varepsilon^{v_1}_s + {}^uC^s_{E_2}\, {}^u\varepsilon^{v_2}_s). \tag{75}$$

Equation (74), its time derivative, and eqn (75) are functions of time, and constitut a system of equations that can be solved to obtain the unscaled control coefficient in terms of the unscaled elasticity coefficients. Integrating the system from tim zero, we obtain:

$$\begin{aligned} {}^uC^s_{E_1} &= {}^uT^s \frac{\displaystyle\int_0^t \frac{{}^u\varepsilon^{(v_1 - v_2)}_s - {}^u\varepsilon^{v_2}_s}{{}^u\varepsilon^{v_1}_s - {}^u\varepsilon^{v_2}_s}\, \mathrm{d}t}{t} \\ \text{and} \qquad {}^uC^s_{E_2} &= {}^uT^s \frac{\displaystyle\int_0^t \frac{{}^u\varepsilon^{v_1}_s - {}^u\varepsilon^{(v_1 - v_2)}_s}{{}^u\varepsilon^{v_1}_s - {}^u\varepsilon^{v_2}_s}\, \mathrm{d}t}{t}. \end{aligned} \tag{76}$$

It will be noted that eqns (73) and (76) are two forms of the same relationship, but the latter exhibits explicitly the concentration control coefficients in terms of the (time-dependent) elasticity and time coefficients.

The control coefficients (scaled) are obtained in terms of the scaled elasticity coefficients applying the appropriate transformations to eqns (76).

$$C^s_{E_1} = T^s \frac{\int_0^t \frac{\varepsilon_s^{(v_1-v_2)} - \varepsilon_s^{v_2}}{\varepsilon_s^{v_1} - \varepsilon_s^{v_2}} \mathrm{d}t}{t}$$

and (77)

$$C^s_{E_2} = T^s \frac{\int_0^t \frac{\varepsilon_s^{v_1} - \varepsilon_s^{(v_1-v_2)}}{\varepsilon_s^{v_1} - \varepsilon_s^{v_2}} \mathrm{d}t}{t}.$$

Once the unscaled concentration control coefficients are known, the unscaled flux control coefficients can be obtained using eqn (61):

$$\begin{aligned}
{}^uC^{J_1}_{E_1} &= J_1 + \frac{\partial v_1}{\partial S}\, {}^uC^s_{E_1} \\
{}^uC^{J_1}_{E_2} &= \frac{\partial v_1}{\partial S}\, {}^uC^s_{E_2} \\
{}^uC^{J_2}_{E_1} &= \frac{\partial v_2}{\partial S}\, {}^uC^s_{E_1} \\
{}^uC^{J_2}_{E_2} &= J_2 + \frac{\partial v_2}{\partial S}\, {}^uC^s_{E_2}.
\end{aligned} \tag{78}$$

The expressions that relate the scaled control coefficients are [see eqn (67)]:

$$\begin{aligned}
C^{J_1}_{E_1} &= 1 + \varepsilon_s^{v_1} C^s_{E_1} \\
C^{J_1}_{E_2} &= \varepsilon_s^{v_1} C^s_{E_2} \\
C^{J_2}_{E_1} &= \varepsilon_s^{v_2} C^s_{E_1} \\
C^{J_2}_{E_2} &= 1 + \varepsilon_s^{v_2} C^s_{E_2}.
\end{aligned} \tag{79}$$

It is worth pointing out that, unlike the steady state solution (see below), we have four different (not two) flux control coefficients. This is because, at any time before the steady state, the two fluxes J_1 and J_2 are not necessarily equal to one another and, as shown here, respond differently to a particular enzyme variation. This is true, not only for the small system considered here [eqn (70)], but for any system of any complexity. The immediate biological relevance of this is that, in e.g. developing systems, different parts can show differential sensitivities even if at steady state no such differences are detectable.

Equations (76)–(79) show how the sensitivity of the metabolite concentration and fluxes to changes in one enzyme concentration are quantitatively affected by the kinetic properties of the individual enzymes, included in the elasticity coefficients.

It is important to note that the elasticity coefficients appear within the time integral. This is so because the effect of the local variations (given by the elasticity coefficients) on the system variations (given by the control coefficients) depend not only on the properties of the individual rates, and the topology of the network, but on the extent of time the system has, in fact, evolved. Furthermore, at the initial condition of time, even if the elasticity coefficients are non-zero, the metabolite concentration control coefficients are all zero since the metabolite concentration has not changed. In these conditions the flux control coefficient (scaled) is one, if the flux considered corresponds to the step where the enzyme was changed, and zero if that flux is affected only by the movement of the metabolite concentration.

If the system is one that approaches a stable steady state, the limit of the functions given in eqns (77), as time goes to infinity, is:

$$(C^{s}_{E_1})_{ss} = \frac{1}{(\varepsilon^{v_2}_{s})_{ss} - (\varepsilon^{v_1}_{s})_{ss}}$$

and (80)

$$(C^{s}_{E_2})_{ss} = \frac{-1}{(\varepsilon^{v_2}_{s})_{ss} - (\varepsilon^{v_1}_{s})_{ss}},$$

the subscript ss indicating steady state values. Substituting eqns (80) into eqns (79) we obtain:

$$(C^{J_1}_{E_1})_{ss} = (C^{J_2}_{E_1})_{ss} = \frac{(\varepsilon^{v_2}_{s})_{ss}}{(\varepsilon^{v_2}_{s})_{ss} - (\varepsilon^{v_1}_{s})_{ss}}$$

$$(C^{J_1}_{E_2})_{ss} = (C^{J_2}_{E_2})_{ss} = \frac{-(\varepsilon^{v_1}_{s})_{ss}}{(\varepsilon^{v_2}_{s})_{ss} - (\varepsilon^{v_1}_{s})_{ss}}. \quad (81)$$

Equations (80) and (81) are the same expressions obtained for the control coefficients in terms of the elasticity coefficients, when the scheme (70) is studied using steady state control analysis (developed in Kacser, 1983).

The equations derived in this section, and the general conclusions obtained hold whatever function the dependence of the rates on the concentration of S is assumed.

Let us now restrict our treatment to the case where the rate laws are monotonic functions in the concentration of S (decreasing for the first and increasing for the second step). With this assumption, the sign of the concentration control coefficients can be studied using eqns (73). If the initial value of S (S_{ini}) is less than the steady state value (S_{ss}), then $J_1 > 0$ and $J_1 > J_2$ for any time. Considering these inequalities together with eqn (73) we conclude that ${}^{u}C^{s}_{E_1} > 0$ for any time. If, in addition, $S_{ini} > X_1/K_2$ (K_2 is the equilibrium constant of the second step), then $J_2 > 0$ and ${}^{u}C^{s}_{E_2} < 0$, for any time. But, if $S_{ini} < X_1/K_2$, J_2 changes sign as time goes from zero to infinity. Then, in this last situation, for small time ${}^{u}C^{s}_{E2} > 0$ and for large time ${}^{u}C^{s}_{E2} < 0$. In the case where $S_{ini} > S_{ss}$, ${}^{u}C^{s}_{E_2} < 0$. If, in addition, $S_{ini} < K_1X_0$ (K_1 is the equilibrium constant of the first step), ${}^{u}C^{s}_{E_1} > 0$. But if $S_{ini} > K_1X_0$, then ${}^{u}C^{s}_{E_1}$ changes sign in the time course, being negative for small time and positive for large time. The sum of the concentration control coefficients, given by eqn (74), is positive or negative depending on $S_{ini} < S_{ss}$ or $S_{ini} > S_{ss}$ respectively. It is obvious that the scaled concentration control coefficients have the same sign as the unscaled ones.

We conclude that for monotonic rate laws, even though at steady state $C^s_{E_1} > 0$ and $C^s_{E_2} < 0$ and their sum is equal to zero [see eqns (80)], the corresponding time dependent coefficients and their sum can be either positive or negative depending on the initial concentration of S and the time. The steady state flux control coefficients, given in eqns (81), are each positive and less than one, their sum being equal to one. In time dependent situations, it may be shown that each of the four flux control coefficients [eqns (79)] and the sum of those corresponding to the same flux, can be either positive or negative for different initial conditions and temporal points. These conclusions are independent of the enzyme mechanisms provided only that we could assume monotonicity. To illustrate one possible pattern of behaviour, in Fig. 1, we show a diagram of the change in time of the two flux control coefficients ($C^{J_1}_{E_1}$ and $C^{J_1}_{E_2}$) and their sum obtained by simulation of system (70) with particular mechanisms, parameters and initial conditions.

Using the assumptions of monotonicity of the rate laws we studied the signs of the time dependent control coefficients, comparing the results with those at steady state. Let us now impose additional restrictions to the rate laws, with the aim of making predictions about the magnitude of the control coefficients. We assume reversible Michaelis-Menten rate laws for both steps of scheme (70). The elasticity coefficients can be written as the sum of two terms, one that depends on the disequilibrium and the other on the saturation (Westerhoff *et al.*, 1984). In this case we consider two extreme situations: (a) the overall reaction (from X_0 to X_1) is near equilibrium, and (b) both reactions are far from equilibrium and substantially

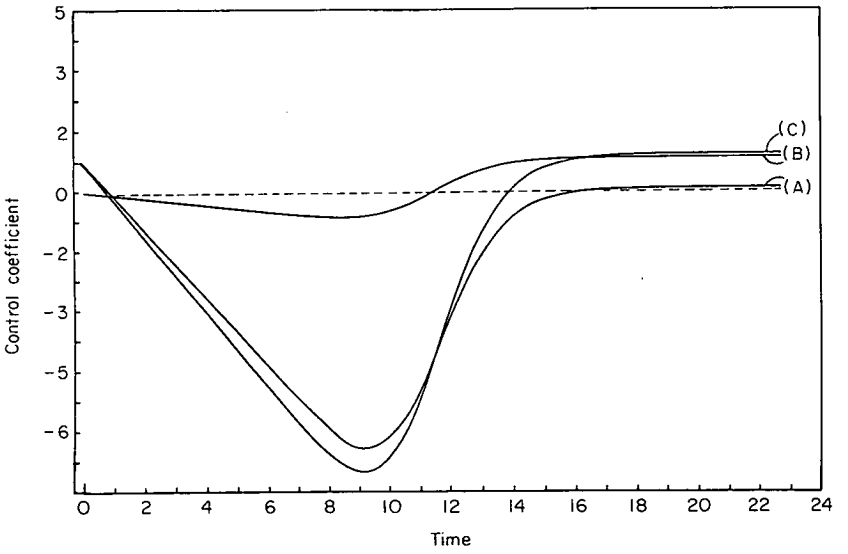

FIG. 1. Time evolution of flux control coefficients in system (70). The rate equations for both steps were represented by: $v_1 = V_1\ (X_0 - S/K_1)$ and $v_2 = V_2(S - X_1/K_2)$ $V_1 = 10$, $V_2 = 1$, $K_1 = 1$, $K_2 = 0{\cdot}1$, $X_0 = 1000{\cdot}1$, $X_1 = 100$ and at $t_0 = 0$, $S_0 = S_{ss}/100$. We show only changes in $C^{J_1}_{E_1}$ (curve *A*), $C^{J_1}_{E_2}$ (curve *B*), and their sum (curve *C*). The latter is also equal to $1 + T^{J_1}$. At large values of time they nearly reach their steady state values.

saturated by the substrate at steady state. In situation (a) the elasticity coefficient at steady state are large (Westerhoff, 1984) and, from eqn (80), the concentratio control coefficients are small. At the initial conditions the concentration contro coefficients are zero. Between the initial conditions and the steady state we can fin points where the interval of time elapsed from the initial conditions is such that th concentration of S can change significantly, but the elasticity coefficients are no large and the time coefficient is not small. At these points the concentration contro coefficients can have values that are not small as they would be in the initial stage and at steady state [see eqn (77)]. Furthermore, when, in addition, unsaturated rat laws are considered, it can be shown (Acerenza, Sauro and Kacser, unpublishe results) that the sum of the time dependent concentration control coefficients (scaled can attain a maximum positive value of one, for cases where $S_{ini} < S_{ss}$ or considerabl large negative values for cases where $S_{ini} > S_{ss}$. Now we consider the extrem situation (b). If both reactions are far from equilibrium and saturated by substrat at steady state, the elasticity coefficients (at steady state) are small and the contro coefficients are large [eqns (80)]. As was pointed out above, the concentratio control coefficients at the initial condition are zero. Then, in this situation, th concentration control coefficients, given by eqns (77), take a wide range of values as the system evolves from the initial condition to the steady state.

10. Discussion

In the definitions of control, time and elasticity coefficients (Section 4), and i the derivation of summation and connectivity relationships several mathematica assumptions were made. The variables and their derivatives were considered to b continuous functions of the parameters and time. A large and simultaneous chang in all enzyme concentrations by the same factor will not change the qualitativ behaviour of the system, the only difference being a constant time scale transforma tion (see Section 3). Other changes, however, in some of the parameters coul change, for example, a stable steady state, into sustained oscillations, if a bifurcatio point is reached. Due to the discontinuities occurring in these points, bifurcatio behaviour is excluded in our treatment. In eqn (1), the temporal change of th metabolite concentrations is written as a linear combination of the kinetic laws o individual enzymes. This implies that, even if the concentrations of metabolites ar changing in time, a quasi-stationary approximation for the different forms unde which each enzyme exists, is plausible. If "hysteretic enzymes" (Neet & Ainslie 1980) are present, however, these can present lags or bursts with relaxation time up to minutes, and in general they do not satisfy the last approximation. We als assumed that each enzyme affects only one step ("independence") and that th reaction rate is of first order with respect to the total concentration of this enzym ("additivity") [see eqn (2)]. There is some experimental evidence suggesting tha enzymes can act non-independently, and that metabolites can be transferred b enzyme-enzyme interaction, but it is not established how frequent this phenomeno is *in vivo* (Srivastava & Bernhard, 1986; Srere, 1987). Enzymes that present associ ation-dissociation mechanisms (Frieden, 1967) do not fulfill the additivity requisite

and are excluded from our treatment. Metabolic Control Analysis for steady state systems that are non-additive and non-independent has been recently developed by Kacser, Sauro and Acerenza (unpublished results). Furthermore, the present treatment excludes metabolic structures, such as conserved cycles, where the concentrations of metabolites are linked by conservation equations [see eqn (53)]. All other systems that do not violate the mathematical assumptions made are, strictly speaking, included in our treatment, while systems which contain some of the above aspects may nevertheless be amenable to the present analysis if, quantitatively, the deviations are not significant.

The time response of (parts of) organisms can be an important factor in the proper functioning of the organism in its environment. Thus, transition from one steady state to another caused by some external stimulus must be under enzymatic (and eventually genetic) control. Similarly, certain periodic phenomena (from high frequency "songs" to circadian rhythms) are known to be genetically determined and must operate through some system of enzyme mediated steps. The distribution of control between the different steps of the network is therefore a critical aspect of the fitness of the organism. It was shown (and supported by one example, Fig. 1) that in a system which had only positive steady state flux control coefficients, the control of the time course can display high negative coefficients. An enzyme (E_1) which had little control over the steady state flux could be seen to exert a mayor (negative) control on the development of the system. The establishment of the relationship between the time-dependent control coefficients and the kinetic nature of the enzymes is a first step towards understanding the molecular basis of these phenomena.

It is known that in many cases (particularly in microorganisms) whole pathways are co-ordinately induced or repressed (see, for example, Stuart *et al.*, 1986). This is very close to the summation transformation, where the control exerted by a simultaneous and equal change in all the enzyme concentrations of a pathway, by the same factor ("co-ordinate control") are considered (see Section 5). It should be noted that the coordinate control is directly related to the time scale transformation of the time course of the metabolite concentrations (see Section 3).

The summation and connectivity relationships, as was stated in Sections 8 and 9, can be used to write the control coefficients in terms of the time and elasticity coefficients, when linear pathways are considered. These equations are not as simple as for steady state situations, because the elasticity coefficients appear inside the time integral [see eqns (74)-(77)]. In spite of this fact, the analysis of these relationships gives insight on the way the main features of the kinetics of each metabolic reaction can affect the control coefficients, even if a complete knowledge of the rate laws is not available (see Section 9). This is one important achievement of Metabolic Control Analysis. Unlike traditional approaches to the problems of "control" which either are concerned with detailed mechanistic arguments or make use of *in vitro* values of parameters for simulations of putative *in vivo* models, Metabolic Control Analysis draws its principal conclusions without such specific assumptions.

The theory developed in the present paper is an extension of traditional Metabolic Control Analysis to time dependent systems. Each of the relationships derived has

its counterpart in the known steady state theory. But even if many of the basic idea here are not new, they are enlarged from the static vision of the stable steady state to a wider domain that includes time evolution, oscillations and chaos.

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REFERENCES

BOITEUX, A., HESS, B. & SEL'KOV, E. E. (1980). *Curr. Topics Cell. Regul.* **17,** 171.
BURNS, J. A., CORNISH-BOWDEN, A., GROEN, A. K., HEINRICH, R., KACSER, H., PORTEOUS, J. W. RAPOPORT, S. M., RAPOPORT, T. A., STUCKI, J. W., TAGER, J. M., WANDERS, R. J. A. & WESTERHOFF, H. V. (1985). *Trends Biochem. Sci.* **10,** 16.
DECROLY, O. & GOLDBETER, A. (1982). *Proc. natn. Acad. Sci. U.S.A.* **79,** 6917.
FELL, D. A. & SAURO, H. M. (1985). *Eur. J. Biochem.* **148,** 555.
FRIEDEN, C. (1967). *J. biol. Chem.* **242,** 4045.
GOODWIN, B. C. (1965). *Adv. Enzyme Regul.* **3,** 425.
HEINRICH, R. & RAPOPORT, T. A. (1974). *Eur. J. Biochem.* **42,** 89.
HIGGINS, J., FRENKEL, R., HULME, E., LUCAS, A. & RANGAZAS, G. (1973). In: *Biological and Biochemical Oscillators* (Chance, B., Pye, E. K., Ghosh, A. M. and Hess, B., eds.). p. 127. New York Academic Press.
HOFMEYR, J., KACSER, H. & VAN DER MERWE, K. J. (1986). *Eur. J. Biochem.* **155,** 631.
HUNDING, A. (1974). *Biophys. Struct. Mech.* **1,** 47.
KACSER, H. (1983). *Biochem. Soc. Trans.* **11,** 35.
KACSER, H. (1987). *Biochem. Plants* **11,** 39.
KACSER, H. & BURNS, J. A. (1973). *Symp. Soc. exp. Biol.* **27,** 65.
KACSER, H. & BURNS, J. A. (1979). *Biochem. Soc. Trans.* **7,** 1149.
KACSER, H. & PORTEOUS, J. W. (1987). *Trends Biochem. Sci.* **12,** 5.
KOHN, M. C. & CHIANG, E. (1982). *J. theor. Biol.* **98,** 109.
KOHN, M. C., WHITLEY, L. M. & GARFINKEL, D. (1979). *J. theor. Biol.* **76,** 437.
MIZRAJI, E., ACERENZA, L. & HERNANDEZ, J. (1988). *Biosystems* **22,** 11.
NEET, K. E. & AINSLIE, G. R., JR. (1980). *Methods Enzymol.* **64,** 192.
NICOLS, G. & PRIGOGINE, I. (1977). *Self-Organization in Non-Equilibrium Systems.* New York: Wiley
PROCACCIA, I. (1988). *Nature, Lond.* **333,** 618.
RAPP, P. E. (1979). *J. exp. Biol.* **81,** 281.
REDER, C. (1988). *J. theor. Biol.* **135,** 175.
SAURO, H. M., SMALL, J. R. & FELL, D. A. (1987). *Eur. J. Biochem.* **165,** 215.
SELWYN, M. J. (1965). *Biochim. Biophys. Acta* **105,** 193.
SORRIBAS, A. & BARTRONS, R. (1986). *Eur. J. Biochem.* **158,** 107.
SRERE, P. A. (1987). *A. Rev. Biochem.* **56,** 89.
SRIVASTAVA, D. K. & BERNHARD, S. A. (1986). *Curr. Topics Cell. Regul.* **28,** 1.
STUART, F., PORTEOUS, D. J., FLINT, H. J. & KACSER, H. (1986). *J. gen. Microbiol.* **132,** 1159.
WESTERHOFF, H. V. & CHEN, Y. (1984). *Eur. J. Biochem.* **142,** 425.
WESTERHOFF, H. V., GROEN, A. K. & WANDERS, R. J. A. (1984). *Bioscience Reports* **4,** 1.
WYMAN, J. (1975). *Proc. natn. Acad. Sci. U.S.A.* **72,** 3983.

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Enzyme-enzyme interactions and control analysis

1. The case of non-additivity: monomer-oligomer associations

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Two usual assumptions of the treatment of metabolism are: (a) the rates of isolated enzyme reactions are additive, i.e. that rate is proportional to enzyme concentration; (b) in a system, the rates of individual enzyme reactions are not influenced by interactions with other enzymes, i.e. that they are acting independently, except by being coupled through shared metabolites. On this basis, control analysis has established theorems and experimental methods for studying the distribution of control. These assumptions are not universally true and it is shown that the theorems can be modified to take account of such deviations. This is achieved by defining additional elasticity coefficients, designated by the symbol π, which quantify the effects of homologous and heterologous enzyme interactions. Here we show that for the case of non-proportionality of rate with enzyme concentration, ($\pi_i^i \neq 1$), the summation theorems are given by

$$\sum_{i=1}^{n} C_{E_i}^{J_j}/\pi_i^i = 1 \quad \text{and} \quad \sum_{i=1}^{n} C_{E_i}^{S_j}/\pi_i^i = 0 .$$

The example of monomer-oligomer equilibria is used to illustrate non-additive behaviour and experimental methods for their study are suggested.

