Analytical Physiology of Cells and Developing Organisms

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Dedicated to

PROFESSOR C. H. WADDINGTON, F.R.S.

Ah, but a man's reach should exceed his grasp,
or what's a heaven for?

"Andrea del Sarto"
R. Browning

PREFACE

My intention in writing this book has been to construct a picture of the physiological behaviour of cells and developing organisms based upon the insights of molecular biology on the one hand and mathematical model-building on the other. The use of the term analytical physiology derives from this two-fold basis, analysis being understood in both its experimental and its mathematical senses, with complementary interaction between them. The first three chapters of the book are dominated by the view of cell behaviour in terms of the control metaphor, wherein homeostasis and adaptation are regarded primarily as manifestations of positive and negative feedback processes, and the stability of the living system is interpreted in informational rather than in energetic terms. This view emerged during the 1950s and developed into a golden age of control theory and cybernetics in biology, but it is now undergoing a rapid transformation into something else, not yet named. This may be the flowering of an indigenously biological metaphor which is dominated by the concepts of structure, form and transformation rather than of purely dynamical stability. This would seem to be an appropriate development in biology, a subject in which morphology has always been a primary feature. The tendency to denigrate comparative anatomy and descriptive embryology during the past few decades is consistent with an analytical approach to the organization of living systems, and was a necessary part of the development of molecular biology. The success of this approach is extremely impressive, and constitutes one of the most exciting developments in the history of biology.

However, the picture of the organism which we get from molecular biology and the control metaphor has its limitations, and these become progressively more evident as the book proceeds. One can get away with very little reference to structure and morphology as long as one is concerned with the purely homeostatic and adaptive properties of cells, although even here it soon becomes apparent that size and shape must be taken into account, if only minimally. When one comes to study the stability of tissues and the phenomena of regeneration and morphogenesis, however, form becomes a primary aspect of the problem and the dynamical aspects of physiology become extended and organized in space. From Chapter 4 onwards, the consideration of such spatio-temporal relationships becomes

progressively prominent. The dynamical models of developmental processes considered in Chapters 5 and 6 still derive from molecular biological postulates, but diffusion and wave propagation begin to emerge as important space-ordering processes. The study of morphogenetic mechanisms is one which is now developing very rapidly, and we shall certainly witness a dramatic transformation of viewpoint in the next few years from the relatively static space-ordering models which still tend to dominate experimental study in this field, to dynamic and flexible space–time processes, some of whose properties I attempt to anticipate in these chapters.

In an effort to unify the ideas developed throughout the book and to provide a context within which they can be transcended and linked with other areas of study which I think have natural affinities with developmental biology, I explore in the last chapter an approach to the study of organismic behaviour in which I attempt to combine a phenomenological attitude with an analytical one, rather in the spirit of contemporary structuralism. Here the organism is regarded as a cognitive system, adapting and evolving on the basis of knowledge about itself and its environment. The dynamical modes of an organism's behaviour are represented as manifestations of co-operative or collective activity among cognitive units, development being seen as the orderly unfolding of these modes within a structurally stable, knowledge-using system.

The general mood of the book is one which regards conceptual (including experimental) and mathematical analysis as continuous and mutually reinforcing, but separable according to the taste of the reader. Unlike my previous book, in which I was preoccupied with the search for a formal treatment of physiological processes within cells, the primary material in this volume is biological, with a variety of mathematical devices employed to analyse in more detail certain aspects of the behaviour of cells and developing systems. Having started my career as an experimental biologist involved in physiological research before being diverted by the formal beauty of mathematics and the elegance of statistical thermodynamics, I feel that the measure in which this book is dominated by experimental problems is the measure of my return to original preoccupations.

As to structure, the organization of the material is hierarchical, as befits a biological analysis. A brief description of pre-control view of the stability properties of biochemical networks is followed by discussions of metabolic regulation, macromolecular regulation, the cell cycle, biological clocks, cell population control and morphogenesis in unicellular and multicellular organisms. Much of this material comes from various courses given to generations of students at the University of Sussex, to whom I am grateful for reactions, corrections and clarifications. The treatment of the material is in no sense comprehensive, the selection being a personal and therefore idiosyncratic one. I attempt to make acknowledgements wherever

