3 Biological applications of control theory

3.1 METABOLIC REGULATION AS A CONTROL SYSTEM

This chapter introduces the concepts and basic techniques of control theory that have been useful in biological research. Being an introduction, it does not present a rigorous and detailed account of the theory. Particular attention has been paid to the definition of systems nomenclature, since, while most biologists are already accomplished at thinking about feedback control systems, they find the mathematician's language uncongenial. Mathematically sophisticated readers may be vexed by an explicit definition of, say, an input, but biological colleagues will be heartened to learn that it means exactly what they thought it did all along. Control theorists will also find these definitions unnecessarily restrictive and valid for only the simplest linear systems. This is necessary, however, since general definitions would assume knowledge of several topics, notably functions of complex variables, that a biological readership would find unfamiliar. In preparing this chapter, several references were found to be particularly valuable. Toates's (1975) *Control Theory in Biology and Experimental Psychology* and Milsum's (1966) *Biological Control System Analysis* are also directed to nonmathematicians and thus of interest to biologists. For the more determined novice, the Schaum Outline, *Feedback and Control Systems* (DiStefano, Stubberud & Williams, 1967) provides valuable drill work. Mathematical readers who require detailed information will find Hsu & Meyer (1968) and Atherton (1975) useful. For anyone considering active research in biochemical systems analysis, Savageau's (1976) book is an excellent introduction. A program that is similar in some respects to that developed by Savageau can be found in the article by Kacser & Burns (1973), who have investigated the control of multi-enzyme systems, and in that by Heinrich, Rapoport & Rapoport (1977), who have published an extended introduction to the mathematical modeling of metabolic regulation. Horrobin (1970) has written a useful nonmathematical introduction to biological control.

Twenty years ago a chapter on the applications of control theory to biology would begin with a brief essay on the importance of control

theory techniques to biological research. This no longer seems necessary since these methods are now used in such a wide range of biological investigations. Control ideas can be examined by two approaches: simulation and analysis. Simulation techniques have been covered in Chapter 2. This paper will be concerned with analytical methods. While preparing and testing a computer model is usually the best first step in analyzing a complex physiological or biochemical process, a successful approach to any problem requires the application of both methods. The difficulties of analytical work has resulted in an overemphasis on computer simulations. The exclusive use of simulations as a means of studying biological systems is not fully satisfactory for several reasons. Any model of a biological process will contain several parameters such as reaction constants, transport rates and initial conditions. Often the numerical value of a parameter is uncertain. Since the behavior of a differential equation is sensitive to parameter variation it is necessary to run the program for several values of each parameter, otherwise important features of the system may be missed. A typical model may contain, say, 30 parameters. Suppose that 10 estimates of each were considered. This would result in 10^{30} separate simulations. This number could be compared with the estimated age of the universe (10^{17} s). Also the behavior of differential equations can be unstable with respect to variation in the form of the governing equations (for example if Michaelian enzyme equations are replaced by allosteric equations, the qualitative behavior of the solution can be very different). Ideally several different sets of governing equation should be tested. The preceding objections would be valid even if numerical methods were perfect. However, they are not and computer programs can yield very misleading results.

Before analytical methods can be applied, a complicated system must be reduced. The object is to produce a model that is simple enough to treat analytically but complicated enough to preserve essential biological behavior. This is the most taxing and important aspect of the biologist–mathematician interaction. The reduction process requires a sophisticated understanding of the biological problem and a good notion of available mathematical tools. No general procedure exists and usually each problem must be treated as a special case. A few general guidelines can be given. The most successful procedure is a reduction of the number of variables by separation of time scales. This is now discussed.

Decomposition by time scales

The recognition of different equilibrium times to effect a simplification of systems of differential equations is well known to those familiar with steady state enzyme kinetics, where two simplifying assumptions are

made. First, it is assumed that total enzyme concentration is constant; this exploits the slow time scale on which enzyme concentrations actually do change. Secondly, it is assumed that the equilibrium between enzyme and substrate has been attained; this exploits the fast time scale for complex equilibration. It is supposed that during the course of reaction rate measurements dC/dt is very small (where C represents the concentration of enzyme substrate complex). The steady state condition is often expressed by the equation $dC/dt = 0$. Strictly speaking this is incorrect. Lin & Segel (1974) and Reich & Sel'kov (1974, Example 2.5) provide a careful derivation of the Michaelis–Menten equations from the point of view of singular perturbation theory. The separation of biochemical processes into different time regimes occurs naturally and is an important idea in the qualitative, as well as quantitative, understanding of metabolic regulation.