In the classical study of the control of metabolism, two fundamental assumptions are usually made regarding the properties of the enzymes which constitute metabolic systems. The first is that the reaction rate of an isolated enzyme is first order with respect to enzyme concentration and the second assumption is that all enzymes are independently acting catalysts.

The first assumption, that of additivity, (i.e. rate $\propto E$) is based on the observations of the kinetics of a wide range of extracted enzymes. However, there are a number of instances where additivity may not apply [1]. For example, if an enzyme monomer-oligomer equilibrium exists and if the overall specific activity of the mix of monomer and homologous oligomer varies with the proportions of the two forms, then deviations from additivity will occur. Similar deviations could occur if the total concentration of a particular enzyme is partitioned between the free enzyme and enzyme bound to a catalytically inert substratum, such as a membrane or cytoskeleton, present in constant amount. If the bound enzyme has different kinetics from the free enzyme, then non-additivity would be observed. Similar arguments apply to enzymes operating in cascades.

The second assumption, that of independence, is usually represented by each enzyme catalysing one step without the enzyme associating with any other. Such associations between some enzymes, however, are known to take place. Nevertheless, there would be no functional consequences of such association if the catalytic activities of the enzymes in the heterologous complex were the same as those of the free enzymes. In the analysis, they would still be treated as independent catalysts, having a total concentration equal to free plus complex. If, however, the interaction by association of two enzymes affects the kinetic parameters of one or both of the constituent enzymes, the independence assumption is no longer valid.

Control analysis [2, 3] has recognised explicitly that intermediate metabolite(s) in all metabolic systems interact with each of their flanking enzymes. Thus adjacent enzymes are coupled via their shared metabolites and it necessarily follows that a perturbation in, e.g. an enzyme activity at any catalytic locus will create a response in reaction rate, the effects of which will spread through the whole system. Thus, although the enzymes may be assumed to act independently of each other, the fluxes sustained by them are not independent. Starting with this recognition, it has been possible to relate local responses (at each individual step in a metabolic system) to the consequential system responses and thus to establish experimentally quantifiable criteria for control of systemic functions such as flux rates and metabolite concentrations. In establishing these quantitative criteria, control analysis of the steady state has made the common assumptions of additivity and independence. It should be noted that in such an analysis, each enzyme in a metabolic pathway may display any form of kinetics, such as cooperative effects, substrate activation, saturation, inhibition, etc. The analysis is not confined to enzymes which obey the simple Michaelis-Menten formulation, as has sometimes been implied [4]. Furthermore, pathways of any network complexity may be analysed. It is not the case that only unbranched chains can be examined by control analysis [5–9]. Finally, whether an enzyme, when acting in a system, has a concentration which is comparable to, or even greater than, its substrate level is immaterial to the conclusions of control analysis of the steady state.

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With the assumptions of independence and additivity it was possible to describe the distribution of control in any system by establishing a number of theorems. These are the summation and connectivity theorems [2, 3, 6, 7, 10, 11]. In this paper we wish to describe the modifications to the theorems of control analysis and the consequences for the control properties of pathways when the assumptions of additivity are violated. We shall deal with the violation of independence in the subsequent paper. We will not be concerned with how the molecular events of complex formation lead to kinetic changes in the constituent subunits. This latter aspect is a strict enzymological problem. We are only concerned with studying the effects on the control properties of systems when such changes do occur.

DEFINITIONS AND THEOREMS

To approach the analysis of metabolic systems we begin by setting up general perturbation equations for the steady state. We shall consider a very simple step, i, in the whole system carrying a steady state flux, J_i and steady state concentrations of the variables, S_1 and S_m

$$\rightarrow \rightarrow S_1 \underset{E_i}{\overset{(J_i)}{\rightarrow}} S_m \rightarrow \rightarrow .$$

The rate of transformation of S_1 to S_m will be given by an equation of the form $v_i = f(S_1, S_m, E_i, k, \ldots)$. Changes from one steady state to another only occur if one (or more) parameters, p, of the system are changed. These may be either external, e.g. nutrient, external effector concentrations, etc., or internal parameters associated with the rate equation of some step, e.g. E, k_{cat}, K_m, K_i, etc. Let us consider for the moment an internal parameter change affecting the rate only at j somewhere in the system, but not a parameter associated with the rate equation of step i. Here we shall not be concerned with transient perturbations but only with permanent ones. Changes at i will occur because the initial change at j will tend to be transmitted right through the system. The system will eventually settle to a new steady state with the changes at i being given by δJ_i, δS_1 and δS_m. In our formulations we will always consider fractional changes, $\delta x/x$, which eliminate units of measurements of x. Because S_1 and S_m occur directly in the rate equation governing step i the system change $\delta J_i/J_i$ can occur in this example if, and only if, there are changes in either or both S_1 and S_m. Each change, $\delta S_1/S_1$ and $\delta S_m/S_m$, will make a separate contribution to the net change $\delta J_i/J_i$. Each of these contributions will be the product of the potential change in the rate, i.e. the elasticity coefficient, and the actual change in the steady state of the particular metabolite concentration. For S_1, for example, this contribution is therefore

$$\varepsilon_{S_1}^{v_i} \times \frac{\delta S_1}{S_1}.$$

where the elasticity coefficient is defined as:

$$\varepsilon_s^v = \frac{s}{v}\left(\frac{\partial v}{\partial s}\right)_{y,z\ldots} = \frac{\partial \ln v}{\partial \ln s}. \tag{1}$$

The elasticity coefficient is the partial derivative of a rate, v with respect to S (multiplied by the scaling factor, s/v). Since the elasticity is defined as the partial derivative, all other quantities are held constant. We therefore have to consider the step as isolated, i.e. all molecular concentrations (including metabolites), clamped at their steady-state values. For purposes of defining (and measuring) these local elasticity coefficients, metabolites are therefore parameters of the isolated rate while they are, of course, dependent variables in the free system.

Considering both contributions from $\delta S_1/S_1$ and $\delta S_m/S_m$ we can write the net change in flux at step i, as

$$\frac{\delta J_i}{J_i} = \varepsilon_{S_1}^{v_i}\frac{\delta S_1}{S_1} + \varepsilon_{S_m}^{v_i}\frac{\delta S_m}{S_m}. \tag{2}$$

This is only true provided the δ changes are small.

We now focus our attention at the source of the perturbation, i.e. the step j. Let the change in a parameter, p_j, associated with the rate at j, be the perturbation imposed on the system. The effect of this on the rate at j will be given by

$$\frac{\delta v_j}{v_j} = \pi_{p_j}^{v_j}\frac{\delta p_j}{p_j} \tag{3}$$

where $\pi_{p_j}^{v_j}$ is a 'parameter-elasticity coefficient'.

Definition:

$$\pi_p^v = \frac{p}{v}\left(\frac{\partial v}{\partial p}\right)_{q,r\ldots} = \frac{\partial \ln v}{\partial \ln p}. \tag{4}$$

Because this elasticity is of a different kind from the metabolite elasticity, ε, (Eqn 1), although of the same form, it is useful to designate it by the symbol π (standing for parameter). The general usefulness of such a notation will become apparent as our treatment proceeds. (Note: this is not the elasticity described by the π elasticity of Heinrich [12].)

We shall term $\delta v_j/v_j$ as the local rate change. It could be considered as measured in isolation or at the moment of the imposed change in p_j. This local rate change, then is the cause of the eventual movement of all metabolites (including those at i and j) to the new steady state.

We can give quantitative expression of the cause (change in local rate v_j) on the effect (change in net flux J_i) by dividing Eqn (2) by $\delta v_j/v_j$

$$\frac{\delta J_i}{J_i}\Big/\frac{\delta v_j}{v_j} = \varepsilon_{S_1}^{v_i}\frac{\delta S_1}{S_1}\Big/\frac{\delta v_j}{v_j} + \varepsilon_{S_m}^{v_i}\frac{\delta S_m}{S_m}\Big/\frac{\delta v_j}{v_j}. \tag{5}$$

We can take the limit as $\delta v_j \rightarrow 0$, to be

$$\frac{\delta J_i}{J_i}\Big/\frac{\delta v_j}{v_j} \rightarrow \frac{v_j}{J_i}\left(\frac{\partial J_i}{\partial v_j}\right) = C_{v_j}^{J_i}$$

symbolised as 'C_v'. We can do a similar operation to the δ terms on the right-hand side of Eqn (5) to obtain the concentration control coefficients measured with respect to rate. In general, we can write the definition for this global control coefficient as

$$C_{v_j}^{Y} = \frac{v_j}{Y}\left(\frac{\partial Y}{\partial v_j}\right), \tag{6}$$

with Y standing for any system variable (metabolite concentrations or fluxes). The usefulness of introducing the C_v notation for these parameter-unspecified coefficients will become apparent below.

We can now write Eqn (5), for infinitesimal changes as

$$C_{v_j}^{J_i} = \varepsilon_{S_1}^{v_i} C_{v_j}^{S_1} + \varepsilon_{S_m}^{v_i} C_{v_j}^{S_m}. \tag{7}$$

This expresses the flux control coefficient for J_i in terms of the concentration control coefficients for the metabolites and their respective elasticity coefficients for changes in a distant parameter modulation at j.

If we now consider, instead, that the change occurs at step i, i.e. a modulation of a parameter, p_i, occurring in the rate equation for v_i, the change in the local rate is

$$\frac{\delta v_i}{v_i} = \pi_{p_i}^{v_i} \frac{\delta p_i}{p_i} .$$

Eqn (2) now becomes

$$\frac{\delta J_i}{J_i} = \varepsilon_{S_1}^{v_i} \frac{\delta S_1}{S_1} + \varepsilon_{S_m}^{v_i} \frac{\delta S_m}{S_m} + \frac{\delta v_i}{v_i} \qquad (8)$$

there now being three direct contributions to the change in flux, J_i.

Dividing this time by $\delta v_i/v_i$ we obtain

$$C_{v_i}^{J_i} = \varepsilon_{S_1}^{v_i} C_{v_i}^{S} + \varepsilon_{S_m}^{v_i} C_{v_i}^{S_m} + 1 . \qquad (9)$$

We can write Eqn (7) and Eqn (9) more neatly as

$$C_{v_j}^{J_i} = \varepsilon_{S_1}^{v_i} C_{v_j}^{S_1} + \varepsilon_{S_m}^{v_i} C_{v_j}^{S_m} + \delta_{ij} , \qquad (10)$$

where the symbol δ_{ij} is the Kronecker delta with values of 1 for $j = i$ and 0 for $j \neq i$. Equations of this type form the basis of the subsequent analysis.

If the reaction considered is more complicated because of, say, bimolecularity or effector participation, additional terms of the same form would appear in the equations. It should be noted that the control coefficients are defined with respect to (infinitessimal) changes in a local rate without specifying the parameter(s) producing the change. The only requirement is to specify at which step in the metabolism the change is made. These parameter-unspecified definitions, C_v values, are the control coefficients from which all particular versions, specifying the modulated parameter, are derived. The theorems which can be obtained apply to these C_v values and are valid irrespective of any assumptions concerning the relationships between parameters and local rates and are therefore quite general. In this paper we are particularly concerned with enzyme concentration as a parameter. We investigate how the theorems must be modified if control coefficients are expressed (and measured) with respect to enzyme concentrations.

THE CASE OF ADDITIVITY AND INDEPENDENCE

When we choose the enzyme concentration, E_i, as the parameter p_i, the local rate change is given by

$$\frac{\delta v_i}{v_i} = \pi_{E_i}^{v_i} \frac{\delta E_i}{E_i} . \qquad (11)$$

With the assumption of additivity, $\delta v_i/v_i = \delta E_i/E_i$, and therefore

$$\pi_{E_i}^{v_i} = 1 \qquad (12)$$

which is a formal restatement of the additivity property. A parameter-specific control coefficient can now be defined for the parameter, enzyme concentration, as:

$$C_{E_i}^{Y} = \frac{E_i}{Y}\left(\frac{\partial Y}{\partial E_i}\right) \qquad (13)$$

and it follows by using Eqn (6) and Eqn (12) that $C_{v_i}^{Y} = C_{E_i}^{Y}$ (see also Burns et al. [13]).

An additional assumption is implicit in this identity which is that no enzyme affects the reaction rate of any other. This means that the elasticity of any rate with respect to changes in enzymes other than its own is zero, that is:

$$\pi_{E_j}^{v_i} = 0 \quad \text{for all } j \neq i . \qquad (14)$$

Eqn (14) represents a formal statement of independence. Non-independence is dealt with in the subsequent paper.

For this case of additivity and independence (and only this case) the C_v terms are equal to the C_E terms. Heinrich et al. [14] gave their definitions in terms similar to C_v terms but also noted the possible formulations of C_E terms. They make, however, no symbolic distinction between the two nor that relations Eqn (12) and Eqn (14) are implied when the two are numerically equal. Higgins [15] was the first to give a general definition. See also Salter et al. [16].

The theorems of control analysis

One of the major contributions of control analysis has been the derivation of theorems (or properties) of metabolic systems. Of these, two classes may be discerned. There is the class of theorem which applies to all pathways no matter what their complexity. These include the summation theorems, which are relationships between the control coefficients, and the connectivity theorems which are relationships between the control coefficients and elasticity coefficients. The second class of theorem relates to particular pathway structures such as branching structures and moiety-conserved cycles [6–8, 10] and are additional relationships which may be regarded as modified summation and connectivity theorems, respectively [17]. By their nature, all the theorems possess both heuristic and computational properties which have been exploited by a number of workers in the field [16, 18]. Computationally, the theorems are important since they can be used to derive the control equations which relate, for particular pathway structures, the elasticities to the control coefficients, i.e. they relate the local enzymological properties at their operating values to the system behaviour. A number of matrix-related techniques have been developed to exploit this [9, 19, 20].

The undernoted theorems Eqn (15) are given in terms of C_v and can be shown to follow necessarily from their definitions and the modulation procedures as given in [2, 3, 11, 21].

Summation theorems

$$\sum_{i=1}^{n} C_{v_i}^{J_j} = 1; \qquad \sum_{i=1}^{n} C_{v_i}^{S_j} = 0 . \qquad (15)$$

Connectivity theorems

$$\sum_{i=1}^{n} C_{v_i}^{J_j} \varepsilon_S^i = 0; \qquad \sum_{i=1}^{n} C_{v_i}^{S_j} \varepsilon_{S_k}^i = -\delta_{jk}$$

where $\delta_{jk} = 1$ for $j = k$, and $= 0$ for $j \neq k$. J_j is any flux and S_j any one metabolite in the whole system having n rates. If conditions of Eqn (12) and Eqn (14) can be assumed, an identical set of theorems apply with all C_v terms replaced by C_E terms. This is the form in which they are most usually presented, e.g. [2, 6, 16, 22], and a distinction between the two formulations is not neccessary for such a case.

If, however, we wish to consider modulations of enzyme concentrations (as we are forced to do when considering certain practical problems of determining the coefficients) in situations where the assumptions Eqn (12) and/or Eqn (14) do not hold, then the theorems Eqn (15) written in terms of changes in enzyme concentration (C_E) are not adequate and must be modified.

The use of perturbation and matrix equations

As an example we may consider a complete system consisting of a three-step unbranched pathway (Scheme 1):

$$X_0 \xrightarrow[(J_1)]{E_1} S_1 \xrightarrow[(J_2)]{E_2} S_2 \xrightarrow[(J_3)]{E_3} X_3 .$$

Scheme 1. *Three-step unbranched pathway for an enzyme system.* X_0 represents a constant source of substrate while X_3 represents a product sink [22]. S_1 and S_2 represent the substrates of enzymes E_2 and E_3, respectively. J is the rate of reaction

Considering all possible changes at each of the three steps leads to the formulation of three perturbation equations of the form shown in Eqn (8).

$$\frac{\delta J_1}{J_1} = \varepsilon_1^1 \frac{\delta S_1}{S_1} + \frac{\delta v_1}{v_1}, \tag{16}$$

$$\frac{\delta J_2}{J_2} = \varepsilon_1^2 \frac{\delta S_1}{S_1} + \varepsilon_2^2 \frac{\delta S_2}{S_2} + \frac{\delta v_2}{v_2}, \tag{17}$$

$$\frac{\delta J_3}{\delta J_3} = \varepsilon_2^3 \frac{\delta S_2}{S_2} + \frac{\delta v_3}{v_3} . \tag{18}$$

We now consider a change in one of the local rates, e.g. v_1 only (i.e. $\delta v_2 = 0$ and $\delta v_3 = 0$). Dividing both sides of each equation by $\delta v_1/v_1$, taking the limit as $\delta v_1/v_1 \to 0$ and solving for the steady state when $J_1 = J_2 = J_3 = J$, leads to three equations for $(v_1/J)\ (\partial J/\partial v_1) = C_{v_1}^J$:

$$C_{v_1}^J = \varepsilon_1^1 C_{v_1}^{S_1} + 1 , \tag{19}$$

$$C_{v_1}^J = \varepsilon_1^2 C_{v_1}^{S_1} + \varepsilon_2^2 C_{v_1}^{S_2} , \tag{20}$$

$$C_{v_1}^J = \varepsilon_2^3 C_{v_1}^{S_2} . \tag{21}$$

Elimination of $C_{v_1}^{S_1}$ and $C_{v_1}^{S_2}$ gives the flux control coefficient with respect to v_1 in terms of elasticities. A similar operation can be carried out using $\delta v_2/v_2$ or $\delta v_3/v_3$ instead of $\delta v_1/v_1$ to obtain expressions for $C_{v_2}^J$ and $C_{v_3}^J$.

Thus, for additivity and independence assumptions, $C_v = C_E$ and the complete set of flux control coefficients for Scheme 1 is,

$$C_{v_1}^J = \frac{\varepsilon_2^3 \varepsilon_1^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} = C_{E_1}^J \tag{22}$$

$$C_{v_2}^J = \frac{-\varepsilon_2^3 \varepsilon_1^1}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} = C_{E_2}^J \tag{23}$$

$$C_{v_3}^J = \frac{\varepsilon_1^1 \varepsilon_2^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} = C_{E_3}^J . \tag{24}$$

By appropriate elimination from Eqns (19–21), it is also possible to obtain expressions for $C_{v_1}^{S_1}$, $C_{v_1}^{S_2}$, etc., in terms of elasticities only. If the assumptions in Eqns (12) and (14) can be made, a direct transformation to C_E values is possible. The procedure just outlined is a derivation from basic equations and does not rely on any theorems. For large systems containing many enzymes, branches and loops, the method is exactly the same but becomes more tedious. A simpler method and one which can be applied to any system is based on a solution to the set of theorems associated with the pathway [6, 10]. In the example here, we can obtain the particular summation theorem and two connectivity theorems Eqn (25). They may be recognised from Eqn (15).

$$\text{Summation theorem} \quad C_{v_1}^J + C_{v_2}^J + C_{v_3}^J = 1 .$$

$$\text{Connectivity theorems} \quad \begin{cases} C_{v_1}^J \varepsilon_1^1 + C_{v_2}^J \varepsilon_1^2 = 0 \\ C_{v_2}^J \varepsilon_2^2 + C_{v_3}^J \varepsilon_2^3 = 0 . \end{cases} \tag{25}$$

The three theorems (Eqn 25) constitute a set of three equations in three unknowns, the $C_{v_1}^J$ values, which may be solved. This is the basis of the matrix method [6, 10]. The above set of equations may be written in matrix form and rearranged to give:

$$\begin{bmatrix} C_{v_1}^J \\ C_{v_2}^J \\ C_{v_3}^J \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_1^2 & 0 \\ 0 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix}^{-1} \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} . \tag{26}$$

The solution to this equation, which involves matrix inversion, yields exactly the same expression as in Eqns (22–24).

The matrix method may also be easily extended to evaluate simultaneously the concentration control coefficients [10, 19] by exploiting the concentration control coefficient theorem (Eqn 15)

$$\begin{bmatrix} C_{v_1}^J & C_{v_1}^{S_1} & C_{v_1}^{S_2} \\ C_{v_2}^J & C_{v_2}^{S_1} & C_{v_2}^{S_2} \\ C_{v_3}^J & C_{v_3}^{S_1} & C_{v_3}^{S_2} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_1^2 & 0 \\ 0 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix}^{-1} \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix} \tag{27}$$

from which all control coefficients can be obtained from the elasticities. With the assumptions, Eqn (12) and Eqn (14), all C_v expressions can be replaced by C_E expressions. It should be noted that other methods [9, 14, 17, 20, 23, 24] of arriving at the theorems (Eqn 15) and the control equations may also be used to achieve the same results (although it should be noted that Reder [17] uses unscaled coefficients in her analysis).