appropriate, but much of my indebtedness to individuals is of an educational nature. However, there is one outstanding personal debt of gratitude which I owe, and this is to the late Professor C. H. Waddington, to whom the book is dedicated. It was his belief in the importance of theoretical analysis and mathematical model-building in biology which first provided me with the opportunity of exploring beyond the conceptual horizons which I had experienced in experimental research. This same belief of Waddington's gave rise to the series of conferences at the Villa Serbelloni on Lake Como which constituted a unique educational experience in interdisciplinary discussion and has resulted in the volumes entitled 'Towards a Theoretical Biology'. The 'Towards' in this title was symptomatic of Waddington's thought, which was always reaching beyond accepted ideas and involved a constant flirtation with the heretical. However, this play with heterodox fire was firmly based upon a deep familiarity with the detailed behaviour of organisms. It is this type of balance between knowledge and vision after which I aspire, and whatever quality this book may have in this respect is due in no small measure to inspiration received directly and indirectly from C. H. Waddington. Another acknowledgement I would like to make is to the Science Research Council of Great Britain, whose generosity in financing an Analytical Biology Research Group at Sussex made possible the appointment of individuals with mixed scientific parentage and creative insight to visiting and research positions in the area of biological analysis of the type described in this book. To my friends and colleagues at the University I am indebted as well, but particularly to Drs John Dowman, Gerald Webster and Keith Oatley.

The last chapter of the book derives from ideas explored during a sabbatical year at the National University of Mexico. The collaborative work carried out there with physicists and biologists was of the greatest value to me, and my thanks for many discussions go to Drs Manuel Berrondo, Germinal Cocho, Octavio Novaro, Rafael Perez-Pascual, Ruy Perez-Tamayo and Gustavo Viniegra-González. To the heads of the Departments of Theoretical Physics and of Biomedical Research, Drs M. Moshinsky and J. Mora, I am indebted for hospitality and assistance; while to the Royal Society I owe a debt of gratitude for a Leverhulme Visiting Prefessorship which made the visit to Mexico a possibility. And lastly, although they are unlikely to be in the least interested, I am grateful for the serene magnificence and beauty of Istaccihuatl and Popocatapetl whenever they were in view from my window in the Torre de Ciencias, for they were a potent source of detached inspiration.

September, 1976

B. C. GOODWIN
Sussex University

The Garden of Forking Paths is an incomplete, but not false, image of the universe as Ts'ui Pen conceived it. In Contrast to Newton and Schopenhauer, your ancestor did not believe in a uniform, absolute time. He believed in an infinite series of times, in a growing, dizzying net of divergent, convergent and parallel times. This network of times which approached one another, forked, broke off, or were unaware of one another for centuries, embraces all possibilities of time. We do not exist in the majority of these times; in some you exist, and not I; in others I, and not you; in others, both of us. In the present one, which a favourable fate has granted me, you have arrived at my house; in another, while crossing the garden, you found me dead; in still another, I utter these same words, but I am a mistake, a ghost.

"Labyrinths"
Jorge Luis Borges

CONTENTS

Chapter 1

STABILITY AND REGULATION IN THE METABOLIC SYSTEM

The Metabolic Network

Our story begins in the years immediately before control theory became dominant, that is, before the early 1950s, when the most obtrusive feature of cellular metabolism was its sheer complexity. Thirty years of biochemistry had been necessary before some coherent picture began to emerge regarding the inter-related pathways whereby one metabolic species is converted into another in living organisms, each reaction being catalysed by a specific enzyme. It had become evident that there must be hundreds of different enzymes in each cell and it was conjectured that the exact metabolic state of any cell was determined by the types and the amounts of each of these organic catalysts, together with the concentrations of precursors or nutrients available to the cell. A useful, but somewhat inaccurate representation of this picture of branching and inter-connected metabolic pathways is given in

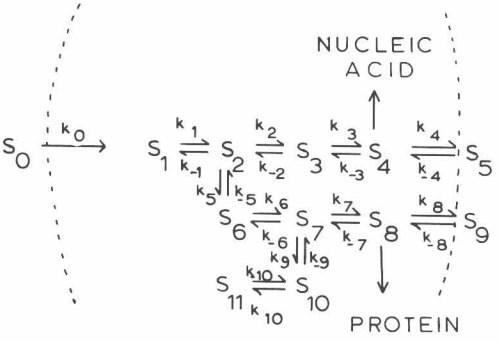

Figure 1.1. A linear picture of the branching pathways characteristic of the metabolic system of a cell. The dotted lines represent the membrane boundaries, S_0, S_5, and S_9 being external nutrients. S_8 and S_4 are precursors for protein and nucleic acid synthesis, respectively.