Following Waddington's (1957) format, Goodwin (1963) has classified biological systems according to their relaxation time. The **relaxation time** of a process can be loosely defined as the time required to return to equilibrium after a 'small' disturbance. The fast **metabolic time scale** consists of rapid events such as the enzymatic conversion of small molecules. The regulatory processes are enzyme activation and enzyme inhibition. Since a single enzyme molecule can convert 10 to 10 000 molecules of substrate per second the relaxation times will fall in the range of 0.1 s to 100 s. The second, slower time scale is the **epigenetic system**. Epigenetic events are defined as processes involving the synthesis and degradation of macromolecules and thus include regulation of enzyme concentration by enzyme induction and enzyme repression. The relaxation time would be on the order of minutes or hours. Goodwin's third time scale is the **genetic** level. The events include the evolutionary process involving the appearance and movement of novel genes in a population. This tertiary structure provides a general decomposition of biological processes. A more detailed breakdown is possible: electromechanical (neural firing and muscular contraction, milliseconds), metabolic (enzyme catalyzed reactions, seconds to minutes), epigenetic (short-term regulation of enzyme concentration, minutes to hours), developmental (control of gene activity during differentiation, hours to years), and evolutionary (movement of genetic material through a population, months or years).

A branch of control theory, called hierarchial systems theory is concerned with the structure and control of multi-level systems (Mesarovic', Macko & Takahara, 1970) but, because the relevant time scales frequently overlap, the decomposition of complex biological processes into smaller, manageable subunits is not always possible. A legitimate reduction can be effected only if the separation between time

scales is sufficiently large. Often this is not the case. The ambiguity inherent in terms such as 'sufficiently large' almost precludes rigorous, general mathematical results. One of the few general theorems is Tikhonov's theorem, which specifies circumstances in which an n-dimensional system is effectively confined to a smaller dimensional subspace. A careful statement of the theorem in English has been given by Plant & Kim (1975). In practice each problem is examined separately. Reich & Sel'kov (1974, 1975) give several carefully presented biochemical examples of this process. The mathematical complexity of these problems unfortunately puts any further discussion of this topic out of the scope of this chapter.

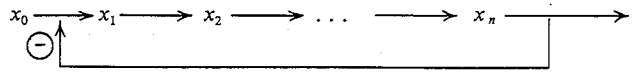
Supposing that a complex system has been reduced to its essential subunits, the next step is to analyze each subunit. *Ab initio* the analysis of local control subunits would seem to present an unlimited number of special cases. However, examination of biological control systems shows that though the processes may be very different one finds that complicated systems are constructed by adding together subunits which are regulated by a comparatively small number of control circuit types. Therefore experience gained in analyzing representative members of each group can be applied to a wide range of problems. The next section gives examples of these archetypal control circuits.

Archetypal control circuits

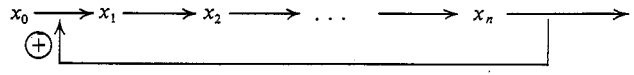
A chemical system is **controlled** if one or more of the chemical components affects the rate of a reaction by some means other than just substrate availability. The simplest form of control is a single loop control system where only one chemical acts as a controller and only one reaction is regulated. The four possible types of single loop control (positive and negative feedback and feedforward control) are shown in Figure 3.1.1. Each type of archetypal control pattern has been found in biological systems.

Single loop negative feedback is the most commonly encountered form of biological regulation. As shown in Figure 3.1.1*a*, feedback metabolite x_n inhibits the x_0 to x_1 reaction; so an increase in x_n decreases the net rate of x_n production while a decrease in x_n deinhibits the first reaction thus, eventually, causing an increase in x_n synthesis. The first experimentally confirmed example of negative feedback control of a metabolic process was the inhibition of threonine deaminase by isoleucine reported by Umbarger (Figure 3.1.2; Umbarger, 1956). Several other examples were soon published. By 1963 enough cases had been established to make several general observations possible (Monod, Changeux & Jacob, 1963). Monod and his colleagues found that a general

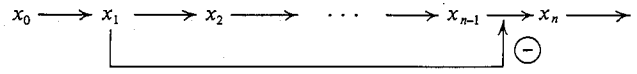
(*a*) Negative feedback control



(*b*) Positive feedback control



(*c*) Negative feedforward control



(*d*) Positive feedforward control

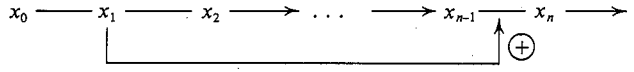


Figure 3.1.1. Four archetypal control loops. The symbol ⊖ indicates that the reaction is inhibited while ⊕ indicates that the reaction is accelerated.