THE CASE OF NON-ADDITIVITY

All approaches to the solution of the control equations yield the same formulations for the C_v expressions. Assuming additivity and independence, modulation of enzyme concentration yield C_E values which are identical in form and value to C_v values. Consider now that the additivity assumption no longer applies say to the second enzyme in scheme 1, i.e. $\delta v_2/v_2 \neq \delta E_2/E_2$. The local rate change is now given by,

$$\frac{\delta v_2}{v_2} = \pi_{E_2}^{v_2} \frac{\delta E_2}{E_2} .$$

The π elasticity defines the potential effect on the isolated rate of a change in the concentration of the enzyme.

$$\pi_{E_2}^{v_2} = \frac{E_2}{v_2} \left(\frac{\partial v_2}{\partial E_2} \right)_{S_1, S_2} (= \pi_2^2) . \tag{28}$$

Thus, for the modified Scheme 1, the second equation, replacing Eqn (17), must be written as:

$$\frac{\delta J_2}{J_2} = \varepsilon_1^2 \frac{\delta S_1}{S_1} + \varepsilon_2^2 \frac{\delta S_2}{S_2} + \pi_2^2 \frac{\delta E_2}{E_2} \tag{29}$$

with π_2^2 no longer necessarily equal to unity. Modulation of enzyme concentration produces the control equations, derived in the same manner as before, and we obtain the equations given below

$$C_{E_1}^J = \frac{\varepsilon_2^3 \varepsilon_1^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} \tag{22}$$

$$C_{E_2}^J = \frac{-\varepsilon_2^2 \varepsilon_1^1}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} \pi_2^2 \tag{30}$$

$$C_{E_3}^J = \frac{\varepsilon_1^1 \varepsilon_2^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} . \tag{24}$$

One may now observe that, algebraically, the equation for $C_{E_1}^J$ and $C_{E_3}^J$ are the same as those derived previously on the

additivity assumption, Eqns (22) and (24). It is only the second equation which is different to the extent that there is an additional multiplier, π_2^2.

It should be pointed out that if one considers a possible experiment where one replaces the simple enzyme E_2 by one showing non-additivity, we would have a completely new system. The rate equation would be of a different form and hence the elasticities would be different. This in turn would affect substrate levels and, hence, fluxes. We end up with formulations which are algebraically the same for two of the control coefficients (Eqns 22 and 24) but with different magnitudes for the elasticities and hence different magnitudes for the control coefficients. Additionally the control coefficient for step 2 will have the π_2^2 multipler. If non-additivity exhibits itself in either the first or third enzyme then the corresponding control equation is modified in a similar manner with a correspondingly appropriate π elasticity. If non-additivity applies to all three steps, then all three control equations are modified by the addition of a multiplier, π_i^i. Individual π values may be greater or less than 1, i.e. rate more or less than proportional to E.

$$C_{E_1}^J = \frac{\varepsilon_2^3 \varepsilon_1^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} \pi_1^1 , \tag{31}$$

$$C_{E_2}^J = \frac{-\varepsilon_2^3 \varepsilon_1^1}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} \pi_2^2 , \tag{32}$$

$$C_{E_3}^J = \frac{\varepsilon_1^1 \varepsilon_2^2}{\varepsilon_2^3 \varepsilon_1^2 - \varepsilon_2^3 \varepsilon_1^1 + \varepsilon_1^1 \varepsilon_2^2} \pi_3^3 . \tag{33}$$

Using Eqns (22 – 24) and (31 – 33) yields the following identities (see also [17, 24]):

$$\begin{aligned} C_{E_1}^J &= C_{v_1}^J \pi_1^1 \\ C_{E_2}^J &= C_{v_2}^J \pi_2^2 \\ C_{E_3}^J &= C_{v_3}^J \pi_3^3 . \end{aligned} \tag{34}$$

Thus there is a direct relationship between the flux control coefficients defined with respect to enzyme concentration and the flux control coefficients defined with respect to rate. It is evident that this must affect the summation theorems. In general, for a system of n enzymes, and using $\sum_{i=1}^{n} C_{v_i}^J = 1$ (from Eqn 15), it follows, from generalising Eqn (34), that:

$$\sum_{i=1}^{n} C_{E_i}^J \neq 1,$$

but

$$\sum_{i=1}^{n} C_{E_i}^J / \pi_i^i = 1. \tag{35}$$

Modulating the enzyme concentrations to obtain the control coefficients would not yield a unit sum if one or more π_i^i are not equal to one. Thus $\sum_{i=1}^{n} C_{E_i}^J = 1 + \text{DEV}$, where DEV is the deviation from unity. DEV may be positive or negative. Similarly it may be shown that:

$$\sum_{i=1}^{n} C_{E_i}^{S_j} / \pi_i^i = 0 . \tag{36}$$

Eqns (35) and (36) are the general summation theorems for non-additive enzyme systems and revert to the classical ones when all values of π_i^i are equal to unity.

The matrix equations (Eqn 27) will also be modified to give

$$\begin{bmatrix} C_{E_1}^J & C_{E_1}^{S_1} & C_{E_1}^{S_2} \\ C_{E_2}^J & C_{E_2}^{S_1} & C_{E_2}^{S_2} \\ C_{E_3}^J & C_{E_3}^{S_1} & C_{E_3}^{S_2} \end{bmatrix} = \begin{bmatrix} \pi_1^1 & 0 & 0 \\ 0 & \pi_2^2 & 0 \\ 0 & 0 & \pi_3^3 \end{bmatrix} \begin{bmatrix} 1 & 1 & 1 \\ \varepsilon_1^1 & \varepsilon_2^1 & 0 \\ 0 & \varepsilon_2^2 & \varepsilon_2^3 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix} \tag{37}$$

or using Eqn (27)

$$\begin{bmatrix} C_{E_1}^J & C_{E_1}^{S_1} & C_{E_1}^{S_2} \\ C_{E_2}^J & C_{E_2}^{S_1} & C_{E_2}^{S_2} \\ C_{E_3}^J & C_{E_3}^{S_1} & C_{E_3}^{S_2} \end{bmatrix} = \Pi \begin{bmatrix} C_{v_1}^J & C_{v_1}^{S_1} & C_{v_1}^{S_2} \\ C_{v_2}^J & C_{v_2}^{S_1} & C_{v_2}^{S_2} \\ C_{v_3}^J & C_{v_3}^{S_1} & C_{v_3}^{S_2} \end{bmatrix} \tag{38}$$

where Π is the diagonal matrix of π coefficients. These will give the same results as Eqns (31 – 34).

In a system with properties of independence and additivity, the distribution of control is constrained according to the classical summation theorem such that a large finite increase in the flux control coefficient of one enzyme (for whatever reason) will be compensated by a decrease in the flux control coefficient of one or more other enzymes (and vice versa). An interesting corollary is that in a system displaying non-additivity, consequential changes in other coefficients (C_E) may not necessarily compensate the effect of the altered flux control coefficient. As a result, the value for the deviation may change and the previously found sum will be different.

For the particular case when only the second enzyme in the three-step pathway exhibits non-additivity, the summation theorem, Eqn (35), gives:

$$C_{E_1}^J + C_{E_2}^J/\pi_2^2 + C_{E_3}^J = 1 \tag{39}$$

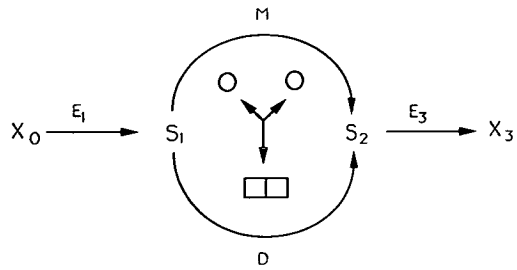
while

$$C_{v_1}^J + C_{v_2}^J + C_{v_3}^J = 1. \tag{40}$$

To summarise, if a particular step in a metabolic pathway exhibits non-additivity (for whatever reason) then, although the sum of flux control coefficients with respect to changes in rate always sums to unity, the sum of control coefficients with respect to changes in enzyme concentration will not. The correct summation theorem for control coefficients is one where each of the C_E^J expressions are scaled by an appropriate π elasticity. Considerations (or measurements) of C_v^J expressions only would therefore miss an important biological aspect of control.

KINETIC MODELS

It is no part of control analysis *sensu stricto* to describe metabolic systems in terms of kinetic mechanism of their reactions. Both systemic control coefficients and local elasticity coefficients are defined algebraically as partial derivatives of unspecified functions, whatever their kinetic formulations may be, or whether explicit expressions are obtainable or not. It will have been noted that the foregoing analysis has been conducted in this manner. The operational equivalents of the coefficients are specified by certain experimental procedures which again, in principle, require no detailed kinetic information. There is, however, a not unnatural interest to link such an analysis to the formulations of kineticists and enzymologists. In the following we shall discuss some kinetic models which may represent particular cases of the general treatment given above.



Scheme 2. *Monomer-dimer model of a three-step unbranched enzyme system.* For definition of the components see the legend to Scheme 1. Monomer, M; dimer, D

Monomer/dimer model (example 1)

Monomer-oligomer association/dissociation is one phenomenon which would show non-additivity of reaction rate against enzyme concentration [1]. A typical example is the case of monomer-dimer association. Let us consider the three-step unbranched pathway as described in Scheme 1 but where the second enzyme (E_2) now consists of two active forms, a dimeric form designated D, and a monomeric form designated M (Scheme 2). We will assume that there is an equilibrium between the two forms such that the equilibrium constant is given by $K = D/M^2$. The equilibrium constant, K, is strictly K_{app} since the effects of S_1 and S_2 may affect its value. For constant, steady-state levels of S_1 and S_2, however, it is fixed. (For a general treatment, see the Appendix.) We will also assume that E_1 and E_3 show no special polymerizing behaviour that affects the kinetic properties of these enzymes. Since the second enzyme consists of two forms there will be two rates, v_m and v_d, by which S_1 is converted to S_2. At steady state, the sum of both rates is equal to the system flux ($J = v_m + v_d$) which is the same through E_1 and E_3.

Although phenotypically the second step is distinguished by two protein forms, monomer and dimer, there is, in reality, only one gene responsible for expressing the two forms and hence one gene product, the monomer. Changes in the expression of this gene, say via some hormone effect, will alter the total concentration of protein (E_2) which then distributes itself between monomer and dimer with a change in their proportions. An interesting control coefficient to consider is $C^J_{E_2}$, i.e. the response of the pathway flux to changes in total protein concentration (E_2). Because many physiological, phenomena involve changes in enzyme concentration (inductions/repressions), C_E coefficients are the biological relevant measures of the effect. They are also experimentally accessible.

Thus the question we wish to ask concerns the nature of $C^J_{E_2}$, how its magnitude is affected by the presence of the monomer-dimer system and how we involve the π elasticities. We begin by considering the conservation between the total protein and the individual protein forms. From the nature of the stoichiometry between monomer and dimer it is evident that, apart from the equilibrium constraint, their concentrations are constrained by mass conservation, according to $E_2 = M + 2D$, where E_2 is expressed in terms of monomer units. Modulation of E_2, however, will change the equilibrium distribution of the two forms and therefore the partition of each flux through each of the arms of Scheme 2. If the specific activity of each catalytic site is not different due to the association, there will be no net effect on the combined rate due to the change in distribution (apart from the effect of changing the total by the modulation). In general, however, we must consider possible differences in the catalysis by monomer and dimer and consequently the π elasticity, $\pi^{v_2}_{E_2}$, will not necessarily be equal to unity. Since $v_2 = v_d + v_m$, the form of expression for π^2_2 which equals $\pi^{v_m+v_d}_{E_2}$, must involve the effects of E_2 modulations on the component rates v_d and v_m. This property may be expressed in the form of the component π elasticities. Thus the component elasticities, $\pi^{v_m}_{E_2}$ may be shown to be equal to (see the Appendix)

$$\pi^{v_m}_{E_2} = \frac{E_2}{v_m}\left(\frac{\partial v_m}{\partial E_2}\right) = \frac{1}{2}\left(1 + \frac{1}{\sqrt{1 + 8KE_2}}\right)$$
$$\pi^{v_d}_{E_2} = \frac{E_2}{v_d}\left(\frac{\partial v_d}{\partial E_2}\right) = \left(1 + \frac{1}{\sqrt{1 + 8KE_2}}\right). \qquad (41)$$

Note that $\pi^{v_d}_{E_2} = 2\,\pi^{v_m}_{E_2}$. If we consider $\pi^{v_m}_{E_2}$ for the moment, it is seen that its value depends in an inverse manner on both the value of the equilibrium constant and the total protein concentration. Thus for values of $K \cdot E_2$ much less than 1, the $\pi^{v_m}_{E_2}$ elasticity approaches 1.0 and for high values of $K \cdot E_2$, $\pi^{v_m}_{E_2}$ approaches 0.5. Simultaneously, the value for $\pi^{v_d}_{E_2}$ varies between 2.0 and 1.0. Both the equilibrium constant and the total protein concentration are independent parameters and so the effect of a small value of, e.g. K, may be compensated by a high value for E_2 and vice versa.

One may now express π^2_2, the overall elasticity with respect to the net velocity through both forms, i.e. $v_m + v_d$, in terms of the components π. It is easily shown that the following relationship is true:

$$\pi^2_2 = \pi^{v_m+v_d}_{E_2} = \pi^{v_d}_{E_2}\frac{v_d}{v_m + v_d} + \pi^{v_m}_{E_2}\frac{v_m}{v_m + v_d} \qquad (42)$$

or

$$\pi^2_2 = \pi^{v_d}_{E_2}\alpha + \pi^{v_m}_{E_2}(1 - \alpha)$$

where α is the fraction of total flux going through the dimer D. The rates, v_m and v_d depend on the kinetic parameters as well as on enzyme and metabolite concentrations.

Eqn (42) may be seen to be made up of two components. The first comprises the values for the component π elasticities, i.e. $\pi^{v_m}_{E_2}$ and $\pi^{v_d}_{E_2}$. These depend on the value of the equilibrium constant and total enzyme concentration, E_2 (Eqn 41); they represent a thermodynamic contribution to π^2_2. The second component, a kinetic factor, is the relative distribution [α and $(1-\alpha)$] of the flux, J, through the individual monomer and dimer enzymes, v_d and v_m, and will depend on kinetic properties of the two forms. The flux, is of course, a systemic property and depends on the kinetics of all the enzymes in the pathway.

Using Eqn (41) we reduce Eqn (42) to:

$$\pi^2_2 = \pi^{v_m}_{E_2}(1+\alpha)\,. \qquad (43)$$

This is the same type of π^2_2 elasticity which appears in Eqn (39) and for a complete control analysis of the system shown in Scheme 2 it would be necessary to determine the magnitude of π^2_2 at the operating point of the system. We note that equations (Eqns 41 and 42) set the limits of π^2_2 between 0.5 and 2.0. (The experimental determination of π^2_2 is discussed in Experimental Methods.)

Since our system obeys Eqns (39) and (40), the flux-control coefficient, $C^J_{E_2}$, is, as before, given by the product, $C^J_{v_2}\pi^{v_m+v_d}_{E_2}$. In the most extreme case, when $C^J_{v_2} \approx 1$, the flux control coefficient, $C^J_{E_2}$, could approach a maximum value of 2.0 or a minimum of 0.5. The sum of the control coefficients, coefficients, could therefore lie between 0.5 and 2.0. The exact value for the control coefficient is however a complex function of a number of factors, including the equilibrium constants

Scheme 3. *Monomer-dimer-tetramer model of enzyme association.* (○) Monomer; (□) dimer; (◇) tetramer

overning the distribution of oligomeric forms, the total level f protein (E_2), the partitioning of flux between the different orms and the values for all the control coefficients (C_v^J).

Monomer-dimer-tetramer model (example 2)

More complex protein polymerizing systems might also be onsidered, for example a monomer-dimer-tetramer system as hown above (Scheme 3).

The equations for $\pi_{E_2}^{v_m}$, $\pi_{E_2}^{v_d}$ and $\pi_{E_2}^{v_t}$ are more complex [see ne Appendix in relation to Eqn (41) and the text of Kurganov 1] for further details]. The equations involve two equilibrium onstants in this case. For any values of the equilibrium con- tants and total protein concentration, the magnitudes of the values are constrained within the following limits:

at high E_2	at low E_2
$0.25 \leqslant \pi_{E_2}^{v_m} \leqslant 1.0$	
$0.5 \leqslant \pi_{E_2}^{v_d} \leqslant 2.0$	
$1.0 \leqslant \pi_{E_2}^{v_t} \leqslant 4.0$.	

t will be noted that the quantitative range within which the elasticities lie is independent of the values of the catalytic onstants between the different forms. They depend only on he stoichiometry of the polymerisation. Since the monomer nd each polymeric form catalyses the same reaction, the π lasticity of the total flux ($v_m + v_d + v_t = J$), i.e. $\pi_{E_2}^{v_m + v_d + v_t}$ s given by

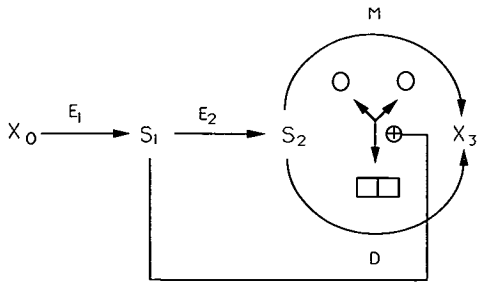
$$\pi_{E_2}^{v_m + v_d + v_t} = \pi_{E_2}^{v_m} \frac{v_m}{v_m + v_d + v_t} + \pi_{E_2}^{v_d} \frac{v_d}{v_m + v_d + v_t} + \pi_{E_2}^{v_t} \frac{v_t}{v_m + v_d + v_t} . \quad (44)$$

From Eqn (44) it can be concluded that the limits of $\pi_{E_2}^{v_m + v_d + v_t}$ are 0.25 and 4.0. Similar to the case of the mono- ner-dimer system, the limits of the sum of the C_E coefficients herefore lie between 0.25 and 4.0.

Coupling of pathway effectors to non-additive enzymes (example 3)

Up to now we have considered how the change in total nzyme concentration affected the association/dissociation listribution. In the study of π_2^2 it was assumed that the specific ctivities of monomer and dimer, and the apparent equilib- ium constant between them, are constant, because all the free netabolite concentrations are fixed.

However, the association/dissociation properties of many nzymes, are greatly affected by the levels of substrates, prod- cts and other effectors. Examples include yeast hexokinase nd rabbit glyceraldehyde-3-phosphate dehydrogenase [25]. n the case of hexokinase it has been reported that a dimer- nonomer equilibration exists which is affected by ATP [26]. A similar case is where an enzyme exists in a phosphorylated nd dephosphorylated form (with different activities) such hat the distribution depends on the concentrations of a path- vay effector. For example, the enzyme, isocitrate dehydroge- ase, which catalyses a step in the citric acid cycle, is known



Scheme 4. *Positive feed-forward mechanism involving enzyme association in a three-step enzyme system.* For definition of the components see the legend to Scheme 1

to exist in two forms in *Escherichia coli*, one of which is inactive and dephosphorylated and the other active but phosphorylated [27]. It is known that the degree of phosphorylation/dephosphorylation is partly determined by the level of isocitrate so that there is a coupling between the pathway and the enzyme-enzyme interaction system. A list of many other cases is given by Kurganov [1].

It is with reference to such examples, that we now wish to ask how the property of non-additivity is affected by coupling to pathway effectors. The general case of ligand-dependent effects is dealt with in the Appendix. Scheme 4 shows a possible specific system. Although we have specified a positive feed-forward mechanism, the general relationships will apply to any interacting system. The assumption is that S_1 somehow affects the equilibrium and thereby the net rate through step 3.