Figure 1.1. This is simply a kinetic description of what one sees on a metabolic pathway chart, with some simplifications that I will consider in a moment.

One can imagine such a network expanded to accommodate several hundred different metabolic species, S_i, with associated rate constants for forward and backward reactions. These rate constants include enzyme concentrations, so that the first assumption made in representing a metabolic network in this form is that these concentrations are not changing. In looking for some analytical insight into the collective behaviour of the variables of intermediary metabolism, the first question we encounter, then, is: for what period of time is it legitimate to assume that enzyme concentrations in the cells will remain constant? The phenomenon of enzyme adaptation, wherein a culture of micro-organisms adapts to a change of carbon source (galactose instead of glucose, say) by the appearance of a new enzyme activity has been known for nearly a century and tells us that enzyme activities in cells do change, but this process requires between 20 minutes and a few hours in organisms such as bacteria or yeast. Thus we can put a very rough upper bound to the time interval over which enzyme activities may be assumed to be constant in such organisms at a few minutes. Since we are interested in variations in S_1, the metabolites, we must choose a lower bound which gives enough time for change in these variables. This we can estimate from enzyme turnover numbers (the average number of molecules of substrates an enzyme molecule can convert into product in one second) which lie in the range 10^2–10^5, with a usual figure about 10^3. For a measurable change in the concentration of some metabolite such as glucose 1,6-diphosphate or aspartate, then, a minimum period of at least a few seconds would be required. So in considering the behaviour of a metabolic network of the type represented in Figure 1.1, we are restricting our attention to processes in the time range of seconds to minutes. This corresponds to the experimental periods used by enzymologists and those studying the processes of intermediary metabolism.

Now we can be somewhat more analytical in our procedure, using little more than the information already introduced. We need to know first what the expected concentration of a metabolite is likely to be in a cell, and this we can deduce simply from the Michaelis constants of enzymes. This is because these constants, telling us the concentration of substrates that give half-maximal velocities of the enzyme-catalysed reactions, may be expected to correspond to the effective metabolite level in the living system where the enzyme is operating. Of course this can only be an estimate, since the properties of a purified enzyme *in vitro* are bound to be different from those of the *in vivo* catalyst, but estimates are all we want at the moment. A typical Michaelis constant is about 10^{-3} M, which we then take to be a mean value of a variable S_1. Next we need an estimate of enzyme concentrations in cells. This, of course, varies widely between different enzymes, but an average value is about 10^{-8} M. Using a mean enzyme turnover number of 10^3

molecules/s, we find that the rate of change of substrate produced by the enzyme is 10^{-8} M $\times 10^{3}$ s$^{-1} = 10^{-5}$ M s^{-1}. Consider now the equation for one of the steps in the reaction sequence of Figure 1.1, say that for the variable S_1:

$$\frac{dS_1}{dt} = k_0 S_0 + k_{-1} S_2 - k_1 S_1. \tag{1.1}$$

We take S_0 to be a constant, representing some nutrient source term (e.g., glucose). Let us take S_2 also to be a constant, an approximation which would be valid if we were perturbing the system slightly from its study state by changing S_1 (say by injection), then following the initial stages of the return to the steady state. In this case we can solve the equation for S_1. Writing $a = k_0 S_0 + k_{-1} S_2$, constant, we get

$$\frac{dS_1}{dt} = a - k_1 S_1, \tag{1.2}$$

whose solution is

$$S_1 = \frac{a}{k_1} + b\, e^{-k_1 t} \tag{1.3}$$

where b is determined by the initial conditions. If the experiment consists in a perturbation of S_1 which causes a transient increase over its steady state value which is a/k_1, the size of this increase is b. Using our previously estimated values of $S_1 = 10^{-3}$ M and the estimated value for the rate of change of substrate, $k_1 S_1 = 10^{-5}$ M s^{-1}, we find that $k_1 = 10^{-2}$ M s^{-1}. We may now ask the more precise question: how long will it take for the initial perturbation to have decreased to e^{-1} of its original value? Evidently this time is given by the value of t for which $k_1 t = 1$, i.e., $t = 1/k_1 = 10^{2}$ s, or about two minutes. This is known as the relaxation time of the system described by equation (1.2). Thus we get a more detailed answer to the question: what is the characteristic time for metabolic experiments? And we may then use this time to define what I will refer to as the metabolic system: that set of interconnected variables undergoing enzyme catalysed or more generally protein-mediated transformations which change significantly in time periods too short for significant variations in macro-molecular concentrations. In the study of a system as complex as the living cell, it is essential to use analytical devices of this kind to dissect the system temporally if we are to get any dynamical insight into its total operation. The traditional biological disciplines have in fact identified themselves by precisely these criteria, reflecting accurately what we presume to be the organizational principles of the living system itself. What has been identified above is the temporal

aspect of the hierarchical organization which has always been recognized as the foundation of biological order. We will see how this dynamical hierarchy unfolds in the course of our analysis, providing insight into adaptive and developmental behaviour.

