Control of Metabolic Processes

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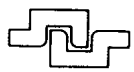

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Preface

THIS BOOK collects together papers given at a NATO Advanced Research Workshop held at Il Ciocco (Lucca), Italy, from the 9th to the 15th April, 1989. It sets out to present the current state of understanding of the principles governing the way fluxes and concentrations are maintained and controlled in metabolic systems. Although this is a topic that has held the interest of biochemists for many years, it is only quite recently that the methods of analysing the kinetics of multi-enzyme pathways developed over the past two decades have come to be widely discussed or applied experimentally. Many biochemists remain sceptical that the new methods offer a real advance (except in complexity) over the landmark discoveries of the 1950s and 1960s relating to inhibition of enzymes at branch-points by the end products of metabolic pathways, and the interpretation of allosteric effects and cooperativity.

Even those who have become convinced that the classical ideas provide only the starting point for understanding metabolic control have been by no means unanimous in their assessment of the direction in which one should advance. In this book we have tried to include all of the current points of view, including the view that the classical theories tell us all that we need to know. We have not seen it as our role as editors to paper over the cracks that exist and to pretend that we can speak to the world with one voice. Nonetheless, at the Workshop that this book records we did try to resolve some of the controversies that were apparent, for example, in the pages of *Trends in Biochemical Sciences* in 1987, and we hope that some progress towards such resolution may be evident in the book.

The Prologue is based on a paper written by Daniel Atkinson without any intention of publication but circulated before the Workshop in the hope of stimulating discussion and focussing the attention of participants on some issues that he believed needed to be addressed. It proved highly successful in doing this, and stimulated a number of additional discussion papers, all of which are also included in the Prologue. We emphasize that this part of the book was written before any of the other chapters and that it has not been edited to take account of anything that may have been written later.

The next part of the book is concerned with general aspects of metabolic control analysis, including discussion of historical and philosophical aspects as well as description of the somewhat different approaches that have developed in Europe and the USA, which cannot yet be considered to have reached a synthesis. This is followed by several chapters dealing with the mathematical basis of control analysis.

Enzymes possessing two forms with different catalytic activity that can be interconverted by covalent modification reactions form a special category of enzymes that need to be studied in relation to metabolic control. The number of experimental examples of these continues to grow rapidly, and we include several chapters discussing their different aspects.

These are followed by some chapters dealing with methods that have been developed for

applying the ideas of control analysis to experimental systems. These methods will, we hope, help to dispel the idea that metabolic control analysis is an abstract subject with little relationship to "real" biochemistry. Control-pattern analysis, discussed in the last chapter of this section, may perhaps in time come to provide the same degree of intuitive understanding of metabolic control that the method of King and Altman has given to enzyme mechanisms.

Direct "channelling" of intermediates from one enzyme to the next in a pathway, other kinds of interactions between enzymes, failure of rates to be strictly proportional to enzyme concentrations, and the fact that real systems do not always operate in the steady state, are all complications that need to be considered before any simple theory of metabolic control can be accepted as providing the whole story. To some degree these complications overlap; in other respects they are quite distinct: we have found it convenient to group together the chapters discussing these various ways in which nature makes real systems more complicated than one might have hoped.

Although in most of these earlier chapters there are sufficient references to experimental systems to show that metabolic control analysis is more than a preserve of pure theory isolated from the real world, the relation between metabolic control analysis and "wet" biochemistry becomes more evident in the final group of chapters in the book. Here it will be seen that the ideas of control analysis are now being applied to all of the classical problems that have engaged the attention of biochemists — erythrocyte metabolism, photosynthesis, amino acid metabolism, and so on. Even gene expression is included, a topic that many of us might have thought too difficult to be yet accommodated in a mathematical treatment of metabolic control. The range of applications will certainly increase in the years to come, and we suspect that it will eventually be thought odd that biochemists could ever have thought that these problems could be addressed without the aid of control analysis.

Readers who see any merit in the lay-out of this book may like to know that it was printed in its entirety from camera-ready copy prepared on an Apple LaserWriter II NT driven by an Apple Macintosh Plus computer: the text was edited using WriteNow for Macintosh (version 2.0, T/Maker Company, Mountain View), and the mathematical expressions were laid out with Expressionist (version 2.0, Allan Bonadio Associates, San Francisco).