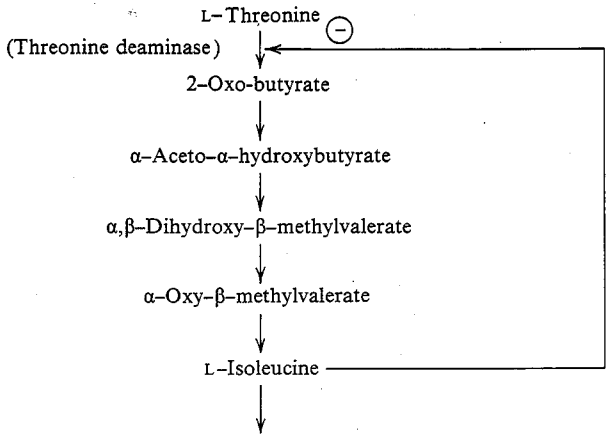


Figure 3.1.2. Feedback inhibition of threonine deaminase by L-isoleucine (Umbarger, 1956).

pattern was followed. Many experimentally investigated systems obeyed the following two rules:

(*a*) 'the regulatory enzymes (each of them acting immediately after a metabolic branching point) are all strongly and specifically inhibited by the terminal metabolite of the pathway in which each of them operates; intermediary metabolites in each pathway do not inhibit the regulatory enzyme;
(*b*) the enzymes which intervene after the regulatory one in each pathway are not significantly sensitive to inhibition by the terminal metabolite.' (Monod *et al.*, 1963).

Stated in the present nomenclature, all of the circuits considered by Monod are single loop negative feedback systems. The predominance of single loop systems is probably not accidental. Savageau (1976) has examined the optimal properties of several possible patterns of negative feedback control in unbranched pathways. He demonstrates that if all of the proposed networks have the same responsiveness to change in initial substrate, then the single loop alternative will be least sensitive to parameter variation. In considering input responsiveness, it is shown that a compromise between responsiveness and desensitization must be made, but, whatever balance is established, single loop end-product inhibition assures the maximum of both. These theoretical results suggest that single loop negative feedback would compete successfully in the natural selection process and accordingly the observed prevalence of this form of control is expected.

Negative feedback control is an intuitively satisfactory form of control since an increase (decrease) in output leads to a decrease (increase) in the rate of synthesis. A comparison with positive feedback (Figure 3.1.1*b*) indicates that positive feedback is unlikely to be useful since an increase in x_n leads to an increase in x_n synthesis. Indeed, Toates (1975, p. 118) suggests that positive feedback is only encountered in pathological conditions where normal homeostatic regulation has broken down. However, as shown in Section 3.8 below, positive feedback loops can be used to construct chemical on–off switches. The best-known examples of positive feedback are associated with processes where a rapid all-or-none switching response is required. For example the generation of a neuronal action potential is the result of a positive feedback loop (Horrobin, 1970; see also Section 7.7 below). A small initial membrane depolarization causes an increase in sodium permeability which permits inward sodium movement. This sodium in turn causes an increased depolarization. Eventually the fibre becomes positive and sodium influx stops (saturation of response). Active pumping moves sodium out of the cell. The saturation of response exhibited here is common in biological systems and

explains why the response generated by a positive feedback loop is finite. Positive feedback in the control system regulating blood clotting provides a rapid response mechanism (Esnouf & MacFarlane, 1968; Davie & Kirby, 1973; Müller-Eberhard, 1975). A similar positive feedback switch generates the large sudden release of luteinizing hormone during ovulation (Figure 3.1.3; Horrobin, 1970).