Stability and Mass Action in the Metabolic System

Equation (1.3) shows us that the steady state of the kinetic system described by equation (1.2) is stable to perturbation, because of the negative exponential term. Graphically, the response is as shown in Figure 1.2. This behaviour arises from a very basic assumption about kinetic reactions, embodied in the law of mass action, and giving rise to the term $-k_1 S_1$ in equation (1.2): the rate of a reaction is proportional to the concentration of

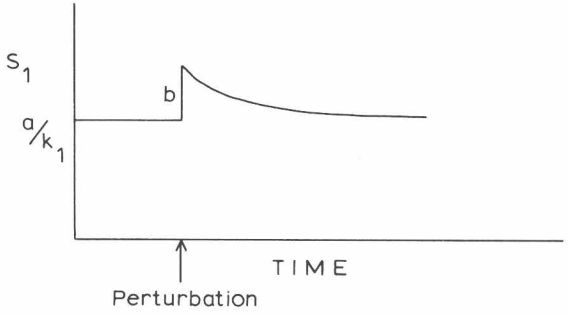

Figure 1.2. The response of a metabolic intermediate, S_1, to a small perturbation of amplitude *b*.

reactants. Thus the more S_1 there is, the faster is it converted to its product. This is true of all steps in the reaction sequence of Figure 1.1 and so one is led to the conjecture that a network of this kind is probably stable to the perturbation of any one of its variables, or indeed to any set of them. This was in fact shown to be true by Hearon (1952), and for precisely the reason conjectured: the law of mass action. However, we must now consider the consequences of a second assumption that has been used in representing intermediary metabolism in the form shown in Figure 1.1, which is that each reaction is regarded as first order in the substrates. We know that many reactions are bimolecular: aspartate and carbamyl phosphate are both involved in the reaction catalysed by aspartate transcarbamylase to produce carbamyl aspartate, for example. It is possible that *in vivo* such reactions are quasi-linear, one substrate being present in virtually saturating concentrations, but it seems unlikely. Therefore the equations we use to represent the kinetics, such as (1.1), will in general include products of concentrations of

two or more variables instead of being linear. Hearon's result was for linear systems for which there is only a single steady-state solution. For the metabolic system generally, with quadratic and higher order terms in the kinetic equations, we cannot draw firm conclusions because it is simply not possible to obtain general solutions and to examine the stability of the different steady states. One encounters immediately severe mathematical difficulties with such non-linear systems. There are, however, some considerations of a general analytic nature which suggest that non-linear networks will still have locally stable steady states. One such is provided by Newman (1971). The older literature on this problem is also of interest (Kacser, 1957; Denbigh *et al.*, 1948), but it is inconclusive as regards the general case. A recent study of the qualitative types of instability which second and third order chemical systems can show is given in an interesting paper by Tyson and Light (1973), in which they demonstrate that only very limited forms of behaviour are available to such systems other than the familiar asymptotically stable states of the type we have been considering, exemplified in the response shown in Figure 1.2. One may say that despite some significant advances the problem of analysing complex non-linear systems remains nearly as intractable now as it did in the early 1950s, and it is fortunate that insight into the organization of metabolic processes in cells was not dependent upon theoretical analysis but upon experiment. In the early 1950s some observations were made on the behaviour of metabolic mutants in bacteria which transformed the picture of Figure 1.1 into a totally new form, ushering in the cybernetic age of cellular physiology.

Feedback Inhibition in Metabolic Pathways

Let us consider what would be the expected behaviour of a system of the type shown in Figure 1.1 in the absence and in the presence of an exogenous source of some metabolite such as S_9. Suppose it to be an amino acid, and our system to be a bacterium, say *Escherichia coli.* If the amino acid is present in the growth medium it will be transported across the bacterial membrane and will enter the internal pool of the amino acid designated S_8, there being available for activation and incorporation into protein. Since metabolic reactions are thermodynamically reversible (the rate constants for forward and backward reactions are non-zero), the amino acid can undergo transformation to S_7, S_6, etc. The only effect of S_9 being present is a mass action effect in the system. All variables will continue to be present within the system, but at an increased steady state value over that when S_9 is absent.