We first consider the resultant new elasticity, ε_1^3, which describes the overall effect of S_1 coupling, irrespective of the nature of the enzyme(s), at step 3. When this elasticity is incorporated in the system equations, the control coefficients (C_v) are given by:

$$C_{v_1(S_1)}^J = \frac{\varepsilon_1^2 \varepsilon_2^3 - \varepsilon_1^3 \varepsilon_2^2}{\varepsilon_1^1 \varepsilon_2^2 - \varepsilon_1^1 \varepsilon_2^3 + \varepsilon_1^2 \varepsilon_2^3 - \varepsilon_1^3 \varepsilon_2^2} \quad (45)$$

$$C_{v_2(S_1)}^J = \frac{-\varepsilon_1^1 \varepsilon_2^3}{\varepsilon_1^1 \varepsilon_2^2 - \varepsilon_1^1 \varepsilon_2^3 + \varepsilon_1^2 \varepsilon_2^3 - \varepsilon_1^3 \varepsilon_2^2} \quad (46)$$

$$C_{v_3(S_1)}^J = \frac{\varepsilon_1^1 \varepsilon_2^2}{\varepsilon_1^1 \varepsilon_2^2 - \varepsilon_1^1 \varepsilon_2^3 + \varepsilon_1^2 \varepsilon_2^3 - \varepsilon_1^3 \varepsilon_2^2} \quad (47)$$

where the subscript, (S_1), denotes the system with S_1 as an effector. Comparing this with Eqns (22–24) it is seen that additional terms, involving ε_1^3, are now present in the control equations. It will be noted that, as before, $\Sigma C_v = 1$. In our oligomeric example, however, we wish to consider that the effect of S_1 is mediated via its effect on the apparent equilibrium constant, K, and not by affecting specific activity. The model we have in mind is one where S_1 acts as a ligand which by some mechanism changes the ratio of dimer/monomer (cf. allosteric effectors in oligomeric systems). This, in turn, will have an effect on the individual rates, v_m and v_d. This allows us to decompose the overall ε_1^3 into three further elasticity components

$$\varepsilon_{S_1}^K = \frac{S_1}{K}\left(\frac{\partial K}{\partial S_1}\right)$$

and

$$\varepsilon_K^{v_m} = \frac{K}{v_m}\left(\frac{\partial v_m}{\partial K}\right)$$

$$\varepsilon_K^{v_d} = \frac{K}{v_d}\left(\frac{\partial v_d}{\partial K}\right).$$

It is easily shown (Appendix) that:

$$\varepsilon_1^3 = \varepsilon_{S_1}^K (\pi_3^3 - 1) . \quad (48)$$

This now gives us the coupling between the π_3^3, previously described, and the coupling of S_1 on K given by $\varepsilon_{S_1}^K$. If the effect of S_1 on the specific activities is as considered, additional terms including $\varepsilon_{S_1}^{a_m}$ and $\varepsilon_{S_1}^{a_d}$ (see the Appendix for and explanation of these terms) will appear in Eqn (48). It can be seen (Eqns 45 – 47) that all three control coefficients contain terms with ε_1^3 and therefore are now all implicit functions of the π_3^3 and $\varepsilon_{S_1}^K$. Inspection of Eqns (45 – 47) in comparison with Eqns (22 – 24), for two systems of equal fluxes and substrate concentrations (and hence equal elasticities) shows that in such a linear chain with 'normal' kinetics and, e.g. positive values of ε_1^3,

$$C_{v_1(S_1)}^J \geqslant C_{v_1}^J$$
$$C_{v_2(S_1)}^J \leqslant C_{v_2}^J$$
$$C_{v_3(S_1)}^J \leqslant C_{v_3}^J .$$

The effect of the loop is therefore to redistribute the values. For the first and second step the C_v expressions are equal to the C_E expressions, but the third step is, as before,

$$C_{E_3(S_1)}^J = C_{v_3(S_1)}^J \pi_3^3 .$$

The effect of such a coupling is therefore to alter the magnitudes of the C_v values (and C_E) and to alter all the algebraic expressions to incorporate additional elasticities. Cases of particular kinetic properties have been dealt *in extenso* by Kurganov [1] concerned with problems of mechanism rather than control properties.

EXPERIMENTAL METHODS

There are three usual experimental methods for determining the control coefficients; (a) specific inhibitor titration which involves a knowledge of the mechanism of inhibition [2, 28]; (b) induction or repression of the enzyme by changes of some external effector [16, 29, 30]; (c) change of gene dose by some genetic or quasi-genetic method [31 – 33]. All three methods have been used. As far as method (a) is concerned, if the inhibition is strictly irreversible and specific to that enzyme only, an estimate of C_E can be obtained [28, 34, 35]. If the inhibitor acts on the activity of the enzyme, then C_v values will be obtained [16]. Methods (b) and (c) will yield C_E values. An experimental method of obtaining the values of elasticity coefficients is given in [21].

Recently a new method for specifically reducing the concentration of gene product has been devised, namely the use of anti-sense RNA (e.g. see the review [36]). This would provide a universal method of reducing specifically any chosen enzyme concentration in any organism. It is the complement of the cloning method of increasing concentrations [32, 33].

The examples of the kinetic models given all show how various kinetic assumptions lead to a variety of non-additive behaviour when enzyme modulations are performed. Although these models may be of interest to those who are concerned with the detailed mechanism, they all suffer from the fact that only in the rarest cases are the kinetic constants accessible and their interpretation are heavily model dependent. Control analysis is not concerned with mechanism. It is, however, essential that any nonlinear properties, such as non-additivity of rates, are discovered and quantified. For this, it is only necessary to establish the value of the π elasticity.

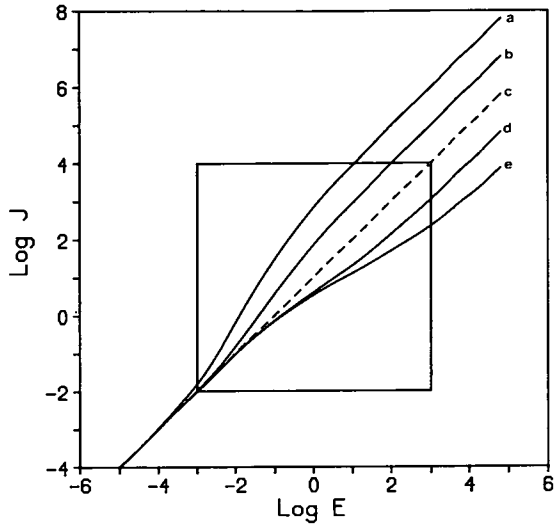


Fig. 1. *Simulation of a monomer-dimer equilibrium.* The model simulated is $X_0 \overset{E}{\rightleftharpoons} X_1$, where the enzyme can exist in two forms, the monomer, M, and the dimer, D. The system is constrained by $E = M + 2D$ and $K = D^2/M$. The overall rate is the sum of the rates through each form, i.e. $v = v_m + v_d$, where $v_m = a_m M$ and $v_d = a_d 2D$ (see Appendix, Eqn A7). The parameters used were $K = 3$ μM; $a_m = 10$ s^{-1} and a_d varying (a) 1000, (b) 100, (c) 10, (d) 1 and (e) 0.1 s^{-1}. Enzyme concentrations (E) were varied from 10^{-5} to 10^5 μM. Values of J are in μM s^{-1}. The slope at any point of the log/log plot shown gives the value of π for the particular enzyme concentration. The dashed line (c) is the simulation when $a_m = a_d$, i.e. when the activity/subunit is unaltered by the association. In this case $\pi = 1$ at all enzyme concentrations. The range over which the nonproportionality occurs depends, of course, on the parameters chosen and the nature of the model (monomer-dimer, monomer-dimer-tetramer, etc.) and is therefore to some extent arbitrary. The inserted square corresponds to the range of the experimental plot of Fig. 2 but no commitment as to the fit of the above model to those data is implied. The curves show the types of non-additive behaviour obtained in a model monomer-dimer system

This can be estimated experimentally even if we have little information about the mechanism or the values of the constants.

Given that π_i^i is defined by

$$\pi_i^i = \frac{E_i}{v_i}\left(\frac{\partial v_i}{\partial E_i}\right)_{S_1, S_2} = \frac{\partial \ln v_i}{\partial \ln E_i}$$

(see Eqn 28), if we study the isolated enzyme, a plot of $\ln v_i$ against $\ln E_i$ will give π_i^i as the slope of this relationship for different values of E_i. This is an experimental method for detecting non-additivity, quantifying its effects and is model independent. Two important qualifications of this method must, however, be stated. Since the π value will depend on the absolute value of the enzyme concentration (see examples 1 and 2), a knowledge of the *in vivo* concentration of the enzyme is required so that the *in vitro* determination of the slope includes this concentration range. Secondly, since π may also be influenced by the concentrations of substrates and effectors, pH etc., a reasonable close approximation of the *in vivo* milieu is required in the experiment in order to obtain physiologically significant values. With these provisos, we show a simulation for a monomer-dimer system (example 1). Fig. 1 gives a number of simulations with parameters as indi-

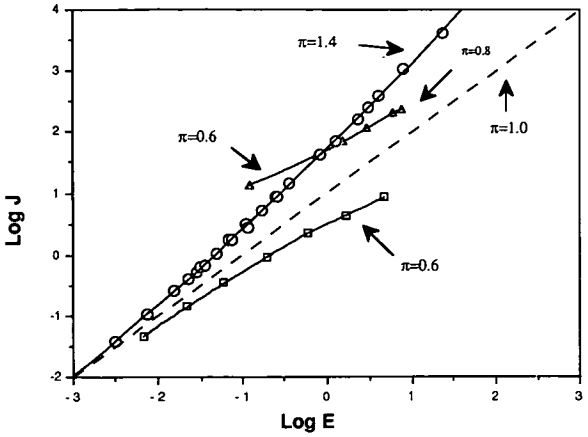


Fig. 2. *Experimental estimates of π values.* A log/log plot of J against E. The lines are least-square fits. We show three sets of data: (□) aspartate aminotransferase (pig heart) from [1]; (○) glycerol-3-phosphate dehydrogenase (rabbit muscle) from [39] (details kindly supplied by Dr Ovadi); (△) glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle) from [25]. The enzyme concentrations (E) are expressed in micromolar and the rates (J) for substrate lost ($\mu M \cdot s^{-1}$). The dashed line is 45° and hence represents π values of unity. The maximum or minimum values of the experimental π values are indicated. No estimate of the *in vivo* concentrations of the enzyme are given nor whether the experimental conditions approach those of the *in vivo* milieu. The range of E values shown are possibly limited by technical problems of rate determination. On the other hand, if the additional information obtainable by extending the range had been realised, a more complete picture may well have been obtained. The magnitudes of the π values so determined are independent of any kinetic assumptions of the mechanism but clearly indicate non-additive behaviour

ated. It will be noted that at both ends of the range, the slope s unity (45°) but at intermediate values of E it is greater (or maller) depending on the specific activity ratio of monomer/ imer. It can be shown (Appendix) that the maximum possible lope is given by $\pi_i^i = 2$ and the minimum by 0.5. Furthermore, he ratio of specific activities of the forms can be obtained by he extreme slope actually obtained (see Eqn A11 in Appendix).

Another method of obtaining the value of a particular π_i^i nay be considered by using the relationship in Eqns (41) and 42). However, this is not a satisfactory method since neither he values of K_{app} nor v_m (or α) are easily determinable. The xperimental method discussed above does not have these isadvantages and is free from model assumptions.

If now the control coefficients (C_E) in the system are determined (by any method) the distribution of control, including he role of any non-additivity can be ascertained.

In Fig. 2 we give experimental estimates of π elasticities rom published data. It is evident that, at low concentrations, oth aspertate aminotransferase and glycerol-3-phosphate dehydrogenase data approach unit slope. This is expected since t low concentrations very little oligomer will be present. At igher enzyme concentrations, the curves diverge. The slope f the aspartate aminotransferase in Fig. 2 declines, indicating hat the oligomeric form(s) has a lower specific activity than he monomer. The opposite is true for glycerol-3-phosphate lehydrogenase which reaches a value of $\pi = 1.4$. Neither set f data, however, covers the range of enzyme concentration vhen the π values would again tend to unity (see Fig. 1). This s probably due to technical reasons, since concentrations above in 10 μM *in vivo* are not unusual. In Fig. 2, the small range of values for the glyceraldehyde-3-phosphate dehydrogenase points suggests that the available data are in the range after the inflexion point of the curve (cf. Fig. 1) while the range of the other two enzymes are clearly before it. The value of our graphical method is of interest not for the magnitudes of the π values here reported but as an indication that it is a relatively simple procedure giving information on an important control aspect. These results may not be immediately related to the *in vivo* milieu since this may be rather different from the experimental conditions used.

Once the π values are ascertained, their effects on the control analysis can be determined (see, e.g. Eqn 35). It may turn out that, in spite of unambiguous evidence of an association mechanism, the value of π_i^i may not differ significantly from unity. The control distribution may therefore be only marginally affected.

GENERAL DISCUSSION

The occurrence of oligomeric forms of enzymes is well documented (see for example the list in Kurganov [1], Table 4.1). Although we have only considered association/dissociation phenomena in our previous examples, the analysis is equally applicable to mechanisms which involve phosphorylation/dephosphorylation of 'inactive' and 'active' forms, as found in many cascade systems [37]. In addition, enzyme forms which are found distributed between free forms and forms bound to membranes, organelles or the cytoskeleton can be treated in an analogous manner.

Occasionally the suggestion is made (see e.g. [14, 23]) that high enzyme concentrations ($E \geqslant S$) result in $\pi_{E_1}^{v_1} \neq 1$. This is a mistaken notion based on the misinterpretation of the assay equation for such a situation (e.g. [38]). Such equations describe the change in rate, given an initial fixed total concentration of substrate. The free concentration will change by sequestration on the enzyme when enzyme concentrations are increased and as a result the rate will not increase proportionally. Elasticities, on the other hand, refer to the behaviour in open systems when the free substrate concentration will readjust consequent to any change is enzyme concentration. Thus, by definition, $\pi_{E_1}^{v_1}$ is measured at constant free substrate concentrations. Irrespective of the relative concentrations of enzyme and substrate, the $\pi_{E_1}^{v_1}$ will be equal to unity unless some unusual mechanism operates. When conserved metabolites are considered (as in moiety conserved cycles [6]) such an unusual situation may arise, where a change in E will result in an alteration in the total free concentrations of the conserved moieties. As a result the elasticity matrix will have a different form. Further discussion of this important problem will be left to a future publication.

It now remains the task of the experimentalist to obtain values for the π elasticities in known cases of oligomeric enzymes or other non-additive systems. We have described an experimental method for determining the value of the π elasticities for such enzymes (Figs 1 and 2). From a control point of view the detailed kinetic description of the enzyme in its various forms is not required. The effect on the control properties are completely defined by the value of π. Since this value depends on the properties of the cellular environment (including the enzyme concentration) a knowledge of the *in vivo* conditions is, however, required. Thereafter the usual procedures of determining the control coefficients can be applied to complete the analysis.

APPENDIX

Monomer/dimer model

We consider a single reaction catalysed by an enzyme that exists in a monomer/dimer equilibrium. The equilibrium constant K_{eq} of dissociation is given by:

$$K_{eq} = \frac{D_0}{M_0^2} \tag{A1}$$

where M_0 and D_0 are the concentrations of free monomer and dimer respectively. In the presence of ligands (substrates, products and effectors), monomer and dimer will exist in a number of different forms which differ either in the conformation or in the level of occupation by ligand. Designating a particular form of monomer or dimer by M_i or D_i, respctively, the total concentration of monomer (M_T) or dimer (D_T) can be expressed as the sum of the corresponding forms:

$$\begin{aligned} M_T &= \Sigma M_i \\ D_T &= \Sigma D_i \end{aligned} \tag{A2}$$

and they satisfy the conservation equation:

$$E_T = M_T + 2\,D_T \tag{A3}$$

where E_T is the total concentration of enzyme in monomer units. Now we define the apparent equilibrium constant as follows:

$$K_{app} = \frac{D_T}{M_T^2}. \tag{A4}$$

By assuming an equilibrium between the different forms of monomer, we can write each M_i as proportional to M_0. Similarly each D_i is proportional to D_0. In both cases the proportionality constants depend only on the free ligand concentrations and equilibrium constants. Then, from Eqns (A1), (A2) and (A3) we conclude that the apparent equilibrium constant (K_{app}) depends only on the concentration of free ligands and is independent of the total enzyme concentration.

Using Eqns (A3) and (A4) we obtain M_T and D_T as functions of E_T and K_{app}.

$$\begin{aligned} M_T &= \frac{-1 + \sqrt{1 + 8\,K_{app}E_T}}{4\,K_{app}} \\ D_T &= \frac{(-1 + \sqrt{1 + 8\,K_{app}E_T})^2}{16\,K_{app}}. \end{aligned} \tag{A5}$$

The overall rate of reaction, v, catalysed by the enzyme can be written as the sum of the rates through the monomer (v_m) and the dimer (v_d)

$$v = v_m + v_d\,. \tag{A6}$$

In turn, each of these corresponding rates, can be expressed as the product of the specific activity (activity/subunit; a_m for monomer and a_d for dimer) and the concentration of enzyme subunits with that specific activity:

$$\begin{aligned} v_m &= a_m\,M_T \\ v_d &= a_d\,(2\,D_T)\,. \end{aligned} \tag{A7}$$

The specific activities, a_m and a_d, are independent of total enzyme concentrations (as for K_{app}). They depend on the free ligand concentrations and can be considered constant whenever these concentrations are not changed.

Since $v_m \propto M_T$ and $v_d \propto D_T$, differentiation of Eqn (A5) give the π elasticities shown in Eqn (41) of the main text.

Now we can calculate $\pi^v_{E_T}$, using Eqns (A5–A7),

$$\pi^v_{E_T} = \frac{E_T}{v}\frac{\partial v}{\partial E_T} = 1 + \frac{1 - A}{\sqrt{1 + 8\,K_{app}E_T}} \cdot \frac{2\,D_T}{2\,D_T + AM_T} \tag{A8}$$

where

$$A = \frac{a_m}{a_d}. \tag{A9}$$

It can be easily shown using Eqn (A8) that $\pi^v_{E_T}$ is greater than, equal to or less than unity if, and only if, A is less than, equal to or greater than unity, respectively, whatever is the concentration of enzyme. For very small or very large concentrations of enzyme, $\pi^v_{E_T}$ tends to unity, whatever the value of A. Thus the deviation of $\pi^v_{E_T}$ from unity is evidence for non-additivity.

Differentiating $\pi^v_{E_T}$ with respect to E_T and equating to zero, it can be shown that this quantity has a relative extreme (maximum if $A < 1$ or minimum if $A > 1$). The value of E_T where the maximum or minimum appears is:

$$(E_T)_{extreme} = \frac{\sqrt{2A} + A}{4\,K_{app}}. \tag{A10}$$

Substituting Eqn (A10) into Eqn (A8) the extreme value of $\pi^v_{E_T}$ is obtained

$$(\pi^v_{E_T})_{extreme} = 1 + \frac{1 - A}{[\sqrt{2A} + 1]^2}. \tag{A11}$$

From this equation, it can be seen that $(\pi^v_{E_T})_{extreme}$ takes values between 0.5 and 2 as A decreases from infinity to zero, and is one when $A = 1$ (as was expected). The expression that relates A with $(\pi^v_{E_T})_{extreme}$ is:

$$A = 1 + \frac{1 - (\pi^v_{E_T})_{extreme}}{(\sqrt{2(\pi^v_{E_T})_{extreme}} - 1)^2}. \tag{A12}$$

The deviation of $\pi^v_{E_T}$ from unity is due to the existence of different specific activities of monomer and dimer. Then A, the ratio of the specific activities, is an alternative measure of departure from additivity. Using Eqn (A12), A can be calculated from the value of $(\pi^v_{E_T})_{extreme}$ determined experimentally.

Derivation of Eqn (48) in the main text

Given that $\varepsilon^{v_d}_{S_1} = \varepsilon^K_{S_1}\varepsilon^{v_d}_K$ and $\varepsilon^{v_m}_{S_1} = \varepsilon^K_{S_1}\varepsilon^{v_m}_K$, the elasticity of the combined rate, $v_m + v_d$, is

$$\varepsilon^3_1 = \varepsilon^{v_d + v_m}_{S_1} = \varepsilon^K_{S_1}[\varepsilon^{v_d}_K\,v_d/(v_d + v_m) + \varepsilon^{v_m}_K\,v_m/(v_d + v_m)]$$

or

$$\varepsilon^3_1 = \varepsilon^K_{S_1}[\varepsilon^{v_d}_K\,\alpha + \varepsilon^{v_m}_K(1 - \alpha)]\,. \tag{A13}$$

For the particular case of the monomer-dimer model, one can show that $\varepsilon^{v_d}_K = \pi^{v_d}_{E_3} - 1$ and $\varepsilon^{v_m}_K = \pi^{v_m}_{E_3} - 1$, so that

$$\varepsilon^3_1 = \varepsilon^K_{S_1}[\pi^{v_d}_{E_3}\alpha + \pi^{v_m}_{E_3}(1 - \alpha) - 1]\,. \tag{A14}$$

By analogy with Eqn (42), $\pi^3_3 = [\pi^{v_d}_{E_3}\alpha + \pi^{v_m}_{E_3}(1 - \alpha)]$ and therefore

$$\varepsilon^3_1 = \varepsilon^K_{S_1}(\pi^3_3 - 1)\,. \tag{A15}$$

We wish to thank Drs Rankin Small and David Fell for their most helpful discussions especially in clarifying some of our early ideas. We would also like to thank Dr Judit Ovadi for helpful comments and the supply of unpublished data, as well as Dr Rick Welch for providing us with a copy of his manuscript before publication. We are indebted to the Wellcome Trust and the European Commission for financial support.