We are very grateful to the Scientific Affairs Division of NATO for the grant that made possible the Workshop on which this book is based, and to Dr Craig Sinclair, the Director of the Advanced Research Workshop programme, for his help and encouragement in the organization. Additional financial support provided by various other bodies was also much appreciated, as it enabled the participation of several scientists who would otherwise have been unable to attend the Workshop. In this connection, we thank Dr Paolo Fasella, for a grant from the Commission of the European Communities; Dr Minor S. Coon, for travel grants from the International Union of Biochemistry and the American Society for Biochemistry and Molecular Biology; and Dr Christopher I. Pogson, for a grant from the Wellcome Foundation Ltd.

Many participants in the Workshop have commented to us afterwards how much they enjoyed the time that they spent at Il Ciocco. A major part of the credit for this must go to Mr Bruno Giannasi and his staff, who went far beyond mere obligation in ensuring that everyone was well provided for. We thank them most sincerely. If only we could have had as much success in planning the weather on the half-day excursion ...

We are grateful to Jacques Ricard for suggesting the Workshop in the first place, and to him and the other the members of the Organizing Committee — Albert Goldbeter, Tamás Keleti and Hans Westerhoff — for many useful suggestions and other invaluable help, and to Brigitte Gontero for all of her assistance as Meeting Secretary — before, during and after the Workshop. We also thank the many authors who delivered their text in machine-readable form, thereby reducing the amount of retyping that we had to do. For the chapters that did need to be retyped, we thank Mme Monique Payan for her highly professional work.

We record with great sorrow that Tamás Keleti died suddenly during the period in which the book was in preparation. Many will remember him for his tireless efforts on behalf of enzymology in Hungary; he will be sadly missed, not only by his colleagues there, but also by many enzymologists around the world, and especially by our daughter Isadora.

Athel Cornish-Bowden
María Luz Cárdenas

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PROLOGUE

Prologue

What Should a Theory of Metabolic Control Offer to the Experimenter?

DANIEL E. ATKINSON

IN HIS PREPARATIONS for this symposium, Athel Cornish-Bowden mentioned a desire to present control theory to experimentalists in such a way as to persuade them that their subject could advance more rapidly with more attention to theoretical ideas. He is also inviting some experimentalists to indicate what they think a useful theory should offer, and has asked me to attempt to assess in the final chapter the extent to which the others have offered experimentalists a workable approach to metabolic control.

I applaud this attempt to bring theorists and experimentalists together not only physically but intellectually. However, I remember all too well previous meetings of this kind that I have attended, in which theorists and experimentalists talked past each other with little or no effective interaction. I am concerned that there might not be much for me to say at the end except to deplore the fact that we had once again done so.

In an attempt to avoid finding myself in that unhappy situation, I am setting forth here some of the points that experimentalists wish theorists would consider. I hope that our chances of meaningful interaction will be enhanced if some participants consider these points in advance.

Firstly, and encompassing many of the other points, what experimentalists desire from theorists is relevance. Many model builders appear to believe that control theory is a discrete area of importance in itself, and that it is unnecessary, if not intellectually demeaning, to take the features of actual systems into account in the design of the models. However, even if an autonomous body of concepts does exist, those concepts are of no use to people dealing with an actual experimental or engineering system until they are adapted to relate to specific features of the system. A steam engine, an internal-combustion piston engine, a Wankel rotary internal combustion engine, a gas or water turbine, and an electric motor all generate power and transmit it by way of a rotating shaft. There may be some rarified level of control theory that applies to all of them. But a theory that ignores the existence of valves, an

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ignition system, and mechanisms for introduction of fuel into the cylinders will not be of any practical value to a design engineer who works on piston engines. Similarly, models that ignore the properties of enzymes cannot be helpful to experimental biochemists.

Enzymes are saturable. Their effective kinetic orders change with the degree of saturation. Most enzymes are non-regulatory in the usual sense, and their behaviour is adequately described by the familiar Michaelis equation. In such cases, the momentary kinetic order equals 1 - v/V_{max}. Regulatory enzymes typically have higher, but still variable, kinetic orders. Some of the more mathematically-oriented models ignore the fact that all real metabolic reactions are catalysed by enzymes, and assume reactions of invariant kinetic order one. Since regulation is necessarily a kinetic phenomenon, models that begin with incorrect assumptions as to the kinetic nature of the reactions cannot be useful.

Consideration of the patterns of metabolic conversions seems to indicate clearly that metabolic control is effected in large part by changes in the properties of enzymes that compete for substrates at branchpoints. On the basis of known metabolic patterns and the behaviour of specific enzymes in kinetic studies *in vitro*, it seems to be well established that the outcomes of such competitions at branchpoints are regulated by changes in the affinities of the enzymes for the branchpoint metabolite. No model that does not incorporate those aspects of real metabolic systems is likely to have sufficient relevance to be potentially useful to experimentalists or to contribute to understanding of metabolic control.