With the discovery of sexuality in bacteria by Lederberg in 1946, genetic techniques of analysis became available for the study of bacterial metabolism and a period of intense and immensely fruitful investigation was begun.

By 1955 it had been observed that mutant strains of bacteria with a block late in a biosynthetic sequence due to the presence of a defective enzyme tended to accumulate metabolic intermediates prior to the block (Roberts *et al.*, 1955). For example, if the mutant enzyme was that catalysing the reaction

$$S_7 \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} S_8$$

so that $k_7 = k_{-7} = 0$, then precursors S_6 and S_7 were observed to be present in relatively large amounts. The bacteria would not, of course, be able to grow in the absence of exogenous amino acids. This observation is perfectly consistent with our picture as far as it goes, suggesting only that the reaction from S_2 to S_6 is an exergonic one (liberating free energy) so that the equilibrium is in favour of an accumulation of the intermediates leading to S_8, the end product of the sequence. However, it was then established that if the end product is provided exogenously the precursors disappear from the bacteria, which were then capable of growing (Novick and Szilard, 1954). This is not consistent with our mass action picture, since the presence or absence of S_8 in the cell should make no difference to S_6 and S_7 when $k_7 = k_{-7} = 0$. It strongly suggested that the end product was having a specific effect on the enzyme catalysing the first step in the reaction pathway, from S_2 to S_6.

Direct confirmation of this followed in 1956 when Umbarger showed that threonine dehydrase, the first enzyme of five in the pathway from threonine to isoleucine, the end product of the sequence, was strongly and specifically inhibited by L-isoleucine. He called this, appropriately, end product inhibition. In the same year, Yates and Pardee (1956) reported the specific inhibition of aspartate transcarbamylase, the first enzyme in the pathway from aspartate to the pyrimidines, by cytidine triphosphate. It is of interest to observe that the forward reactions in both these instances are strongly exergonic, making them physiologically irreversible (i.e., essentially irreversible under physiological time scales and conditions). Exit of end product from the pathway is either by incorporation into macromolecules (protein or nucleic acid for the respective enzymes), or by degradation, thus producing a one-way flux along the biosynthetic sequence. It is this unidirectional property which makes control of flow rate by a single enzyme a possibility in these cases. The exergonic nature of the first step makes this the thermodynamically efficient place to exercise control. Metabolic logic leads to the same conclusion, since if the end product is available from an alternative source (e.g. from the nutrient outside the cell), then none of the metabolic intermediates is required along the pathway in so far as their only metabolic

purpose is to provide end product. It is gratifying to find organisms behaving logically as well as efficiently according to our lights, suggesting that we have grasped at least one of their essential principles of organization. One could see what the consequence would be if this principle were not followed. Consider what would happen if, for example, lactate dehydrogenase were inhibited by lactate, product of pyruvate reduction by reaction with NADH. This reaction is physiologically reversible, allowing lactate to accumulate under certain circumstances, such as strenuous muscular activity, and then to be oxidized when conditions allow. Since there is no metabolic exit for lactate other than via pyruvate, lactate inhibition of LDH would create a pool of trapped product causing considerable physiological distress. Thus we see that physiologically reversible metabolic steps are there for good reasons and that reversibility is incompatible with control, which provides a throttle for a one-way process.