REFERENCES

1. Kurganov, B. I. (1978) *Allosteric enzymes: kinetic behaviour.* John Wiley & Son.
2. Kacser, H. & Burns, J. A. (1973) *Symp. Soc. Exp.. Biol. 27*, 65–104.
3. Heinrich, R. & Rapoport, T. A. (1974) *Eur. J. Biochem. 42*, 89–95.
4. Savageau, M. A. (1987) *Trends Biochem. Sci. 12*, 219–220.
5. Kacser, H. (1983) *Biochem. Soc. Trans. 11*, 35–40.
6. Fell, D. A. & Sauro, H. M. (1985) *Eur. J. Biochem. 148*, 555–561.
7. Hofmeyr, J.-H. S., Kacser, H. & van der Merwe, K. J. (1986) *Eur. J. Biochem. 155*, 631–641.
8. Small, R. J. & Fell, D. A. (1989) *J. Theor. Biol. 136*, 181–197.
9. Chen, Y.-D. & Westerhoff, H. V. (1986) *Math. Modelling. 7*, 1173–1180.
10. Sauro, H. M., Small, J. R. & Fell, D. A. (1987) *Eur. J. Biochem. 165*, 215–221.
11. Westerhoff, H. V. & Chen, Y.-D. (1984) *Eur. J. Biochem. 142*, 425–430.
12. Heinrich, R. (1985) *Biomed. Biochim. Acta 44*, 914–927.
13. Burns, J. A., Cornish-Bowden, A., Groen, A. K., Heinrich, R., Kacser, H., Porteous, J. W., Rapoport, S. M., Rapoport, T. A., Stucki, J. W., Tager, J. M., Wanders, R. J. A. & Westerhoff, H. V. (1985) *Trends Biochem. Sci. 10*, 16.
14. Heinrich, R. Rapoport, S. M. & Rapoport, T. A. (1977) *Prog. Biophys. Mol. Biol. 32*, 1–82.
15. Higgins, J. (1963) *Ann. N.Y. Acad. Sci. 108*, 305–321.
16. Salter, M., Knowles, R. G. & Pogson, C. I. (1986) *Biochem. J. 234*, 635–647.
17. Reder, C. (1988) *J. Theor. Biol. 135*, 175–202.
18. Groen, A. K., van Roermund, C. W. T., Vervoorn, R. C. & Tager, J. M. (1986) *Biochem. J. 237*, 379–389.
19. Westerhoff, H. V. & Kell, D. B. (1987) *Biotechnol. Bioeng. 30*, 101–107.
20. Cascante, M., Franco, R. & Canela, E. I. (1988) in *Le contrôle du métabolisme* (Mazat, J. P. & Reder, C., eds) pp. 53–63, Université de Bordeaux, Bordeaux.
21. Kacser, H. & Burns, J. A. (1979) *Biochem. Soc. Trans. 7*, 1149–1161.
22. Kacser, H. & Porteous, J. W. (1987) *Trends Biochem. Sci. 12*, 5–14.
23. Giersch, C. (1988) *Eur. J. Biochem. 174*, 509–513.
24. Meiske, W. & Reich, J. G. (1987) *Syst. Anal. Model. Simul. 4*, 423–435.
25. Ovadi, J., Batke, J., Bartha, F. & Keleti, T. (1979) *Arch. Biochem. Biophys. 193*, 28–33.
26. Derechin, M., Rustum, Y. M. & Barnard, E. A. (1972) *Biochemistry 11*, 1793–1797.
27. Nimmo, H. G. (1987) in Escherichia coli *and* Salmonella typhimurium (Neidhardt, F. C., ed.) vol.1, pp.156–169, American Society of Microbiology, Washington D.C.
28. Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., van der Meer, R. & Tager, J. M. (1982) *J. Biol. Chem. 256*, 2754–2757.
29. Stuart, F., Porteous, D. J., Flint, H. J. & Kacser, H. (1986) *Biochem. J. 132*, 1159.
30. Walsh, K. & Koshland, D. E. (1985) *Proc. Natl Acad. Sci. USA 82*, 3577–3581.
31. Flint, H. J., Tateson, R. W., Barthelmess, I. B., Porteous, D. S., Donachie, W. D. & Kacser, H. (1981) *Biochem. J. 200*, 231–246.
32. Brindle, K. (1988) *Biochemistry 27*, 6187–6196.
33. Niederberger, P., Aebi, M., Furter, R., Prantl, F. & Hûtter, R. (1984) *Mol. Gen. Genet. 195*, 481–486.
34. Gellerich, F. N., Bohensack, R. & Kunz, W. (1983) *Biochim. Biophys. Acta 722*, 381–391.
35. Westerhoff, H. V. & Kell, D. B. (1988) *Comments Mol. Cell. Biophys. 5*, 57–107.
36. Lichtenstein, C. (1988) *Nature 333*, 801–802.
37. Small, R. J. (1988) Ph.D. thesis, Oxford Polytechnic.
38. Fisher, R. B. (1963) *Nature 197*, 799.
39. Batke, J., Asboth, G., Lakatos, S., Schmitt, B. & Cohen, R. (1980) *Eur. J. Biochem. 107*, 389–394.

Chapter 25

Temporal Aspects of the Control of Metabolic Processes

LUIS ACERENZA

A METABOLIC system can be defined as composed of metabolites that are interconverted by enzyme reactions. The change of each metabolite concentration with time (dS_i/dt) depends on the balance between the rates v_j of production and consumption of the metabolite:

$$\frac{dS_i}{dt} = \sum_j n_{ij} v_j, \quad i = 1, \cdots, m \tag{1}$$

in which n_{ij} is the stoicheiometric coefficient of S_i in the reaction j and m is the number of metabolites.

Metabolic control analysis as proposed by Kacser & Burns (1973) and Heinrich & Rapoport (1974) studies the case where the metabolite concentrations are constant in time ($dS_i/dt = 0$ for all i), i.e. the steady state. Most of the contributions to the field, including most of the work described in other chapters of this book, deal with this particular case. Metabolic control analysis of the steady state is principally concerned with the effects of small changes in parameters (concentrations of enzymes, external effectors, etc.) on the steady-state values of the variables (metabolite concentrations and fluxes). In operational terms, this aspect of metabolism may be described by the following basic recipe: "Measure the steady-state value of the variable (reference state). Modify one parameter by a small relative amount. Wait until the system settles to a new steady state. Measure the final steady-state value of the variable." The quantitative description is summarized by the *control coefficient* (Burns *et al.*, 1985):

$$C_p^Y = \frac{\delta Y}{Y} \Big/ \frac{\delta p}{p} \tag{2}$$

where $\delta Y/Y$ is the small relative change in the steady-state value of a particular variable Y induced by the small relative change $\delta p/p$ in the parameter p, when all the other

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parameters are maintained at their reference values. In the basic recipe of steady-state control analysis the time is considered implicitly. After the change in a parameter we assume that the system will reach a new steady state. This is an asymptotic state, and therefore we need, in theory, an infinite time to attain it. In practice we wait a "long enough" but finite period of time to reach a good approximation of the new steady-state value. To estimate what is "long enough" one needs to know the order of magnitude of the relaxation time of the variable to the new steady state, but a detailed knowledge of the instantaneous values of the variable during the transition is not required..

The steady-state treatment is a good description for answering some questions, but there are many biological phenomena at different structural levels of organization where the steady-state assumption is not a good approximation. At the molecular level, metabolic systems may exhibit temporal behaviour that ranges from simple monotonic transients to oscillations and deterministic chaos (Higgins, 1967; Higgins *et al.*, 1973; Kohn *et al.*, 1979; Easterby, 1981; Decroly & Goldbeter, 1982; Kohn & Chiang, 1982; Markus *et al.*, 1984; Pachot & Demongeot, 1987; Mizraji *et al.*, 1988; see also Chapter 26 by Markus and Hess in this book). To study the effect of parameters on different variables of a time-dependent metabolic system, control analysis should be extended. We recently developed the basic definitions and relationships to analyse some control features of the instantaneous values of metabolite concentrations of metabolic concentrations and fluxes (Acerenza *et al.*, 1989). Here I shall outline some aspects of this work, and use them to analyse some control properties of time-invariant variables of time-dependent systems.

Returning to eqn. (1), describing the metabolic system, the solutions of this system of differential equations are the values that each S_i can take in time. From these values and the rate equations, the instantaneous fluxes J_j can be calculated. These time courses depend on the values of the parameters. Then, considering a reference time course (generated by a set of reference parameters), we may pose the following question: how are the reference values of a variable modified when one or more parameters are changed at the initial time? In what follows I am interested in one particular change of the parameters. I shall study the case where all the enzyme concentrations are simultaneously altered from their reference values by the same factor α. I shall call this simultaneous change of parameters the *coordinate-control operation*. If E_j are the reference values of the enzyme concentrations, the values after the coordinate-control operation are as follows:

$$E_{j,\alpha} = \alpha E_j \tag{3}$$

I shall use the subscript α to indicate the value of a parameter or variable after the coordinate-control operation. From now on, I assume that the rates v_j are proportional to the corresponding total enzyme concentrations:

$$v_j = E_j f_j \tag{4}$$

where f_j is a function of some metabolite concentrations and parameters, but not of enzyme concentrations and time. Under this hypothesis, if we apply the coordinate-control operation the new rates are given by:

$$v_{j,\alpha} = \alpha v_j \tag{5}$$

and, combining eqns. (1) and (5), the system of differential equations that describes the new situation is as follows:

$$\frac{dS_{i,\alpha}}{d\tau_\alpha} = \sum_j n_{ij} v_j, \quad i = 1, \cdots, m \tag{6}$$

where $\tau_\alpha = \alpha t_\alpha$. As the right-hand sides of eqns. (1) and (6) are identical, the only effect of the coordinate-control operation is to produce a change in the time scale given by

$$\tau = \alpha t \tag{7}$$

Therefore, the solutions of eqns. (1) and (6), $S_i(t)$ and $S_{i,\alpha}(J_\alpha)$ respectively, are identical if τ_α and t are numerically equal. Since $\tau_\alpha = \alpha t_\alpha$ [eqn. (7)], this condition is fulfilled when

$$\tau_\alpha = t/\alpha \tag{8}$$

This means that if for a time t the metabolite concentrations obtained from eqns. (1) have certain values, the solutions of eqns. (6) exhibit the same values at a time t/α:

$$S_{i,\alpha}(t/\alpha) = S_i(t) \tag{9}$$

For the fluxes, we obtain

$$J_{j,\alpha}(t/\alpha) = \alpha J_j(t) \tag{10}$$

When the system approaches a stable steady state, the variables attain approximately constant values in time, and eqns. (9-10) take the following forms:

$$S_{i,\alpha}^{ss} = S_i^{ss}$$

$$J_{j,\alpha}^{ss} = \alpha J_j^{ss} \tag{11}$$

where the superscript ss denotes steady-state values. The results given in eqns. (9-11) are illustrated in Fig. 1 with a simple example. As an immediate consequence of the change in time scale, if one plots the metabolite concentration after the coordinate control operation against time multiplied by α, the resulting curve should coincide with the reference curve. A similar procedure is used as a test for inactivation of an enzyme during assay (Selwyn, 1965; see also Cornish-Bowden, 1979).

In time-dependent systems there are variables which, although having the dimensions of time, have time-invariant values, for example relaxation time, period of oscillation, etc. These time-invariant variables that characterize the time course of the metabolite

$$X_0 \xrightarrow{E_1} S \xrightarrow{E_2} X_2$$

Scheme 1. Model used to obtain the results shown in Fig. 1.

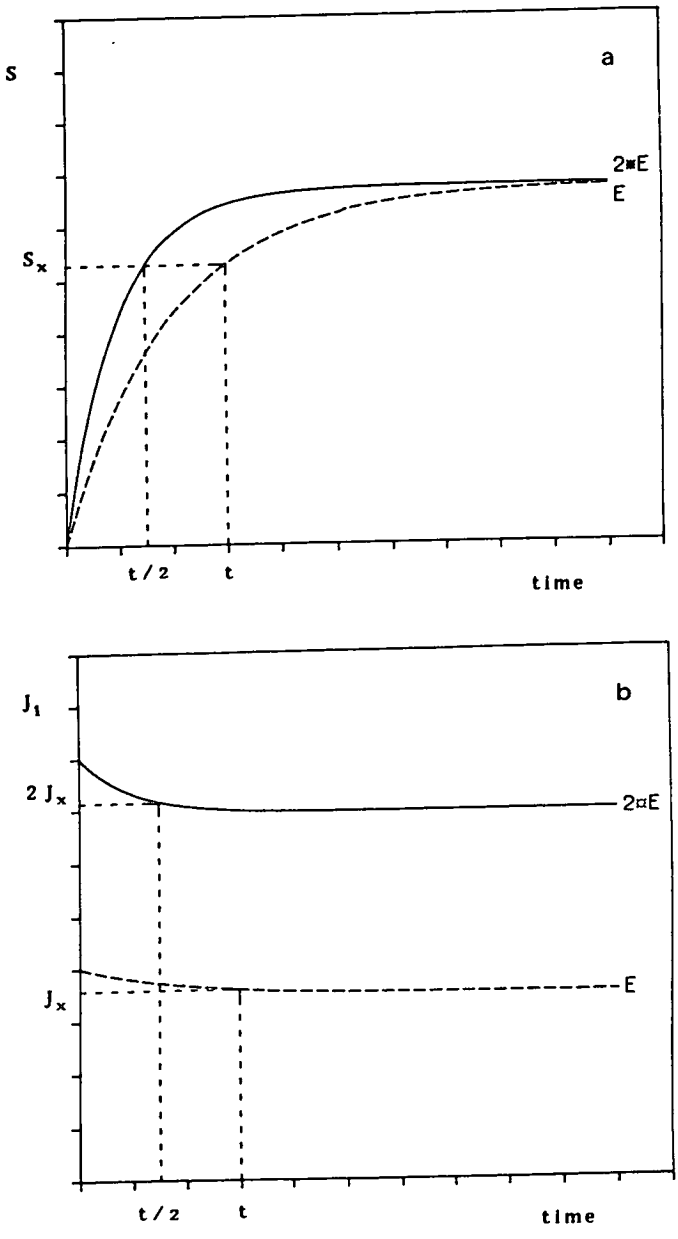
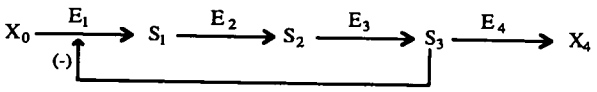


Figure 1. Effect of the coordinate-control operation on the instantaneous values of (*a*) metabol concentration and (*b*) flux. The model used is shown in Scheme 1, and the rate laws for the two steps are follows: $v_1 = V_1(X_0 - S/K_1)$ and $v_2 = V_2(S - X_2/K_2)$, with $X_0 = 1$, $X_2 = 0.1$, $K_1 = 1$, $K_2 = 1$. For t reference curve (dashed), $V_1 = 0.2$ and $V_2 = 5$, whereas for the curve after the coordinate-control operati (continuous), $V_1 = 0.4$ and $V_2 = 10$, i.e. in this case $\alpha = 2$. The reference values of the metabol concentration and flux 1 at time t (arbitrarily chosen) are S_x and J_x respectively. After the coordinate cont operation (doubling both V_1 and V_2 at time zero) we obtain the same value of the metabolite concentrati S_x, at time $t/2$ (Fig. 1*a*), but at the same time the flux has twice its reference value (Fig. 1*b*).



Scheme 2. Model used to obtain the results shown in Fig. 2.

concentrations, T, satisfy eqn. (8), i.e.

$$T_\alpha = T/\alpha \tag{12}$$

This is shown for a particular example in Fig. 2, where the variable T is the period of oscillation. Eqn. (12) may be used as the starting point for obtaining the summation relationship for changes in the variable T with the enzyme concentrations E_j. The result is as follows:

$$\sum_j C_{E_j}^T = -1 \tag{13}$$

where

$$C_{E_j}^T = \frac{E_j}{T}\frac{\partial T}{\partial E_j} \tag{14}$$

The general summation relationship given by eqn. (13) has previously been given for particular definitions of transition time (Heinrich & Rapoport, 1975; Torres *et al.*, 1989;

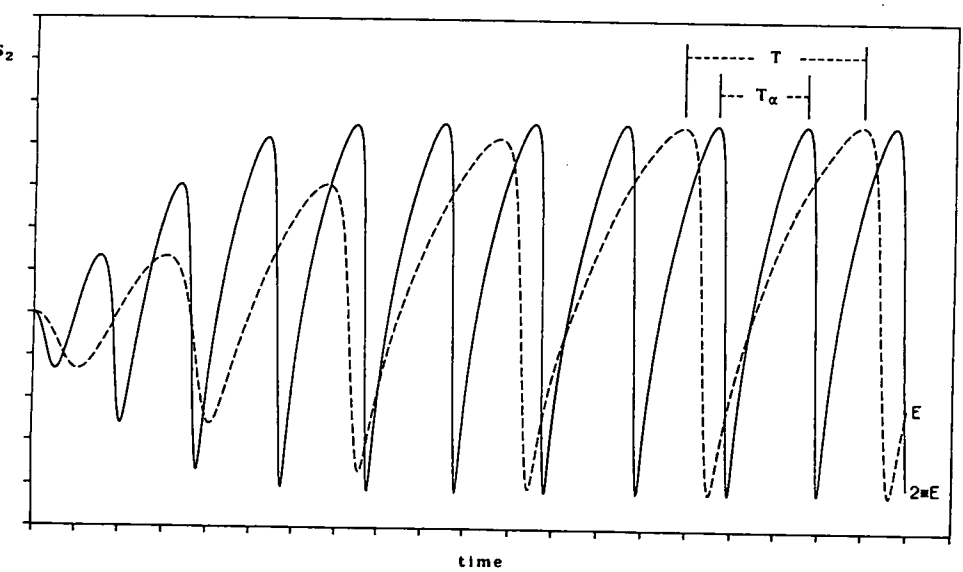


Figure 2. Effect of the coordinate-control operation on the period of oscillation. For the model shown in Scheme 2, the rate law for the first step is $v_1 = V_0X_0/\{X_0 + K_0[1 + L(1 + S_3/K_i)]\}$, and for the other three is $v_i = VS_i/(K + S_i)$, for i = 1, 2, 3, with parameters X_0 = 5, K_0 = 0.02, K_i = 0.2, L = 10, K = 5. The reference values (dashed curve) of the maximum rates are V_0 = 50 and V = 5. After the coordinate-control operation (continuous line) we use V_0 = 100 and V = 10 (α = 2). One may see from the plots that doubling all the maximum rates causes the period T to decrease to half its reference value, $T_\alpha = T/2$. Numerical simulations were carried out using the program SCAMP (Sauro, 1986).

Meléndez-Hevia *et al.*, 1990). The same relationship applies to the period of oscillation. One quantitative measure for characterizing chaotic behaviour is the Lyapunov exponent λ (see Chapter 26 by Markus and Hess in this book): this quantity has the dimensions of inverse of time, and it may be shown that its reciprocal $1/\lambda$ satisfies eqns. (12-13).

Eqns. (9-13) are some of the consequences of the effect that the coordinate-control operation has on the variables of the metabolic system. In their derivation, some assumptions were made (see Acerenza *et al.*, 1989). One of the most important is that all the rate laws are proportional to the corresponding enzyme concentrations. If the deviations from this assumption are significant (see Chapter 20 by Kacser, Sauro and Acerenza in this book), the equations obtained are not fulfilled. The departures from the expected results may give some information about the control properties of the system. For example if we apply the coordinate-control operation (with a small α) to an oscillatory system (see Chapter 26 by Markus and Hess), and we find that the period does not satisfy eqn. (12), then we may conclude that there are deviations from the assumed hypothesis that contribute significantly to the control of the period. This operation may be applied relatively easily to a biological extract. Therefore, the coordinate-control operation may be used as a simple experimental strategy to gain some insight into the control features of metabolic systems.

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REFERENCES

Acerenza, L., Sauro, H. M. & Kacser, H. (1989) *J. Theor. Biol. 137*, 423-444
Burns, J. A., Cornish-Bowden, A., Groen, A. K., Heinrich, R., Kacser, H., Porteous, J. W., Rapoport S. M., Rapoport, T. A., Stucki, J. W., Tager, J. M., Wanders, R. J. A. & Westerhoff, H. V (1985) *Trends Biochem. Sci. 10*, 16
Cornish-Bowden, A. (1979) *Fundamentals of Enzyme Kinetics*, pp. 49-51, Butterworths, Londo and Boston
Decroly, O. & Goldbeter, A. (1982) *Proc. Natl. Acad. Sci. USA 79*, 6917-6921
Easterby, J. S. (1981) *Biochem. J. 199*, 155-161
Heinrich, R. & Rapoport, T. A. (1974) *Eur. J. Biochem. 42*, 89-95
Heinrich, R. & Rapoport, T. A. (1975) *BioSystems 7*, 130-136
Higgins, J. (1967) *Ind. Eng. Chem. 59*, 19-62
Higgins, J., Frenkel, R., Hulme, E., Lucas, A. & Rangazas, G. (1973) in *Biological and Biochemica Oscillators* (Chance, B., Pye, E. K., Ghosh, A. M. & Hess, B., eds.), pp. 127-175, Academi Press, New York
Kacser, H. & Burns, J. A. (1973) *Symp. Soc. Exp. Biol. 27*, 65-104
Kohn, M. C., Whitley, L. M. & Garfinkel, D. (1979) *J. Theor. Biol. 76*, 437-452
Kohn, M. C. & Chiang, E. (1982) *J. Theor. Biol. 98*, 109-126
Markus, M., Kuschmitz, D. & Hess, B. (1984) *FEBS Lett. 172*, 235-238
Meléndez-Hevia, E., Torres, N. V., Sicilia, J. & Kacser, H. (1990) *Biochem. J. 265*, 195-202
Mizraji, E., Acerenza, L. & Hernandez, J. (1988) *BioSystems 22*, 11-17
Pachot, P. & Demongeot, J. (1987) in *Contrôle du Métabolisme Cellulaire* (Mazat, J.-P. & Rede C., eds.), pp. 121-137
Sauro, H. M. (1986) PhD Thesis, Oxford Polytechnic
Selwyn, M. J. (1965) *Biochim. Biophys. Acta 105*, 193-195
Torres, N. V., Souto, R. & Meléndez-Hevia, E. (1989) *Biochem. J. 260*, 763-769

Biochem. J. (1990) 269, 697–707 (Printed in Great Britain)

Enzyme kinetics and metabolic control

A method to test and quantify the effect of enzymic properties on metabolic variables

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It is usual to study the sensitivity of metabolic variables to small (infinitesimal) changes in the magnitudes of individual parameters such as an enzyme concentration. Here, the effect that a simultaneous change in all the enzyme concentrations by the same factor α (Co-ordinate-Control Operation, CCO) has on the variables of time-dependent metabolic systems is investigated. This factor α can have any arbitrary large value. First, we assume, for each enzyme measured in isolation, the validity of the steady-state approximation and the proportionality between reaction rate and enzyme concentration. Under these assumptions, any time-invariant variable may behave like a metabolite concentration, i.e. $S_\alpha = S_r$ (S-type), or like a flux, i.e. $J_\alpha = \alpha J_r$ (J-type). The subscripts r and α correspond to the values of the variable before and after the CCO respectively. Similarly, time-dependent variables may behave according to $S_\alpha(t/\alpha) = S_r(t)$ (S-type) or to $J_\alpha(t/\alpha) = \alpha J_r(t)$ (J-type). A method is given to test these relationships in experimental systems, and to quantify deviations from the predicted behaviour. A positive test for deviations proves the violation of some of the assumptions made. However, the breakdown of the assumptions in an enzyme-catalysed reaction, studied in isolation, may or may not affect significantly the behaviour of the system when the component reaction is embedded in the metabolic network.