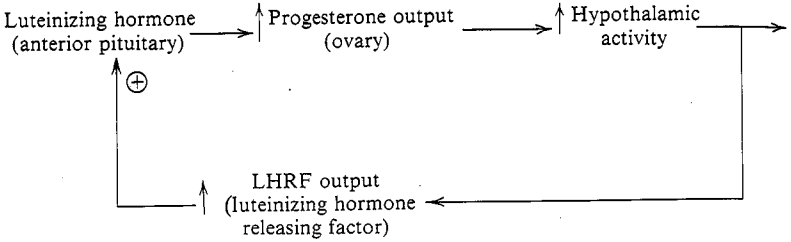


Figure 3.1.3. Positive feedback control of luteinizing hormone during the human female sex cycle (after Horrobin, 1970).

Besides forming chemical switches, positive feedback loops can be used to produce chemical oscillators. As shown in Section 4.1 below, positive feedback has been implicated in the cyclic AMP (cAMP) oscillation in *Dictyostelium* (Goldbeter & Segel, 1977) and in oscillations of the glycolytic pathway (Boiteux & Hess, 1974). It is interesting to speculate about the biological role of these oscillations. The cAMP oscillation in *Dictyostelium* has a well-understood purpose in controlling the aggregation process but the function, if any, of the glycolytic oscillation is unclear. Comparing the positive feedback loop in the glycolytic pathway with the preceding examples, it is possible to speculate that this control circuit primarily exists to fulfill the orthodox purpose of positive feedback, namely producing a chemical switch. The oscillations, which only exist for a comparatively narrow range of glucose entry rate, may be a switch defect. Switch 'chatter' in the form of oscillations is common in physical control systems.

Negative feedforward control is the forward control analog to positive feedback and few examples have been described in the biological literature. A common special case of negative feedforward control is the inhibition of an enzyme by high concentrations of its substrate. Examples include the inhibition of liver carboxylesterase by ethyl butyrate (Murray, 1930), the inhibition of xanthine oxidase by hypoxanthine (Dixon & Thurlow, 1924) and the inhibition of snake venom L-aminoacid oxidase by leucine (Dixon, Massey & Webb, 1964). The kinetic equations describing this inhibition have been published by Haldane (1930) and by

Dixon & Webb (1964, pp. 75–81). Inhibition of enzyme activity by high substrate concentrations prevents an unnecessary synthesis of intermediate compounds.

Positive feedforward control is the forward control analog of negative feedback. If a disturbance can be measured or predicted, this information can be passed by a forward control loop effecting necessary adjustments before a feedback controller would have time to operate. In metabolic systems, positive feedforward control adjusts the demand in the form of enzyme activity to meet the supply expressed as precursor concentration. A familiar class of examples is substrate activation of enzymes such as the activation of fumarate hydratase (Massey, 1953*a*,*b*) and the activation of phosphodiesterase by cAMP (Wang, Teo & Wang, 1972). Other examples include the control of cortisol secretion after hemorrhage (Blessner, 1969, pp. 528–31), cardiovascular and thermoregulatory response to exercise (Clynes & Milsum, 1970, p. 242) and in autonomic involuntary sensory motor systems (Talbot & Gessner, 1973, p. 222). The connection between prediction and feedforward control is especially apparent in man–machine coordination (Clynes & Milsum, 1970) and in the control of physiological processes by circadian rhythms. For example, petal movement in plants is necessarily slow. If the orientation to the sun did not begin until sunrise, several hours of sunlight would be lost. The circadian oscillator predicts dawn and a feedforward control loop begins the petal movement prior to sunrise. The generalization of this example is stated by Hoffman (1976): 'In general it is assumed that the adaptive significance of such endogenous rhythms lies in the fact that by proper phase adjustment, the organism is prepared to meet the challenge of cyclic changes in the environment; the organism can prepare in advance while a stimulus–response system might not allow sufficient time for the appropriate morphological, physiological and behavioral changes.'

If all biological control systems fell into one of these four classes of archetypal control system, the mathematical study of biological regulation would be far easier than it is. However, most biological control networks are multiple loop systems composed of archetypal subunits. Multiple loop systems are briefly considered in the next section.