Designers of mathematical models tend to aim for generality. Some describe their models as capable of dealing with all possible situations, and it is even claimed by some workers that they begin from first principles. Such aims are illusory and unattainable, and they may lead model designers away from concern with real properties of real systems.

It has been clear for 130 years, since publication of *The Origin of Species*, that there are no general principles, of the type sought by some model builders, in biology. The properties of organisms have been determined historically by selection of beneficial changes from among the vast range of choices made available by random mutation. It is totally and unequivocally impossible to predict the consequences of a long evolutionary history from first principles, or indeed from any mathematical, physical, or chemical principles whatever. Chemical and physical considerations constrain the possibilities — metabolic processes, like all others, must entail a decrease in Gibbs free energy if they occur at constant temperature and pressure, for example — but they cannot lead to specific predictions. The strange sequence of reactions by which glucose is oxidized to carbon dioxide in typical living cells, by way of phosphorylated intermediates and a series of di- and tricarboxylic acids, is a consequence of evolutionary history and could not, even in principle, be predicted. The same considerations apply to the use of the ATP-ADP-AMP pool as the primary transducer of metabolic energy, and to thousands of other features of metabolism. Mechanisms of correlation and control have evolved along with the sequences themselves, and hence are also shaped by the blind trial-and-error processes of evolution. Each enzyme has evolved independently (although in most cases probably by modification from a pre-existing enzyme), and both its catalytic activities and its regulatory characteristics are consequences of its own history rather than features imposed by a grand design. The evolution of each enzyme was guided and constrained by the evolving properties of many others, but such

influences were exerted by functional selection of the whole organism rather than by any general integrating plan. Models, if they are to be potentially useful in the study of metabolic regulation, must follow that same pattern of building up from actual functional relationships rather than attempting to impose a system that is derived from cogitation and based on what are taken to be basic principles.

I think that the most important thing that experimentalists have to say to theorists is to urge them to accept the universe rather than redesign it.

Specific Comments

Kacser & Burns (1973), Heinrich & Rapoport (1974) and their colleagues deserve much credit for emphasizing that enzymes interact in the cell, and that pathways must be considered as functional units. However, they do not emphasize what is possibly the most functionally important type of such interactions, that between the initial enzymes of different pathways that compete for common metabolites. Nor does this model incorporate the fact that most regulatory modifications of enzyme action involve changes in the affinities of enzymes for substrates rather than in catalytic activities or maximal velocities. Similar comments apply to the other major models. Incorporation of those features of real enzymes into models would vastly increase the likelihood that they could prove useful to experimentalists.

More disturbing to experimentalists than models that seem to be irrelevant because of inappropriate assumptions are those that reach specific conclusions about regulation, purportedly on the basis of rigorous analysis, that are self-evidently incorrect. Two recent examples will be offered as illustrations.

Crabtree & Newsholme (1987) maintain that the first enzyme of a metabolic sequence must be saturated with its substrate. That surprising conclusion is based on the argument that the concentration of the initial substrate of the sequence will fall continuously, and that saturation of the first enzyme is a means by which flux through the sequence can be independent of that concentration change. The fact that all of us have survived for decades rather than hours is convincing evidence that concentrations of initial reactants (or any others) do not fall continuously. Metabolism is a quasi-steady-state system. Compounds, such as glycogen and fats, that are cyclically stored and depleted in response to varying rates of supply and demand for energy are insoluble, so that their chemical activities do not change as they are generated or degraded. (The location of phosphorylase in the glycogen granule probably eliminates even the small effect that might be thought to result from a decrease in the surface area of the glycogen particle that is available to the enzyme.) Not only is the premise on which the conclusion is based invalid, the conclusion flies in the face of nearly all of our knowledge of metabolic organization. As well be discussed later, it seems to be well established that the degree of saturation of the first enzyme is the most important determinant of the flux through most metabolic pathways.

A recent short paper (Sauro & Fell, 1987) points out that variation in the amount of an enzyme that exhibits zero-order kinetics would control the rate of the sequence in which it

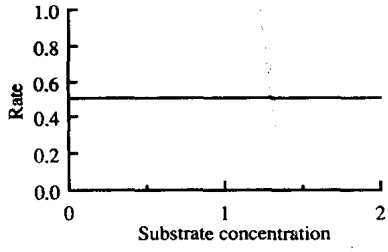

Figure 1. Rate as a function of substrate concentration for a hypothetical enzyme of kinetic order zero.