1. INTRODUCTION

Enzymologists have been studying the kinetic properties of isolated enzyme-catalysed reactions for many years. In the great majority of the reactions the steady-state rate is proportional to the total enzyme concentration. Many exceptions to this property are reported, and this fact is often connected with the existence of subunit associations (see, e.g., Kurganov, 1978). Another common characteristic of most enzyme assays is that the rate remains constant during the early course of the reaction. Some examples are, however, known where the 'initial' rate increases (lag) or decreases (burst) in time (see, e.g., Neet & Ainslie, 1980). In these cases the steady-state assumption for the concentrations of the different enzyme forms is violated.

Enzyme-catalysed reactions are the building blocks of metabolism, and the knowledge of their kinetic features is an important step towards understanding how metabolic networks behave. Nevertheless, we must note that these individual reactions are part of a system where the components influence each other in intricate ways. In metabolic systems the metabolite concentrations are not held constant, as in traditional enzyme kinetic assays. In these systems the rates (i.e. fluxes) affect the metabolite concentrations, and these in turn affect the rates (Kacser, 1987). We must conclude that all the components contribute to the system behaviour to some extent. However, if a component is replaced by a different one, are the properties of the system significantly changed? Or, to particularize the question, is a particular kinetic feature of an enzyme-catalysed reaction (e.g. rate non-proportional to enzyme concentration) relevant to the behaviour of a metabolic variable when the enzyme concentration is changed? As we show in the present paper, the existence of strong deviations from proportionality between rate and enzyme concentration (in a traditional assay) may be almost irrelevant when the enzyme concentration is changed within a metabolic network, whereas in other cases small deviations from proportionality (measured in isolation) may be greatly amplified in the system. Similar conclusions apply to enzymes that exhibit lags or bursts. Furthermore, we show how the quantitative importance of the effects that these kinetic properties of enzymes have on a metabolic variable may be experimentally determined. The relationships and methods developed in the present paper apply to time-dependent metabolic systems. They enable one to analyse the properties of the instantaneous values of a time-dependent variable as well as the properties of the time-invariant variables that may be defined from the time course (e.g. steady-state values).

2. PARAMETERS AND VARIABLES

A metabolic system is, basically, a network constituted of molecules, x_i, 'connected' by chemical reactions. This is usually represented by a 'static' metabolic map, but in our treatment we wish to study some quantitative aspects of its dynamical behaviour. The rates of interconversion between each pair of molecules are given by the rate laws, v_k. These may be functions of the concentrations, x_i, involved (free metabolites, free enzymes, enzyme–metabolite complexes, enzyme–enzyme complexes etc.), temperature, pressure, pH, ionic strength etc.

The 'parameters' of the system are the quantities that can be manipulated independently of each other. Once their values are fixed at the initial point of time, they remain constant during the whole interval of time that the system is studied. In what follows, we consider as parameters the total concentration of each enzyme (free plus complex forms) and physicochemical quantities such as temperature and pressure. The fluxes or free concentrations that act as inputs of the system (e.g. sources and sinks of matter and external effectors) are either held constant or changed in time in

Abbreviations used: CCO, Co-ordinate-Control Operation; D-plot, Direct co-ordinate-control plot; R-plot, Rescaling co-ordinate-control plot; RPS-plot, Reference-Point Sensitivity co-ordinate-control plot; PPS-plot, Point-to-Point Sensitivity co-ordinate-control plot.

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some pre-determined way (an example of the latter situation is given in Markus *et al.*, 1984).

The 'variables' of the system are the quantities whose values depend on the values of the parameters. We may define two types of variables, namely time-dependent and time-invariant, whether their magnitudes do or do not change with time respectively. The instantaneous concentrations x_i, mentioned above, and other quantities that are functions of these concentrations (e.g. fluxes) are time-dependent variables. The successive values that they take in time depend on the values to which the parameters and initial concentrations are set at the initial time. Examples of time-invariant variables are the stable steady-state concentrations and fluxes, the transition time of a metabolite (Heinrich & Rapoport, 1975) or a pathway (Easterby, 1981, 1986) to a stable steady state, the period and amplitude of variables that exhibit sustained oscillations (Hofmann *et al.*, 1985; Goldbeter & Moran, 1987; Mizraji *et al.*, 1988) and the maximum Lyapunov exponent that characterizes a chaotic regime (Hess & Markus, 1987).

First, we centre the attention on the time-dependent variables x_i. The change of each x_i with time, dx_i/dt, may be written as the balance of all the rates, v_k, that affect its concentration directly:

$$\frac{dx_i}{dt} = \sum_{k=1}^{p} n_{ik} \cdot v_k, \qquad i = 1, \ldots, q \tag{1}$$

Here n_{ik} is the stoichiometric coefficient of the molecule x_i in the reaction k. It is positive, negative or zero if x_i is produced, consumed or not altered directly by the rate v_k respectively. For a given set of values of the parameters and initial concentrations, the solutions of eqns. (1) constitute the time courses of the concentrations: $x_i(t)$, $i = 1, \ldots, q$. Introducing these $x_i(t)$ into the rate laws v_k, we obtain the time courses of the fluxes: $J_k(t)$, $k = 1, \ldots, p$.

The values of the time-invariant variables may be obtained from the time course of the time-dependent variables. For example, when a system settles to a stable steady state, an estimate of the steady-state values of metabolite concentrations and fluxes may be obtained from the time courses, by waiting a 'long enough' period of time. In a system that exhibits sustained oscillations in time, the period of oscillation (the interval of time between two consecutive maxima in the time course) is such a time-invariant variable.

3. THE CO-ORDINATE-CONTROL OPERATION (CCO)

It has been traditional to investigate systems by a sensitivity analysis of the variables with respect to specific parameters. Thus control analysis (Kacser & Burns, 1973; Heinrich & Rapoport, 1974) considers the responses of metabolic concentrations and fluxes to modulations of any one of the parameters of the system. Some progress has been made to use this approach to detect deviations from the assumption of proportionality between rate and enzyme concentration in steady-state systems (Kacser *et al.*, 1990; Sauro & Kacser, 1990). In what follows, a different method from modulating individual parameters is described. It applies to time-dependent systems and has the advantage of not being restricted to small (infinitesimal) changes.

Changes in the values of the parameters affect, to various degrees, the values of the variables (control of variables by parameters). For a time-dependent variable, one may define a 'reference time course' generated by a chosen set of values of the parameters, the 'reference parameter values'. If one or more of the reference parameter values are altered at the initial time, the resulting time course may be significantly different from the reference one. In what follows, we study the control of the variables by a particular group of parameters, namely the total enzyme concentrations, E_j. We assume that all the m enzyme concentrations are simultaneously changed by the same arbitrary factor α (not necessarily ≈ 1). If $E_{j,r} (j = 1, \ldots, m)$ are the values of the enzyme concentrations that generate the reference time course, then the new time course is obtained by using enzyme concentrations $E_{j,\alpha} (j = 1, \ldots, m)$ given by:

$$E_{j,\alpha} = \alpha E_{j,r} \tag{2}$$

We call this equal and simultaneous change in all the enzyme concentrations: the Co-ordinate-Control Operation (CCO) (briefly introduced in Acerenza *et al.*, 1989; Acerenza, 1990). The subscripts r and α are used to indicate the value of a parameter or variable before (reference) and after the CCO is applied respectively. We use this operation throughout the following treatment. This approach reveals certain simple properties of time-dependent metabolic systems, when some assumptions are made (see below). Furthermore we suggest how the resulting relationships, and hence the assumptions made, may be experimentally tested in reconstituted systems or biological extracts. We discuss the practical problems associated with attempting to apply a CCO in a subsequent section.

4. ASSUMPTIONS

We now make some assumptions concerning the properties of the metabolic system. These are used to derive some theoretical consequences of the CCO in sections 5 and 6. The analysis of cases where there is a breakdown of the assumptions is considered in sections 9–11.

In the general case (see section 2) the q concentrations x_i that appear in eqns. (1) may be classified in two groups: n free metabolite concentrations, S_i, and $q-n$ enzyme concentration in their different forms (free or combined with metabolites), C_i. If the steady-state approximation for the concentrations C_i is plausible, then $dC_i/dt = 0$ for each C_i (Segel, 1988). Applying these conditions to eqns. (1), the reduced resulting system of differential equations:

$$\frac{dS_i}{dt} = \sum_{j=1}^{m} n_{ij} \cdot v_j, \qquad i = 1, \ldots, n \tag{3}$$

involves only the free metabolite concentrations as variables. In addition, we assume that the rates v_j are proportional to the corresponding total enzyme concentrations E_j:

$$v_j = E_j \cdot f_j, \qquad j = 1, \ldots, m \tag{4}$$

where f_j are functions of the concentrations S_i and parameters, and are independent of enzyme concentrations and time. The Michaelis–Menten rate equation, for example, fulfils eqn. (4).

Applying the CCO to a metabolic system whose rates are given by eqn. (4), the resulting rates, $v_{j,\alpha}$, are related to the reference rates, $v_{j,r}$ (see eqn. 2), as follows:

$$v_{j,\alpha} = \alpha v_{j,r}, \qquad j = 1, \ldots, m \tag{5}$$

Then, under the assumptions described by eqns. (3) and (4), the first important consequence of the CCO is to multiply each term of the right-hand member of eqns. (3) by the same factor α. It is important to note that, if matter is introduced into the system via one or more constant input fluxes, these should also be modified according to eqn. (5) when the CCO is applied. However, any constant (input) concentrations, if present, should not be modified when the rate that transforms them is given by eqn. (4). The discussion of the case where the inputs are changed in time, by the experimentalist, is postponed to section 5.

5. CO-ORDINATE CONTROL OF TIME-DEPENDENT VARIABLES

In this section we outline some consequences of the CCO, related to the control of time-dependent variables. Some mathematical details of this treatment are given in Acerenza *et al.* (1989).

Combining eqns. (3) and (5), we obtain the relationship between the derivative of S_i with respect to time after and before (reference) the CCO:

$$\left(\frac{dS_i}{dt}\right)_\alpha = \alpha\left(\frac{dS_i}{dt}\right)_r \tag{6}$$

The only effect of a simultaneous change in all the enzyme concentrations by a factor α is to make the metabolite concentrations change at a rate that is α times the original one. Then, the CCO is equivalent to a change in the time scale of the time courses of the metabolite concentrations. For each time t_r of the reference time courses [the reference time courses are the functions of time, $S_i(t)$, obtained with the reference parameter values] there exists one time t_α, in the new time courses, at which all the metabolite concentrations have the same values as in the reference state at time t_r. The value of t_α is given by:

$$t_\alpha = \frac{t_r}{\alpha} \tag{7}$$

and hence:

$$S_{i,\alpha}(t_r/\alpha) = S_{i,r}(t_r) \tag{8}$$

From eqns. (5) and (8) we obtain the analogous relationship for the fluxes:

$$J_{j,\alpha}(t_r/\alpha) = \alpha J_{j,r}(t_r) \tag{9}$$

Eqns. (8) and (9) tell us that, when the CCO is applied to a time-dependent metabolic system, which satisfies the assumptions made in eqns. (3) and (4), the instantaneous values of the metabolite concentrations are 'shifted' from the time t to t/α, while the instantaneous values of the fluxes are multiplied by the factor α and 'shifted' from t to t/α. It is important to note that, if matter is introduced into the system via concentrations or fluxes that change in time, these inputs should be altered in the same way as shown by eqns. (8) and (9) respectively when the CCO is applied.

An immediate consequence of eqn. (8) is that if one plots the metabolite concentrations after the CCO against α multiplied by time the resulting curve should coincide with the reference time course (see Figs. 1*a* and 1*b*). This result is used below in section 8. [A similar procedure is used as a test for inactivation of a single enzyme during assay (Selwyn, 1965); (see also Cornish-Bowden, 1979).]

6. CO-ORDINATE CONTROL OF TIME-INVARIANT VARIABLES

A. Co-ordinate control of time-invariant variables with dimension of time

Time-invariant variables with dimension of time, which characterize some temporal aspect of the time course of the metabolite concentrations, satisfy eqn. (7). Examples of these variables are transition times, period of oscillation and the reciprocal of the maximum Lyapunov exponent (mentioned in section 2). Then, if T_r is the value of such a time-invariant variable with dimension of time, obtained from the reference time course, after the CCO the new value of the variable, T_α, is given by:

$$T_\alpha = \frac{T_r}{\alpha} \tag{10}$$

that is a simultaneous increase (decrease) in all enzyme concentrations by a factor α causes a decrease (increase) in the value of T by the same factor.

B. Co-ordinate control of a transition to a stable steady state

If the metabolic system is one that approaches a stable steady state, after a long enough period of time the variables exhibit

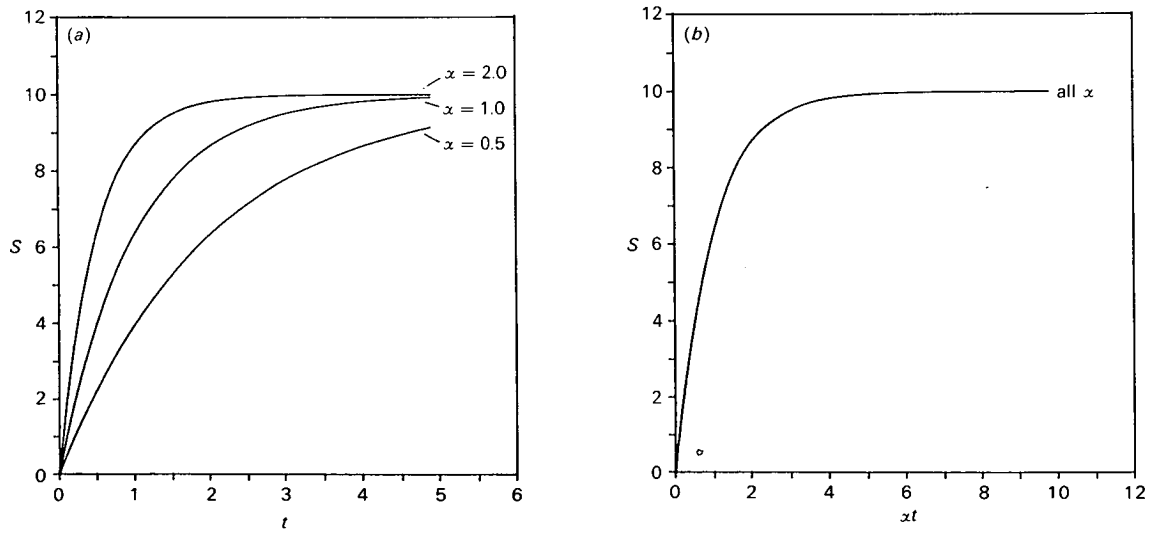

Fig. 1. Example where assumptions eqns. (3) and (4) are valid: (*a*) concentration of S versus time and (*b*) R-plot

In (*a*) we show time courses of the concentration of S (Scheme 1 in section 11) corresponding to different α values. The concentrations of X_0 and X_1 are constant. The rate laws v_1 and v_2 are proportional to the corresponding enzyme concentrations. In (*b*) we plot the same ordinate values as in (*a*), but against α multiplied by time. In this rescaling plot the three curves coincide. It should be noted that, as a result of the rescaling procedure, the curves in (*a*) corresponding to $\alpha = 0.5$, 1.0 and 2.0 end in (*b*) at $\alpha t = 2.5$, 5.0 and 10.0 respectively. [In Figs. 3(*a*) and 3(*b*) we show the same types of plots in a situation where assumption eqn. (4) is not valid.]

approximately constant values independent of time. Therefore eqns. (8) and (9) take the form:

$$S^{ss}_{i,\alpha} = S^{ss}_{i,r} \tag{11}$$

$$J^{ss}_{j,\alpha} = \alpha J^{ss}_{j,r} \tag{12}$$

where the superscript ss indicates steady-state values. Eqns. (11) and (12) show the effect that the CCO has on the steady-state metabolite concentrations and fluxes.

To characterize the transition between the initial conditions and the steady state one may use the transition time as defined by Easterby (1981). This is, of course, a time-invariant variable that behaves according to eqn. (10) when the CCO is applied.

C. Co-ordinate control of sustained oscillations

Here we consider the situation where the time-dependent variables (metabolite concentrations and fluxes) exhibit stable oscillations in time. In this type of behaviour, the values of the time-dependent variables repeat at constant intervals of time, T (period of oscillation). Two time-invariant variables are frequently used to characterize oscillatory phenomena, namely the period and the amplitude. The period is a time-invariant variable with dimension of time, and when the CCO is applied it behaves as shown in eqn. (10). The amplitude of oscillation (A_{S_i}) for a metabolite concentration S_i may be defined as half the difference between the maximum ($S_i^{max.}$) and minimum ($S_i^{min.}$) values: $A_{S_i} = (S_i^{max.} - S_i^{min.})/2$. The maximum and minimum values, and hence the amplitude, depend on the reference parameter values. If we apply the CCO, and wait until the system settles to a new stable oscillation, even though the value of the period is modified as described by eqn. (10), the maximum and minimum values of the metabolite concentrations are not altered (see eqn. 8):

$$\begin{aligned} S^{max.}_{i,\alpha} &= S^{max.}_{i,r} \\ S^{min.}_{i,\alpha} &= S^{min.}_{i,r} \end{aligned} \tag{13}$$

Introducing eqns. (13) into the definition of amplitude, we immediately obtain:

$$A_{S_i,\alpha} = A_{S_i,r} \tag{14}$$

i.e. the value of the amplitude is unaffected by the CCO. The fluxes J_j may be calculated by introducing the metabolite concentrations into the rate equations given in eqn. (4). If the metabolite concentrations are periodic functions of time, with period T, the corresponding fluxes are periodic functions of time with the same period. Therefore when the CCO is applied the period of these fluxes is also modified according to eqn. (10). The metabolite concentrations corresponding to the maximum and minimum fluxes are not modified, and therefore the same applies to the values of f_j at these points, introduced in eqn. (4). However, as the rates are proportional to enzyme concentration, even if the values of f_j are unaltered, the new maximum and minimum values of the flux are α times those of the reference oscillation:

$$\begin{aligned} J^{max.}_{j,\alpha} &= \alpha J^{max.}_{j,r} \\ J^{min.}_{j,\alpha} &= \alpha J^{min.}_{j,r} \end{aligned} \tag{15}$$

The amplitude of oscillation (A_{J_j}) for a flux J_j may be defined as: $A_{J_j} = (J_j^{max.} - J_j^{min.})/2$. Combining eqns. (15) with this definition, the relationship between the flux amplitude before and after the CCO is obtained:

$$A_{J_j,\alpha} = \alpha A_{J_j,r} \tag{16}$$

Besides the period and amplitude, another quantity that may be used to characterize an oscillatory regime is the mean value in a cycle. The mean value of a time-dependent variable Y in an interval of time T is defined by the expression:

$$\bar{Y}_r = \frac{\int_0^T Y_r \cdot dt}{T} \tag{17}$$

It is important to note that we evaluate the mean value in an interval of time equal to the period of oscillation. When the CCO is applied the resulting mean value can be written as follows:

$$\bar{Y}_\alpha = \frac{\int_0^{T_\alpha} Y_\alpha \cdot dt}{T_\alpha} \tag{18}$$

In the following, we discuss the relationship between $\bar{Y}_r$ and $\bar{Y}_\alpha$ when the variable is a metabolite concentration or flux that shows stable oscillations. As was mentioned above, the period of oscillation, T, satisfies eqn. (10). If Y is a metabolite concentration, eqn. (8) is fulfilled and can be written in an equivalent way: $S_{i,\alpha}(t) = S_{i,r}(\alpha t)$. Using this equation together with eqns. (10), (17) and (18), and properties of integrals, we finally obtain:

$$\bar{S}_{i,\alpha} = \bar{S}_{i,r} \tag{19}$$

In the case where Y is a flux, eqns. (9), (10), (17) and (18) are used to obtain:

$$\bar{J}_{j,\alpha} = \alpha \bar{J}_{j,r} \tag{20}$$

In other words, the simultaneous change of all enzyme concentrations by a factor α does not affect the mean values of the metabolite concentrations, but alters all the mean fluxes by the same factor (being the mean values evaluated in a period of oscillation). Eqns. (19) and (20) may be seen as equivalent to the steady-state conditions (11) and (12) when stable oscillations are considered.