There are, of course, metabolic sequences along which flow can occur in either direction according to physiological demand. A classic example is glycolysis-gluconeogenesis. One might suppose that in such an instance, no control would be exercised and that mass action would be allowed to determine the net flux rate according simply to pool sizes of the metabolites at either end of the pathway. This would work in its own rather sluggish way, but clearly it would be very inefficient. This is because some of the steps in glycolysis, for example, are necessarily exergonic, such as the phosphorylation of fructose 6-phosphate by phosphofructokinase (using ATP) to give the product fructose-1,6-diphosphate (FDP). Reversal of this reaction by mass action alone would require the accumulation of very large amounts of FDP, which would disturb cellular pH and osmotic values as well as accumulating a great deal of energy in one component of the system. Therefore an alternative strategy is called for, which is to transform FDP in another way which avoids these disadvantages. Cells do so by exploiting the alternative pathway from FDP to F6P, which proceeds by hydrolysis and so is again an exergonic reaction. The enzyme which catalyses this transformation is phosphatase. Thus it appears that the price paid in the form of a free energy loss due to the hydrolysis of FDP releasing inorganic phosphate instead of ATP as would occur in the other reaction, is readily paid by the cell in order to achieve efficient reversal of this step in the sequence. The enzymes phosphofructokinase and phosphatase then become potential sites of metabolic control by processes such as end product inhibition. We will see later that this is precisely what one finds. Thus regulation can occur in a reversible sequence of metabolic steps, but it operates according to the same principles as those operating in an irreversible or one-way sequence such as the biosynthetic pathways involving threonine dehydrase and aspartate transcarbamylase, as discussed above.

Allosteric Behaviour of Enzymes

It was recognized immediately after the discovery of end product inhibition that an essentially new principle of enzyme behaviour must be involved. Inhibition of enzyme activity by competitive or non-competitive inhibitors had been known and studied for many years, and both were reasonably well understood. A classical case of competitive inhibition is the effect on succinate dehydrogenase activity of malonic acid, sterically similar to succinic acid as we see from Figure 1.3. The kinetic treatement of the interaction between an enzyme, E, and its substrate, S, and such a competitive

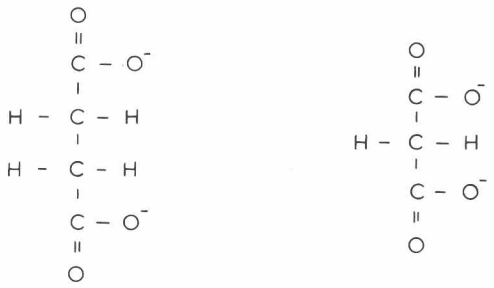

Figure 1.3. Succinic and malonic acids are sterically very similar and so compete for the active site on the enzyme succinic dehydrogenase.

inhibitor, I, is as follows. E combines with S to give the intermediate complex ES, which then undergoes transformation to $E+P$, enzyme plus product, according to the scheme

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E+P \tag{1.4}$$

where we are considering only the initial stages of the reaction before sufficient P has accumulated to make the back reaction to ES appreciable. Enzyme also combines with inhibitor to give an inactive form, according to the scheme

$$E+I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI. \tag{1.5}$$

From these reactions we may write the following kinetic equations:

$$\frac{\mathrm{d}[ES]}{\mathrm{d}t} = k_1[E][S] - (k_{-1}+k_2)[ES] \tag{1.6}$$

and

$$\frac{d[EI]}{dt} = k_3[E][I] - k_{-3}[EI]. \tag{1.7}$$

There is also a conservation condition on the enzyme, viz.,

$$[E]_0 = [E] + [ES] + [EI] \tag{1.8}$$

where $[E]_0$ is the total amount of enzyme initially added to the reaction. At the steady state of the reaction we may take

$$\frac{d[ES]}{dt} = \frac{d[EI]}{dt} = 0$$

and solve the three equations (1.6), (1.7) and (1.8) for $[ES]$, as a function of S and I. From (1.6) we get

$$[ES] = \frac{k_1}{k_{-1} + k_2}[E][S] = K_1[E][S] \qquad \text{where } K_1 = \frac{k_1}{k_{-1} + k_2},$$

while from (1.7) we find

$$[EI] = \frac{k_3[E][I]}{k_{-3}} = K_2[E][I], \qquad \text{where } K_2 = \frac{k_3}{k_{-3}}.$$

Using these in equation (1.8) we have the result

$$\begin{aligned}[E]_0 &= \frac{[ES]}{K_1[S]} + [ES] + K_2[E][I] \\ &= \frac{[ES]}{K_1[S]} + [ES] + \frac{K_2[ES][I]}{K_1[S]},\end{aligned}$$

whence

$$\begin{aligned}[ES] &= \frac{[E]_0}{1/K_1[S] + 1 + K_2[I]/K_1[S]} \\ &= \frac{K_1[E]_0[S]}{1 + K_1[S] + K_2[I]}.\end{aligned}$$

Since the velocity of the reaction is $V = k_2[ES]$, we find that

$$V = \frac{k_2 K_1[E]_0[S]}{1 + K_1[S] + K_2[I]}. \tag{1.9}$$

Assuming that there is a great excess of substrate so that it does not change