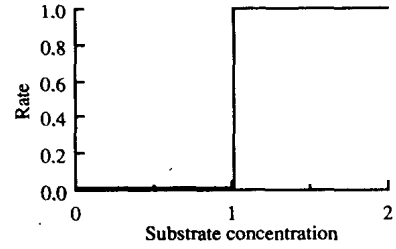

Figure 2. Rate as a function of substrate concentration for a hypothetical enzyme of kinetic order infinity.

occurred, and that variation of the activity of an enzyme that exhibited infinite cooperativity (kinetic order infinitely large) would not have any effect on flux. In their words, the flux control coefficient for the enzyme tends to 1 as the kinetic order approaches zero and tends to zero as the order approaches infinity. They say that they arrived at this conclusion by use of the rigorous theory of Kacser & Burns (1973), implemented in a computer model using a special simulator language, and note that they could alternatively have supplied a formal mathematical proof.

Neither of those approaches was necessary, however. The conclusions are intuitively evident, and can be supported as rigorously by a simple graphical argument as by formal mathematical analysis or computer models. The rate-vs-concentration curves for the (unattainable) boundary conditions of kinetic order zero and of order infinity are shown in Figs. 1 and 2. Zero order (Fig. 1) means that the velocity of the reaction is equal at all finite concentrations of substrate (it must, of course, be zero at zero concentration). An infinitely high order (Fig. 2) means that the concentration of substrate is equal at all rates of reaction. The $S_{0.5}$ value and the saturating concentration are identical in this extreme case. The rate will increase or decrease in such a way as to hold the substrate concentration invariant. It follows that the rate of a reaction catalysed by an enzyme of zero kinetic order will depend only on the catalytic activity of the enzyme. If a sequence with no branches or additional points of input is at steady state, the rate of each reaction is equal to the system flux; thus the amount (or catalytic activity) of a zero-order enzyme would uniquely determine flux. (That would be nearly true also for a real enzyme that was nearly saturated, since the kinetic order of a real enzymic reaction approaches zero as the velocity of the reaction approaches V_{max}.) A large change in the amount of an enzyme of infinite kinetic order (Fig. 2) would not affect the rate of the reaction at all (as long as V_{max} exceeded the flux). Thus such changes could not affect flux through the sequence; the flux control coefficient of the enzyme, as defined by Burns *et al.* (1985), would be zero. For enzymes of high kinetic order, the actual order changes sharply with small changes in rate; if the order were infinite, the change would be abrupt. If the amount of the enzyme were reduced continuously, there would be a point at which the potential rate of supply of substrate exceeded the V_{max} of the enzyme, and at that point the kinetic order would change instantaneously to zero and the flux control coefficient would jump from zero to 1.

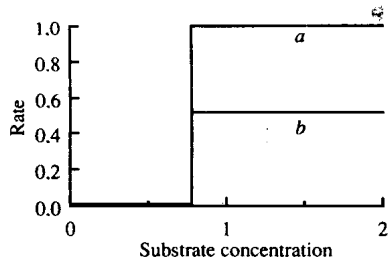

Figure 3. Rate as a function of substrate concentration for a hypothetical enzyme of kinetic order infinity with maximal velocity modulated by a negative modifier metabolite or effector. *a*, rate in absence of modifier; *b*, rate in presence of modifier.

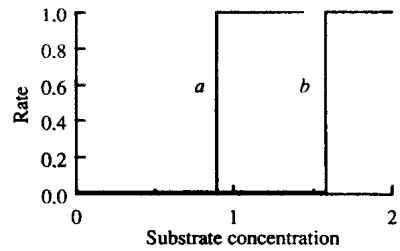

Figure 4. Rate as a function of substrate concentration for a hypothetical enzyme of kinetic order infinity with the affinity of the enzyme for substrate modulated by a negative modifier metabolite or effector. *a*, rate in absence of modifier; *b*, rate in presence of modifier.

From those simple kinetic considerations, Sauro & Fell (1987) point out correctly that enzymes that display positive cooperativity cannot simultaneously have high flux control coefficients as defined. They further assume that this means that such enzymes cannot control fluxes through sequences. They note that this conclusion is in complete contrast to the traditional point of view, according to which allosteric enzymes, which usually possess cooperative kinetics, are the sites of highest flux control.

This example illustrates how the use of erroneous assumptions can lead to egregiously erroneous conclusions. The conclusion that an enzyme with high kinetic order could not have an important effect on flux would be valid if the enzyme were regulated by modulation of its maximal velocity (Fig. 3). A 50% decrease in V_{max}, from *a* to *b*, would have no effect on flux, as long as the new value of V_{max} exceeded the flux allowed by other components of the sequence.