7. CLASSIFICATION OF THE VARIABLES

In sections 5 and 6 we considered the effects that the CCO has on time-dependent and time-invariant variables respectively. In each one of these groups we may distinguish variables that behave like a metabolite concentration (S-type) or like a flux (J-type). Such a classification may serve as a summary of the relationships established and constitutes the basis of experimental tests.

A. Time-invariant variables

All the time-invariant variables, Y, considered in section 6, may be classified in two groups, S-type and J-type, according to the expected response when the system is subject to the CCO. We define as S-type time-invariant variables those that remain unaltered after the CCO:

$$S_\alpha = S_r \tag{21}$$

Examples of this type of variable are S_i^{ss} (eqn. 11), $S_i^{max.}$ and $S_i^{min.}$ (eqn. 13), A_{S_i} (eqn. 14) and $\bar{S}_i$ (eqn. 19). J-type time-invariant variables appear multiplied by the factor α when the CCO is applied:

$$J_\alpha = \alpha J_r \tag{22}$$

and examples of this type are J_j^{ss} (eqn. 12), $J_j^{max.}$ and $J_j^{min.}$ (eqn. 15), A_{J_j} (eqn. 16) and $\bar{J}_j$ (eqn. 20). The reciprocal of T ($1/T$) also belongs to this type (see eqn. 10).

It should be noted that, if Y is a J-type time-invariant variable, then Y/α behaves like an S-type time-invariant variable (see eqns. 21 and 22).

B. Time-dependent variables

The time-dependent variables may also be classified as *S*-type or *J*-type depending on the predicted behaviour when the CCO is applied (see section 5). The effect of the CCO on an *S*-type time-dependent variable is simply expressible as an alteration in the time scale of its time course:

$$S_\alpha(t/\alpha) = S_r(t) \tag{23}$$

The time-dependent metabolite concentrations are *S*-type variables (eqn. 8). In the case of *J*-type variables, the CCO scales simultaneously the time and the variable according to the following relationship:

$$J_\alpha(t/\alpha) = \alpha J_r(t) \tag{24}$$

Examples of *J*-type variables are the time-dependent fluxes (eqn. 9).

We therefore see that the values of an *S*-type (*J*-type) time-dependent variable, corresponding to different α and identical αt, behave like the values of an *S*-type (*J*-type) time-invariant variable. Similarly to the case of time-invariant variables, if Y is a *J*-type time-dependent variable, Y/α behaves like an *S*-type time-dependent variable.

8. TEST OF THE GENERAL RELATIONSHIPS

The data obtained from a CCO experiment may be used to test the general relationships summarized in section 7 (eqns. 21–24). Here we propose simple plots to test these relationships. Depending on whether the variables analysed are time-dependent or time-invariant, the procedures are slightly different.

A. Time-invariant variables

To test the behaviour of a time-invariant variable, Y, the basic experimental information needed is a table with the values of the variable corresponding to different α values (Y_α versus α).

The 'Direct co-ordinate-control plot' (D-plot) is simply the plot Y_α/Y_r against α. Y_r is the value of the variable when $\alpha = 1$ (reference point). The expected result of a D-plot for an *S*-type variable is a straight line where the quotients, Y_α/Y_r, are equal to 1 for all α (see eqn. 21 and Fig. 2). *J*-type variables should give a straight line, with tangent 1 (45°), that, extrapolated to α equal to zero, passes through the origin (see eqn. 22 and Fig. 2). If the D-plot is not as expected, the variable shows departures from the theoretical relationship (eqn. 21 for *S*-type and eqn. 22 for *J*-type variables). Provided that no systematic errors in applying the CCO have been introduced (see section 12), this result should be interpreted as a breakdown of the assumptions introduced in section 4. On the other hand, if the D-plot is as expected, the deviations from the assumptions, if they exist, do not contribute significantly to the behaviour of the variable when the enzyme concentrations are changed.

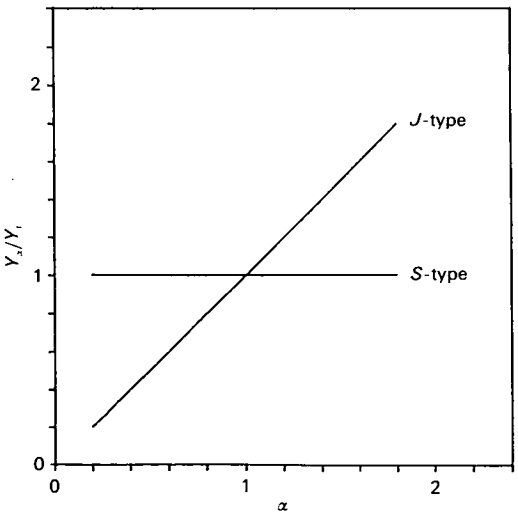

Fig. 2. D-plots, Y_α/Y_r against α, when eqns. (3) and (4) apply

The horizontal straight line is the D-plot of an *S*-type variable and the straight line of 45° corresponds to a *J*-type variable.

B. Time-dependent variables

To test eqns. (23) and (24) the experimental information needed is the time courses for different values of α, namely $Y_\alpha(t)$. On the basis of the properties of time-dependent variables, discussed in section 7, we suggest to plot the data in a 'Rescaling co-ordinate-control plot' (R-plot). The R-plot for *S*-type variables is $S_\alpha(t)$ against αt. If the variable behaves according to eqn. (23), the plots for different α should appear superimposed on the reference curve ($\alpha = 1$) (see Fig. 1). Similarly, in the case of *J*-type variables the R-plot is $J_\alpha(t)/\alpha$ against αt. As a consequence of eqn. (24), the curves corresponding to the different time courses $J_\alpha(t)$ should appear superimposed on the reference time course $J_r(t)$ (where $\alpha = 1$) in the R-plot (not shown). If such an R-plot does not give coincident curves, this would be an indication that the assumptions made in section 4 are not fulfilled, and that these deviations contribute significantly to the behaviour of the system variable when the enzyme concentrations are modified.

As was mentioned in section 7, the values of an *S*-type (*J*-type) time-dependent variable, corresponding to different α and identical αt, behave like the values of an *S*-type (*J*-type) time-invariant variable. Therefore a D-plot may be constructed with these values.

What we have called the R-plot, for *S*-type time-dependent variables, is similar to a test used to detect inactivation of an enzyme during assay (Selwyn, 1965).

9. BREAKDOWN OF THE ASSUMPTIONS

The relationships derived in sections 5 and 6, and summarized in section 7, are based on the assumptions introduced in eqns. (3) and (4). If the steady-state approximation for the different forms of the enzyme concentrations is not valid or the rates are not proportional to the corresponding enzyme concentrations, the system variables may exhibit significant deviations from the predicted behaviour (eqns. 21–24) when the CCO is applied. Here we enumerate some enzyme mechanisms that are known to violate those assumptions and, when embedded in a metabolic network, are potential generators of deviations.

Many proteins described in the literature have a quaternary structure. Depending on the experimental conditions, more than one polymeric form may coexist in significant amount. If a protein with catalytic function shows these structural features, it constitutes a source for the generation of rate laws that do not behave as eqn. (4) (see, e.g., Kurganov, 1978). The simplest example of association–dissociation between homologous subunits is the monomer–dimer equilibrium. In section 11 below we analyse some effects of this type of mechanism on transients to a stable steady state. The existence of associations between different enzymes (heterologous associations) may generate rate laws that depend on more than one enzyme concentration, showing departures from eqn. (4). Some consequences of homologous and heterologous associations on the control properties of steady-state metabolic concentrations and fluxes have recently been addressed (Kacser *et al.*, 1990; Sauro & Kacser, 1990).

The time courses of some enzymic reactions show 'lags' or 'bursts' under normal assay conditions (see, e.g., Neet & Ainslie, 1980). This phenomenon is associated with the existence of slow conformational transitions in the enzyme mechanism. In these cases the steady-state approximation is not valid, and therefore it is not possible to express the behaviour in time by eqns. (3). The existence of slow conformational changes may have major effects on the control of the time course of a variable, while having no effect on the control properties of the steady state of the system.

Some concentrations of metabolites within a system appear to be linked by conservation constraints (e.g. $[NAD^+]+[NADH]$ = constant). If the total concentrations of enzymes are negligible with respect to the concentrations of conserved metabolites to which they bind, the steady-state approximation is valid. In this frequently considered situation, as there is no significant sequestration of the conserved metabolites by the enzymes, when the CCO is applied eqn. (4) is also valid. Even if the total concentrations of the enzymes are of the same order as the conserved metabolite concentrations, the steady-state assumption may still be satisfied, provided that those concentrations are much smaller than the Michaelis constant (see Segel, 1988). Owing to the low 'affinity' (large Michaelis constant) between enzyme and metabolite, the fraction of the metabolite in complexed form is still small. If, however, the total concentrations of the enzymes and the metabolites are of the same order, but greater than the Michaelis constant, the validity of the steady-state assumption is no longer ensured. Furthermore, in this condition there is considerable sequestration of the conserved metabolites, and we may expect significant deviations in the system variables when the CCO is applied (see Fell & Sauro, 1990).

The enzyme mechanisms mentioned above may be responsible for the appearance of departures from the quantitative relationships derived in sections 5 and 6. Furthermore, they may be the cause of a qualitative change in the dynamics of the system if a 'bifurcation point' is reached when the CCO is applied. Such situations, for example, may transform a sustained oscillation into a stable steady state, or vice versa.

10. QUANTIFICATION OF THE DEVIATIONS

The D-plots and R-plots may be used to test the existence of deviations from the predicted relationships (eqns. 21–24). Such a case is illustrated in Figs. 3(*a*) and 3(*b*). Here we introduce additional plots to assess the quantitative importance of the deviations. These plots constitute a phenomenological description of the deviations. Furthermore, as we show below, they may be useful in the search of the origin of the deviations.

In section 7 we discussed two properties of metabolic variables: (*a*) if Y is a J-type variable (time-dependent or time-invariant), then the values of the variable divided by α, Y/α, behave like an S-type variable (time-dependent or time-invariant respectively); (*b*) if $Y(t)$ is a time-dependent variable (S-type or J-type), then the values of the variable for the same αt and different α, $Y_\alpha(t)$, behave like a time-invariant variable (of the same type). These properties allow us to transform the values of any of the variables described in section 7 into the values of an S-type time-invariant variable. If we want, for example, to compare the deviations of an S-type variable with those of a J-type variable, or to compare the deviations of a time-dependent variable corresponding to different time points, such transformations should be applied. The plots, which we introduce in this section, are defined for S-type time-invariant variables. However, they may also be used in the analysis of other types of variables, with application of the appropriate transformations described above.

To quantify deviations, the data from a CCO experiment may be plotted in, at least, two different ways. In what follows we define and discuss two plots, which we call 'Reference-Point Sensitivity co-ordinate-control plot' (RPS-plot) and 'Point-to-Point Sensitivity co-ordinate-control plot' (PPS-plot).

A. Reference-Point Sensitivity co-ordinate-control plot (RPS-plot)

The RPS-plot is established to characterize the changes of a variable with respect to a unique reference point (point corresponding to $\alpha = 1$). We define a deviation function d_r (for an S-type time-invariant variable) as $d_r = [(S_\alpha - S_r)/S_r]/(\alpha - 1)$. A plot of d_r against α represents the relative change in the value of the variable, with respect to the reference value (S_r), per relative change in the enzyme concentrations, $\alpha - 1$ (see eqn. 2), when the CCO is applied. The ordinate values in this plot would constitute a measure of the quantitative importance of the deviations for different α changes. The sign of the ordinate values is positive or negative if the change in the variable is in the same or opposite direction to the change in the enzyme concentrations respectively. In the absence of deviations the ordinate values d_r are equal to zero for all α.

B. Point-to-Point Sensitivity co-ordinate-control plot (PPS-plot)

In the previous (RPS) plot we used a unique reference point. Alternatively, it is possible to establish a plot where each set of enzyme concentrations serves successively as the reference point.

Let E_{n-1}, E_n and E_{n+1} ($E_{n-1} < E_n < E_{n+1}$) be three consecutive values of the concentration of any one enzyme, and S_{n-1}, S_n and S_{n+1} the corresponding values of an S-type time-invariant variable, resulting from the application of the CCO. The point E_n is momentarily considered as the reference point. The relative change in the variable per relative change in the enzyme concentration from E_n to E_{n+1} is: $d_{+1} = [(S_{n+1} - S_n)/S_n]/[(E_{n+1} - E_n)/E_n)]$. Similarly, the relative change in the variable per relative change in the enzyme concentration from E_n to E_{n-1} is: $d_{-1} = [(S_{n-1} - S_n)/S_n]/[(E_{n-1} - E_n)/E_n]$. If the increase and decrease of the enzyme concentration from the reference point are equal (i.e. $E_{n+1} - E_n = E_n - E_{n-1}$), then the relative change in the variable per relative change in the enzyme concentration at the reference point may be estimated by the simple arithmetic mean: $d_n = (d_{+1} + d_{-1})/2$. From p experimental points, $p-2$ values of d_n may be calculated (d_2 to d_{p-1}).

We define the PPS-plot as d_n against E_n. The ordinates in this plot may be considered as an estimate of the deviation in the variable corresponding to each E_n when the CCO is applied. In the absence of deviations the ordinates are equal to zero. The signs of the ordinates are positive or negative if the change in the variable is in the same or the opposite direction to the change in the enzyme concentrations respectively.

It should be noted that if the experimental data are given as S_α against α the relative changes in the enzyme concentrations may be calculated directly from the values of α: $(E_{n+1} - E_n)/E_n = (\alpha_{n+1} - \alpha_n)/\alpha_n$ and $(E_{n-1} - E_n)/E_n = (\alpha_{n-1} - \alpha_n)/\alpha_n$ (see eqn. 2). In addition, the value of d_n may be plotted against α_n.

The arithmetic mean used above to calculate d_n may not be a good estimation when $E_{n+1} - E_n \neq E_n - E_{n-1}$. In this case we propose to use $d_n = [(E_{n+1} - E_n)d_{-1} + (E_n - E_{n-1})d_{+1}]/(E_{n+1} - E_{n-1})$. This weighted mean is equivalent to obtaining the value of d_n by linear interpolation between d_{+1} and d_{-1}. Here the enzyme concentrations may also be substituted by the corresponding α values without changing the results.

There is a link between the values of the ordinates in a PPS-plot and the summation relationships of control analysis. This is given in the Appendix. The construction of these plots is illustrated in section 11.

11. EXAMPLE

Here we show, by way of simulation, how the proposed plots can be used to test and quantify deviations. Although the example chosen is of a monomer–dimer equilibrium (eqn. 4 is violated), the same treatment can be applied to any of the deviations discussed in the preceding section.

We consider one metabolic pathway, whose structure is represented in Scheme 1. The first step is catalysed by an enzyme that presents a monomer–dimer equilibrium. X_0 and X_1 are the constant source and sink concentrations respectively. S is the only metabolite whose concentration is free to alter. The rate for the first step is:

$$v_1 = a_m \cdot M + 2 a_d \cdot D \qquad (25)$$

where a_m and a_d are the specific activities of the monomer and dimer subunits respectively. The total concentrations of monomer and dimer, M and D, appearing in eqn. (25), are given by $M = [-1+(1+8K_{app.}E_1)^{\frac{1}{2}}]/(4K_{app.})$ and $D = K_{app.}M^2$. E_1 is the total enzyme concentration expressed in monomer units ($E_1 = M + 2D$). $K_{app.}$ (the apparent equilibrium constant), a_m and a_d depend on the concentration, X_0, of the substrate X_0, and are independent of E_1. It should be noted that if $a_m = a_d$ then v_1 is proportional to E_1, and eqn. (4) is fulfilled. Here we consider situations where this is not the case.

The second step in Scheme 1 is catalysed by an irreversible Michaelis–Menten enzyme:

$$v_2 = k_{cat.}E_2\frac{S}{K_m + S} \qquad (26)$$

E_2 is the total enzyme concentration and $k_{cat.}$ and K_m are constants.

The time course of the metabolite concentration is obtained solving the differential equation:

$$\frac{dS}{dt} = v_1 - v_2 \qquad (27)$$

$$X_0 \xrightarrow{v_1} S \xrightarrow{v_2} X_1$$

Scheme 1.

where v_1 and v_2 are given in eqns. (25) and (26) respectively. Fig. 3(*a*) shows the reference time course, $\alpha = 1$, obtained for a particular set of reference parameter values (given in the legend to Fig. 3) and the time courses after application of the CCO using values of α different from 1. The corresponding curves (not shown) for the flux carried by the second step (flux 2) are obtained by substituting the instantaneous values of the metabolite concentration into eqn. (26).

The R-plots corresponding to Fig. 3(*a*) are shown in Fig. 3(*b*). The five curves in each R-plot do not coincide, and this fact is a positive test for the existence of deviations from the predicted relationship (eqns. 23). Similar results are obtained in the R-plot for flux 2 (not shown), which reveals significant deviations from eqn. (24). We here characterize and quantify these deviations at two different points of time, namely $\alpha t = 1$ and the steady state. The values $S_\alpha^{(1)}$, appearing in Table 1, are the ordinates corresponding to the abscissa $\alpha t = 1$ in the plot of Fig. 3(*b*). The values $J_\alpha^{(1)}$ are calculated from the ordinates $(J_\alpha^{(1)}/\alpha)$ corresponding to the abscissa $\alpha t = 1$ of the R-plot corresponding to flux 2 (not shown). The steady-state values (S_α^{ss} and J_α^{ss}) are the constant values attained after a 'long enough' time. From the steady-state values another time-invariant variable, namely the transition time of the system, can be calculated (Easterby, 1981, 1986): $\tau = S^{ss}/J^{ss}$. Table 1 shows how this value changes with α.

The PPS-plot, calculated from the data of Table 1, appears in Fig. 4. Here the deviations are different, for the different variables (concentration of S, flux 2 and τ) and αt ($\alpha t = 1$ and steady state, for time-dependent variables). Because of the values chosen for the parameters, the deviations are positive. In the case of the metabolite concentrations (S-type variables) positive deviations mean that, when the CCO is applied, the variable moves in the same direction as the enzyme concentrations. For the fluxes and the reciprocal of τ (J-type variables) positive deviations indicate that the change in the variable is greater than the proportional increase expected when the CCO is applied with α greater than unity. It is important to note that the deviation for S^{ss} increases with α, whereas the deviations for the other metabolite concentration and fluxes decrease. These properties of the PPS-plot

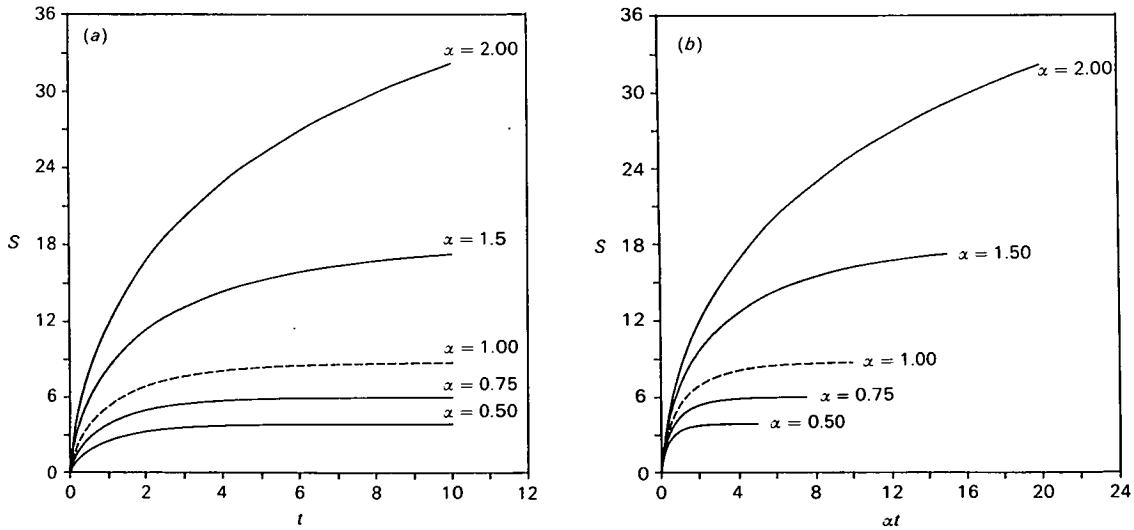

Fig. 3. Example where assumption eqn (4) is not valid, namely monomer–dimer equilibrium: (*a*) concentration of S versus time and (*b*) R-plot

In the example considered in section 11 the reference parameter values used to generate the reference time course ($\alpha = 1$, broken line) are $a_m = 1$, $a_d = 10$, $K_{app.} = 0.1$, $K_A = 3$, $E_1 = 4$ and $V_A = 22$ and the concentration of S at the initial time zero ($S^{in.}$) is zero. The same values for a_m, a_d, $K_{app.}$, K_A and $S^{in.}$ are used to calculate the curves for $\alpha = 0.5$, 0.75, 1.5 and 2.0, and the values of E_1 and V_A are multiplied by the corresponding α (see eqn. 2). The time courses for different α are given in (*a*). In (*b*) the same concentrations of S are plotted against α multiplied by time. The numerical simulations were carried out by using the program SCAMP (Sauro, 1986).