Multiple loop biological control systems

In the context of metabolic regulation a **multiple loop system** is one in which several reactions are inhibited or activated and/or several metabolites are regulators. In Figure 3.1.4*a* a special case is shown in which several reactions are regulated by only one feedback metabolite and in Figure 3.1.4*b* the contrasting case is given where one reaction is regulated by several reactants. The usual biological case contains both

forms of regulation (Figure 3.1.4*c*). Mathematical difficulties have limited theoretical investigations of multiple loop biochemical systems. A partial resolution for systems like Figure 3.1.4*a* has been published (Mees & Rapp, 1978) and work on the related system of Figure 3.1.4*b* is in progress. The details are beyond the scope of this chapter.

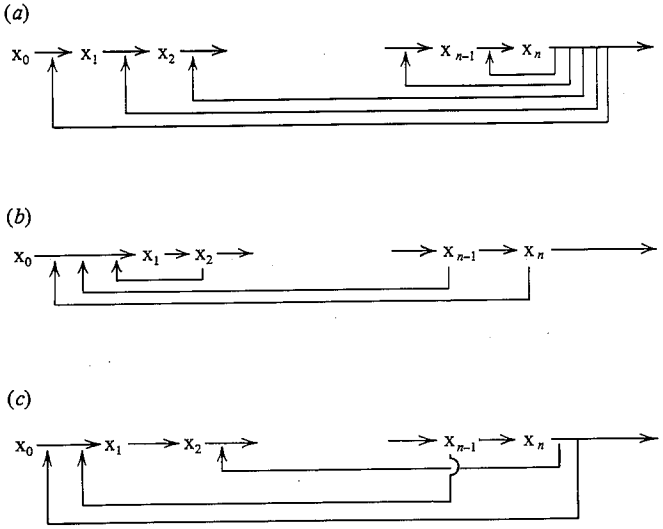


Figure 3.1.4. Three examples of multiple loop control systems: (*a*) The terminal metabolite either inhibits or activates several of the enzymes in the intermediate sequence. (*b*) Several different reactants affect the rate of the first reaction. (*c*) An example of a mixture of both of the previous forms of control (this is the most frequently encountered case).

Biological examples of multiple loop systems are commonplace. As a simple example, acetate in *Pseudomonas fluorescens* represses seven enzymes in the linear mandelate–acetate pathway (Mandelstam, 1968, p. 463). Examples of intermediate complexity include Toates's (1972) model of a division of the autonomic nervous system and the King-Smith & Morley model of granulocyte production (Figure 3.1.5, King-Smith & Morley, 1970).

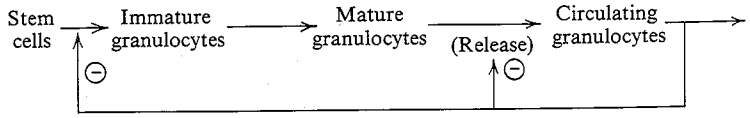


Figure 3.1.5. A specific example of a multiple loop control system is the King-Smith and Morley model of granulocyte differentiation and release (King-Smith & Morley, 1970; diagram redrawn from Tyson & Othmer, 1978).

Detailed models of biological processes inevitably involve highly interconnected multiple loop systems (e.g. the control of cortisol secretion: Gann, 1973, p. 224; Blessner, 1969, pp. 528–31). A particularly famous multiple loop system is Hill's hydrodynamic model of the nerve membrane which includes positive feedback and negative feedforward control (Talbot & Gessner, 1973, p. 82).

In practical terms additional loops complicate the analysis of control systems because their presence prevents the legitimate decomposition of a large system into smaller subunits. Parallel activation and inhibition between pathways (Figure 3.1.6) causes the same mathematical difficulties. Cross-pathway interactions coordinate the output of pathways that have a common source or converge to a common product. A biological example is provided by Stadtman's experimental analysis of aspartate metabolism in *Pseudomonas* (Stadtman, 1963).

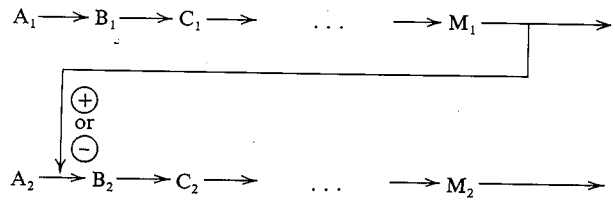


Figure 3.1.6. A hypothetical example of the cross coordination of two unbranched reaction sequences. A control system of this form would be expected if M_1 and M_2 ultimately formed a single product.