However, as far as I know, no enzyme that catalyses a reaction of high kinetic order has been observed to be regulated by modulation of V_{max}. They all respond to modifiers by changes in the value of $S_{0.5}$, the concentration of substrate at which the reaction velocity is half of that at saturating concentration. (For reasons of logical consistency and dimensional accuracy, the symbol K_m is not appropriate for enzymes with kinetic orders either larger or smaller than 1.) If the $S_{0.5}$ value of the hypothetical infinitely cooperative enzyme is increased by a factor of 2 (from *a* to *b* in Fig. 4), the reaction will stop completely. If the sequence were sealed off from either gains or losses from or to the outside, and if this enzyme catalysed a reaction late in the sequence, as assumed by Sauro & Fell (1987), the concentration of substrate would merely build up to the new value of $S_{0.5}$, after which the enzyme would again have no effect on flux. But no known metabolic sequence is like that. Enzymes with high-order kinetics are always, as far as I know, immediately at branch points (or are one step away from a branchpoint and are linked to the branchpoint by a reaction that is in equilibrium, so that in effect the regulatory enzyme is at the branchpoint even in such cases). If the concentration is at a value between the vertical parts of curves *a* and *b* in Fig. 4, it is obvious that a small change in $S_{0.5}$ can cause the flux to change from zero to V_{max}.

Rather than the step function illustrated in Figs. 3 and 4, real regulatory enzymes exhibit sigmoid curves of rate as a function of substrate concentration, as in Fig. 5. The orders are

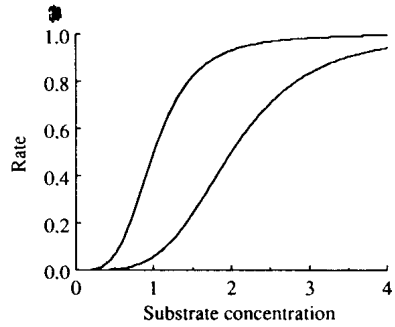

Figure 5. Rate as a function of substrate concentration for an enzyme that binds substrate with a high degree of cooperativity to four catalytic sites, with the affinity of the enzyme for substrate modulated by a negative modifier metabolite or effector. *a*, rate in absence of modifier; *b*, rate in presence of modifier.

typically between 2 and 4; the curves of Fig. 5 are calculated for fourth-order reactions. It is evident that small changes in $S_{0.5}$ can still cause very large changes in reaction rate in such cases. Although the flux control coefficient as defined by Burns *et al.* (1985) is low for such enzymes (since a change in amount of enzyme would have little effect on flux), the flux of the sequence is probably controlled almost entirely by modulation of the $S_{0.5}$ value for the first enzyme. The flux control coefficient of an enzyme, defined in terms of changes in V_{max} or amount of enzyme, is almost entirely unrelated to its regulatory importance — that is, to the extent to which it actually controls flux. Sauro & Fell's erroneous conclusion resulted from their assumption that flux control coefficients as defined in the model are measures of the extent of control. Since very few enzymes are modulated by change in V_{max} or catalytic activity, which is the parameter on which flux control coefficients are based, an enzyme might have a flux control coefficient close to zero but still exert nearly 100% of the control of the flux through the sequence. That indeed is probably the usual situation for nearly all enzymes of regulatory importance. It is noteworthy that high kinetic order, the feature that allows an enzyme to function as a powerful and sensitive determinant of flux when its $S_{0.5}$ is modulated, is the same property that causes an enzyme to have a low value of the flux control coefficient of Burns *et al.* (1985), which is based on the assumption that V_{max} is the modulated parameter.

A kinetic order of zero, or some low finite value, would be useful for stabilizing rates at the expense of wide excursions in concentrations. A high kinetic order is useful for minimizing changes in concentrations while allowing for large fluctuations in flux. Since the need in metabolism, as well as the observable reality of metabolic regulation, is for velocities to be regulatable over wide ranges, as in the transition between rest and heavy exercise, while concentrations are stabilized closely to avoid disruptions of related sequences, it is obvious why regulatory enzymes in general are characterized by high kinetic orders.

Because they are located at the beginnings of sequences (that is, at branchpoints) and because they are regulated by modulation of $S_{0.5}$ rather than V_{max}, high-order enzymes are the main control elements of metabolic sequences. The fact that the erroneous conclusion of Sauro & Fell (1987) was reached with the aid of the model of Kacser & Burns (1973) illustrates the fundamental differences between the assumptions of that model and the characteristics of real metabolic regulatory systems. The assumption that all enzyme modulations are equivalent to changes in amount of enzyme sharply differentiates that model, and also many others, from actual metabolic systems.