Table 1. Concentration of S and flux 2 (J) versus α

The concentration appearing in this Table may be obtained from Figs. 3(*a*) and 3(*b*). The plots for the fluxes are not shown. The values given correspond to the steady state, ss, and $\alpha t = 1$, (1). τ_α is equal to $S_\alpha^{ss}/J_\alpha^{ss}$.

α	S_α^{ss}	J_α^{ss}	$S_\alpha^{(1)}$	$J_\alpha^{(1)}$	τ_α
0.50	3.90	6.22	3.31	5.77	0.63
0.75	6.02	11.01	4.37	9.78	0.55
1.00	8.76	16.39	5.29	14.04	0.53
1.50	18.12	28.31	6.79	22.88	0.64
2.00	45.77	41.29	7.94	31.94	1.11

constitute a quantitative phenomenological description of the deviations.

In what follows, we analyse how the properties of the component rates (v_1 and v_2) affect the resulting behaviour of the variables when the CCO is applied. This analysis is based on infinitesimal changes ($\alpha \approx 1$) as used in control analysis. It provides us with an interpretation of the deviations appearing in Fig. 4. It can be shown that, in the simple example under consideration, the ordinates of the PPS-plot for the metabolite concentration may be estimated by:

$$D_S(t) = (\pi_{E_1}^{v_1} - 1)C_{v_1}^S \qquad (28)$$

Here, $\pi_{E_1}^{v_1} = (E_1/v_1)(\partial v_1/\partial E_1)$. This π elasticity is equal to unity when the rate v_1 is proportional to the enzyme concentration E_1. $C_{v_1}^S = (v_1/S)/(\partial S/\partial v_1)$ is the Control Coefficient. In general, this Control Coefficient is time-dependent, though in the limit it represents the usual steady-state value (see the Appendix and Kacser *et al.*, 1990). The analogous equation for flux 2 is:

$$D_{J_2}(t) = (\pi_{E_1}^{v_1} - 1)C_{v_1}^{J_2} \qquad (29)$$

It is important to note that eqns. (28) and (29) are valid in this particular example, because $\pi_{E_1}^{v_1}$ is independent of time (in a more general case they must be substituted by expressions that involve integrals). Therefore for this particular example the signs and magnitudes of the deviations in a PPS-plot depend on the product of two factors: (*a*) the sign and magnitude of the deviation of the rate $(\pi_{E_1}^{v_1} - 1)$ and (*b*) the sign and magnitude of the effect that a change in the rate has on the variable ($C_{v_1}^S$ or $C_{v_1}^{J_2}$). The values of these quantities are given in Table 2. In the case studied both factors (*a* and *b*) are positive, which results in a positive deviation in the PPS-plot. The deviation of the rate $(\pi_{E_1}^{v_1} - 1)$ decreases with α. $C_{v_1}^{J_2}$ ($\alpha t = 1$) shows the same tendency, whereas $C_{v_1}^{J_2}$ (steady state) is constant and equal to 1 (first step is irreversible) and $C_{v_1}^S$ ($\alpha t = 1$) increases slightly with α. This dependence on α explains the decreasing curves exhibited by the three variables under consideration (Fig. 4). On the other hand, $C_{v_1}^S$ (steady state) increases with α in such a way that the product $C_{v_1}^S$ $(\pi_{E_1}^{v_1} - 1)$ increases too, being the cause of the increase in the deviation with α in the PPS-plot. It should be pointed out that the increase in $C_{v_1}^S$ is due to an increase in the saturation of the second enzyme with α. However, as S is built up from zero, the saturation effect is not important at the early stages of the time course ($\alpha t = 1$).

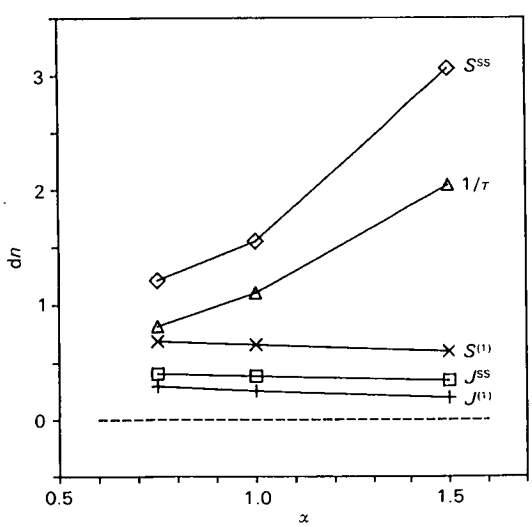

Fig. 4. PPS-plot, d_n against α, in a case where assumption eqn. (4) is not valid: behaviour of time-invariant and time-dependent variables

These plots are constructed by using the data from Table 1. The ordinates corresponding to d_n, S_α^{ss} (◇), $S_\alpha^{(1)}$ (×), J_α^{ss} (□), $J_\alpha^{(1)}$ (+) and $1/\tau_\alpha$ (△) are calculated as described in section 10. The broken line indicates the plot of all variables expected in the absence of deviations.

Table 2. Control Coefficients and deviations

The values of the parameters used to generate the coefficients appearing in this Table are given in Fig. 3. Deviations D_S and D_J were obtained by using eqns. (28) and (29). They are evaluated at two different points: $\alpha t = 1$ and ss (steady state). The values of d_n were obtained from the PPS-plot of Fig. 4. The numerical simulations were carried out by using the program SCAMP (Sauro, 1986).

	α	$C_{v_1}^S$	$C_{v_1}^J$	$\pi_{E_1}^{v_1} - 1$	D_S	d_n^S	D_J	d_n^J
ss	0.75	3.01	1.00	0.39	1.19	1.21	0.39	0.40
	1.00	3.92	1.00	0.37	1.45	1.55	0.37	0.38
	1.50	7.04	1.00	0.33	2.30	3.06	0.33	0.34
$\alpha t = 1$	0.75	1.73	0.70	0.39	0.68	0.68	0.28	0.29
	1.00	1.75	0.63	0.37	0.65	0.65	0.23	0.25
	1.50	1.76	0.54	0.33	0.58	0.59	0.18	0.19

It is important to note that, even though the values of α used to construct the PPS-plot are relatively large, the deviations calculated with eqns. (28) and (29), which are based on infinitesimal changes are in reasonable agreement (see Table 2) with the values of the ordinates, d_n, in Fig. 4, although it is recognized that this need not generally be the case.

Eqns. (28) and (29) illustrate that the existence of strong deviations from proportionality between rate and enzyme concentration (e.g. $\pi_{E_1}^{v_1} \gg 1$) may be irrelevant to the behaviour of a metabolic variable if the magnitude of the Control Coefficient ($C_{v_1}^Y$) is small. However, in other cases the deviation from proportionality in the rate equation may be greatly amplified if the variable shows a high value of the Control Coefficient (e.g. S^{ss} in the situation shown above).

12. DISCUSSION

The ideal CCO consists in the change of all the enzyme concentrations by the same factor, without any alteration in the other parameters of the experiment. [Exceptions are time-invariant input fluxes and time-dependent input metabolite concentrations and fluxes (see sections 4 and 5).] It now remains to discuss how far this operation can be applied to experimental systems. As always, there are special problems that will be encountered in particular applications.

The CCO may be applied to reconstituted systems. These

systems are built up by the use of component molecules that had been previously purified. They are, of course, much more simple than the biological systems that they are intended to mimic. However, they may give insight concerning, for example, the main components and conditions needed to obtain a certain behaviour [see, e.g., Eschrich *et al.* (1980), oscillations; Torres *et al.* (1989), transition time; Salerno *et al.* (1982), transition time]. The composition of a reconstituted system is known and under the control of the experimentalist. Therefore the CCO may, in principle, be applied to reconstituted systems in a simple way. These seem to be the most immediate experimental applications.

In the case of a biological extract, many aspects of the composition are probably not known, which makes it more difficult to apply the CCO. One way to approach this goal might be to take a fraction of the extract and make a complete enzyme inactivation (e.g. denaturation by heating or proteolytic enzymes). By mixing the original extract and the one subjected to inactivation in different proportions, we might obtain dilutions of the active enzyme concentrations without altering the concentrations of the other components of the system. In the ideal conditions the inactivator and the products of inactivation must not react with non-enzymic components of the system. It is evident that the agent used to inactivate the enzymes should be totally removed before the mixing is done. If there is considerable enzyme inactivation during the experiments (spontaneous or induced by unremoved inactivator), the total enzyme concentrations may not be treated as parameters and will constitute a source of deviations. An alternative method consists of successive dilutions of the extract, which would decrease all enzyme concentrations by the same factor. It is, however, necessary to supplement with all the metabolites that are not generated in the system in order to maintain the original concentrations. Such an attempt was made by Das & Busse (1985) in studying glycolytic oscillations in yeast extracts. Although the $[NAD^+]+[NADH]$ and the $[ATP]+[ADP]+[AMP]$ were maintained constant, other cofactors may have been altered by the dilutions. The PPS-plot for the period, which can be constructed with the data obtained from the above publication, shows both positive and negative deviations. This suggests a change of sign of the Control Coefficient, but, in view of the experimental difficulties referred to above, this interpretation may be questionable. If it is desired to extrapolate from experiments on biological extracts to the situation *in vivo*, it is important to note that in the preparation of the extract a dilution takes place. In so doing the quantitative importance of the deviations may be modified.

The application of the CCO to a system *in vivo* appears to be difficult. One might think that the use of, for example, haploid, diploid and tetraploid yeast cells could be a way to achieve this goal. However, in these series the volume increases proportionally to the gene ploidy, leaving most of the enzyme concentrations approximately unchanged (Mortimer, 1958; Ciferri *et al.*, 1969). On the other hand, some enzyme concentrations (e.g. enzymes bound to membranes) may suffer significant changes (Hilger, 1973). This situation is therefore far from what we define as CCO.

A method where enzyme concentrations can be manipulated *in vivo* consists in using conditions when co-ordinate repressions/inductions of pathways occur. These are well known in both fungal and bacterial micro-organisms. By definition the CCO requires the concentrations of all enzymes in the system to be simultaneously altered, and this is certainly not the case in the above systems. Nevertheless, such studies may approach the requirements of a CCO if the system outside the pathway does not interact significantly with it when such repressions/inductions are effected. It is an almost universal observation that single null mutants in one pathway do not impose double (or multiple) requirements on other pathways. This argues against important interactions between pathways. The absence of such interactions, however, will have to be established rigorously or the system will have to be manipulated to eliminate them. An approach to this has been achieved in studying co-ordinate de-repression in the arginine pathway of *Neurospora crassa* (Stuart *et al.*, 1986). Introduction of a regulatory mutant (*cpc-1*) decreases the concentrations of the enzymes by about 3-fold compared with their 'basal' (reference) concentrations in the wild-type. The effects on the flux to arginine of this substantial factorial change, however, are virtually buffered by a strong negative feedback inhibiting an early enzyme of the pathway. When grown in minimal medium, a comparison of the two strains shows only a 16% decrease in the flux in the mutant. This feedback effect can, however, be abolished by growth on citrulline-supplemented medium, which effectively 'shortens' the pathway to the last three steps. When this is done, it is found that the 3-fold decrease in enzyme concentrations results in a 3-fold decrease in flux. In this instance, therefore, no evidence of deviations due to the last three enzymes is observed.

The non-existence of deviations, as a result of a CCO experiment, is informative by itself. This fact indicates that either the assumptions (eqns. 3 and 4) are fulfilled or their violation is unimportant concerning the behaviour of the variables. On the other hand, the discovery of deviations strongly suggests that the properties of one or more components of the network do not coincide with the assumptions made. Furthermore, a positive test for deviations shows that these features of the components have a significant effect on the behaviour of the variables when the enzyme concentrations are changed. The experimental design for performance of the CCO does not necessarily rely on a detailed knowledge of the structure of the metabolic system. However, if we want to have an interpretation of the deviations, the existing profuse amount of information concerning the structure of metabolic systems and the kinetic properties of its component reactions may be useful. This information (e.g. non-proportionality between a rate, v_i, and an enzyme concentration, E_i) may suggest candidates for the 'cause' of the deviations in a variable of the system (Y). To test the candidate, the values of the Control and Elasticity Coefficients (e.g. $C^Y_{E_i}$ and $(\pi^{v_i}_{E_i}-1)$ should be experimentally obtained, in the same conditions used when the CCO was applied (see section 11). We conclude that the CCO and co-ordinate-control plots may be used as a first approach to study the control properties of time-dependent metabolic systems. They constitute a possible way to obtain relevant information and may guide the design of later experiments, leading to a deeper understanding of how metabolic networks work.

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REFERENCES

Acerenza, L. (1990) in Control of Metabolic Processes (Cornish-Bowden, A. & Cardenas, M. L., eds.), pp. 251–257, Plenum Press, New York
Acerenza, L., Sauro, H. M. & Kacser, H. (1989) J. Theor. Biol. **137**, 423–444
Ciferri, O., Sora, S. & Tiboni, O. (1969) Genetics **61**, 567–576
Cornish-Bowden, A. (1979) Fundamentals of Enzyme Kinetics, Butterworths, London
Das, J. & Busse, H.-G. (1985) J. Biochem. (Tokyo) **97**, 719–727
Easterby, J. S. (1981) Biochem. J. **199**, 155–161
Easterby, J. S. (1986) Biochem. J. **233**, 871–875

Eschrich, K., Schellenberger, W. & Hofmann, E. (1980) Arch. Biochem. Biophys. **205**, 114–121
Fell, D. A. & Sauro, H. M. (1990) Eur. J. Biochem., in the press
Goldbeter, A. & Moran, F. (1987) in The Organization of Cell Metabolism (Welch, G. R. & Clegg, J. S., eds.), pp. 291–305, Plenum Press, New York
Heinrich, R. & Rapoport, T. A. (1974) Eur. J. Biochem. **42**, 89–95
Heinrich, R. & Rapoport, T. A. (1975) BioSystems **7**, 130–136
Hess, B. & Markus, M. (1987) Trends Biochem. Sci. **12**, 45–48
Hilger, F. (1973) J. Gen. Microbiol. **75**, 23–31
Hofmann, E., Eschrich, K. & Schellenberger, W. (1985) Adv. Enzyme Regul. **23**, 331–362
Kacser, H. (1987) in The Organization of Cell Metabolism (Welch, G. R. & Clegg, J. S., eds.), pp. 327–337, Plenum Press, New York
Kacser, H. & Burns, J. A. (1973) Symp. Soc. Exp. Biol. **27**, 65–104
Kacser, H., Sauro, H. M. & Acerenza, L. (1990) Eur. J. Biochem. **187**, 481–491
Kurganov, B. I. (1978) Allosteric Enzymes: Kinetic Behaviour, Jol Wiley and Sons, Chichester
Markus, M., Kuschmitz, D. & Hess, B. (1984) FEBS Lett. **172**, 235 238
Mizraji, E., Acerenza, L. & Hernandez, J. (1988) BioSystems **22**, 11–1
Mortimer, R. (1958) Radiat. Res. **9**, 312–326
Neet, K. E. & Ainslie, G. R., Jr. (1980) Methods Enzymol. **64**, 192–22
Salerno, C., Ovádi, J., Keleti, T. & Fasella, P. (1982) Eur. J. Biochem **121**, 511–517
Sauro, H. M. (1986) Ph.D. Thesis, Oxford Polytechnic
Sauro, H. M. & Kacser, H. (1990) Eur. J. Biochem. **187**, 493–500
Segel, L. A. (1988) Bull. Math. Biol. **50**, 579–593
Selwyn, M. J. (1965) Biochim. Biophys. Acta **105**, 193–195
Stuart, F., Porteous, D. J., Flint, H. J. & Kacser, H. (1986) J. Ger Microbiol. **132**, 1159–1166
Torres, N. V., Souto, R. & Meléndez-Hevia, E. (1989) Biochem. J. **260** 763–769

APPENDIX

Relationship of the CCO to control analysis: summation relationships

(*a*) The assumptions of eqns. (3) and (4) of the main paper are valid

Let us consider a time-invariant variable Y. A small relative change in Y, dY/Y, originated by simultaneous small relative changes in all the enzyme concentrations, $dE_j/E_j(=\alpha-1)$, can be written as the sum of the contributions of each individual enzyme:

$$\frac{dY}{Y}=\sum_{j=1}^{m} C_{E_j}^{Y}\cdot\frac{dE_j}{E_j} \qquad (A1)$$

where $C_{E_j}^{Y}$ is the Control Coefficient of the variable Y, by the enzyme concentration E_j, and it is defined as follows:

$$C_{E_j}^{Y}\equiv\frac{E_j}{Y}\cdot\frac{\partial Y}{\partial E_j} \qquad (A2)$$

When the changes in E_j correspond to a CCO (see eqn. 2) $dE_j/E_j=\alpha-1$ (for $j=1,\ldots,m$). If the assumptions introduced in eqns. (3) and (4) of the main paper are valid, the relative change in the variable, dY/Y, is zero in the case of an S-type time-invariant variable, S, and $\alpha-1$ for a J-type time-invariant variable, J (see eqns. 21 and 22 of the main paper). Introducing these results into eqn. (A1), we obtain:

$$\sum_k C_{E_k}^{S}=0 \qquad (A3)$$

$$\sum_k C_{E_k}^{J}-1=0 \qquad (A4)$$

Eqns. (A3) and (A4) are the summation relationships for S-type and J-type time-invariant variables respectively. Examples of these variables are given in part A of section 7 of the main paper. Particular cases of eqns. (A3) and (A4) are the summation relationships for the Control Coefficients of the steady-state metabolite concentrations and fluxes (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). The reciprocal of a time-invariant variable with dimension of time, $1/T$, fulfils eqn. (A4). Note that $C_{E_k}^{1/T}=-C_{E_k}^{T}$ and therefore, for these variables:

$$\sum_k C_{E_k}^{T}=-1$$

This general relationship was previously obtained for particular definitions of transition time (see Heinrich & Rapoport, 1975; Torres *et al.*, 1989; Meléndez-Hevia *et al.*, 1990), but is general for any variable that obeys the transformation eqn. (10) of th main paper.

The summation relationships for the Control Coefficients o S-type and J-type time-dependent variables can be written a follows:

$$\sum_k C_{E_k}^{S}-T^{S}=0 \qquad (A5$$

$$\sum_k C_{E_k}^{J}-1-T^{J}=0 \qquad (A6$$

The 'Time Coefficients' T^S and T^J are defined by $T^Y=(t/Y)/(\partial Y/\partial t)$, where Y stands for S or J (see Acerenza *et al.* 1989).

(*b*) Deviations from the assumptions of eqns. (3) and (4) of the main paper

Eqns. (A3)–(A6) are derived by using the assumptions introduced in eqns. (3) and (4) of the main paper. If these assumptions are not fulfilled, the left-hand members of eqns. (A3)–(A6) are not equal to zero. It may be shown that, when the CCO is applied with small changes ($\alpha\approx 1$), those left-hand members are not equal to zero but are approximately equal to a deviation term (D) given by eqns. (A7)–(A10) respectively:

$$D_S=\frac{\left(\frac{S_\alpha-S_r}{S_r}\right)}{\alpha-1} \qquad (A7)$$

$$D_J=\frac{\left(\frac{(J_\alpha/\alpha)-J_r}{J_r}\right)}{\alpha-1} \qquad (A8)$$

$$D_S(t)=\frac{\left(\frac{S_\alpha(\alpha t_\alpha=t)-S_r(t)}{S_r(t)}\right)}{\alpha-1} \qquad (A9)$$

$$D_J(t)=\frac{\left(\frac{[J_\alpha(\alpha t_\alpha=t)/\alpha]-J_r(t)}{J_r(t)}\right)}{\alpha-1} \qquad (A10)$$

It is important to note that these D values are the better approximations to the left-hand members of eqns. (A3)–(A6) the closer α tends to 1.

The deviations given in eqns. (A7)–(A10) are those plotted against α in a PPS-plot (see section 10 of the main paper). The only difference is that in the PPS-plot we use the mean between positive and negative $\alpha-1$ values to compensate (partially) the

error introduced by the use of relatively large changes. It is easy to show that all D values are zero when the assumptions eqns. (3) and (4) of the main paper apply.

REFERENCES

Acerenza, L., Sauro, H. M. & Kacser, H. (1989) J. Theor. Biol. **137**, 423–444

Heinrich, R. & Rapoport, T. A. (1974) Eur. J. Biochem. **42**, 89–95

Heinrich, R. & Rapoport, T. A. (1975) BioSystems **7**, 130–136

Kacser, H. & Burns, J. A. (1973) Symp. Soc. Exp. Biol. **27**, 65–104

Meléndez-Hevia, E., Torres, N. V., Sicilia, J. & Kacser, H. (1990) Biochem. J. **265**, 195–202

Torres, N. V., Souto, R. & Meléndez-Hevia, E. (1989) Biochem. J. **260**, 763–769

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