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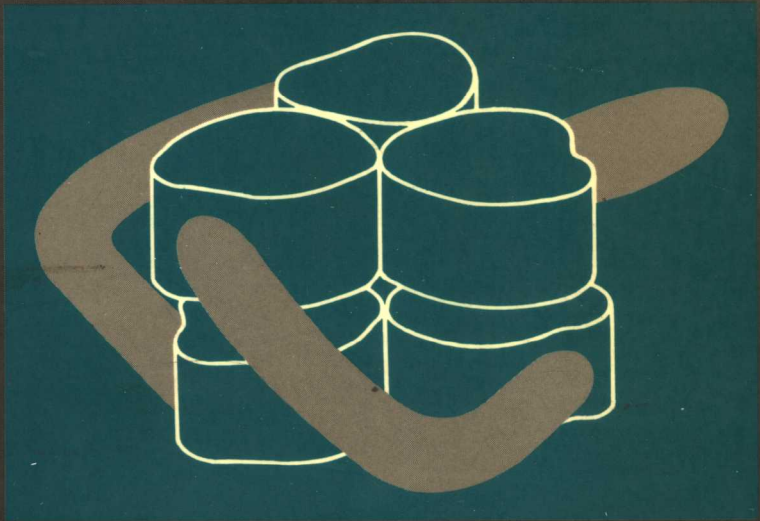
REGULATION OF ENZYME ACTIVITY

J H OTTAWAY



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J.H.Ottaway

University of Bradford, School of Studies in Environmental Science,
Bradford, West Yorkshire BD7 1DP, UK

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Preface

This book is not a guide to metabolic regulation, of which many good examples exist; those quoted in Chapter 1, Section 5.2 may be especially recommended. It is, instead, a book on the ways in which enzymes may be, and are, reversibly regulated, by ligand binding, by covalent modification, or by other means. I had hoped to include a description of the ways in which the activity of enzymes may be regulated by changes in their rate of synthesis or degradation. However, the need to provide generous illustration of the text made it impossible, within the planned compass of the book, for even a brief survey of this very important mode of enzyme regulation.

A good deal of trouble has been taken to make the review wide-ranging, by including as much information as possible, within the limits of space imposed by the publishers, about enzyme regulation in plants and bacteria. In order to do this, it has consequently been necessary to omit a good deal of information of the sort usually found in books of this type; for example the details of glycogen metabolism is not described in its entirety because admirable summaries can be found in the books recommended at the end of Chapter 1. On the other hand, the survey is as topical as possible, in a rapidly expanding field, so that many of the references, often to reviews, date from the last few years. It is hoped that readers will find the examples that have been chosen helpful and illuminating.

I would like to dedicate this book to my first—and, sometimes, during the last 15 years, my only—convert to the delights and rigours of metabolic flux analysis, Dr Linda Saunderson (née McMinn). My thanks to my daughter Sabina for typing the manuscript.

J.H.Ottaway

Abbreviations

Ala	alanine
AMP	adenosine 5′ monophosphate
ADP	adenosine 5′ diphosphate
Asp	aspartate
ATP	adenosine 5′ triphosphate
$\mathbf{c}_{E_i}$	flux control coefficient for the *i*th enzyme in a pathway
CaM	calmodulin
cAMP	cyclic AMP
CAT	carbamoylphospate – aspartate transferase
cAMP – PK	cAMP-dependent protein kinase
cGMP	cyclic GMP
CoA	coenzyme A
CRP	cAMP receptor protein
CTP	cytidine 5′ triphosphate
DAG	diacylglycerol
dATP	deoxyadenosine 5′ triphosphate
dNTP	(unspecified) deoxynucleoside 5′ triphosphate
F-1,6-BP	fructose-1,6-bisphosphate
F-2,6-BP	fructose-2,6-bisphosphate
F-6-P	fructose-6-phosphate
FBPase 1(2)	fructose-1(2),6-bisphosphatase
G-6-P	glucose-6-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
Glc	glucose
Gln	glutamine
Glu	glutamate
GS	glutamine synthase
His	histidine
HMG-CoA	hydroxymethylglutaryl-CoA
IDH	isocitrate dehydrogenase
K_{eq}	equilibrium constant
K_m	Michaelis constant
lac	lactose
MDH	malate dehydrogenase
MLCK	myosin light chain kinase

$NAD(P)^+$	nicotinamide-adenine dinucleotide (phosphate)
NAD(P)H	reduced nicotinamide – adenine dinucleotide (phosphate)
NTP	(unspecified) nucleoside triphosphate
αOG	α-oxoglutarate
OAA	oxaloacetate
OADH	oxoacid dehydrogenase
P_i	inorganic phosphate
PP_i	inorganic pyrophosphate
PDE	(cyclic nucleotide) phosphodiesterase
PDRP	pyruvate dikinase regulatory protein
PEP	phosphoenolpyruvate
PFK1(2)	phosphofructokinase producing F-1,6-BP or F-2,6-BP, respectively
PGA	phosphoglyceric acid
PIP_2	phosphatidylinositol bisphosphate
PI_3	inositol triphosphate
PK	protein kinase
PK-C	protein kinase C
Pyr	pyruvate
redox	oxidation/reduction
mRNA	messenger RNA
Ser	serine
$t_{1/2}$	time taken for a reaction to reach 50% completion
TDO	tryptophan-2,3-dioxygenase
THFA	tetrahydrofolic acid
UDP-Gal	uridine diphosphate – galactose
UDP – Glc	uridine diphosphate – glucose
UMP	uridine monophosphate
v	velocity of an (enzyme-catalysed) reaction
V, V_{max}, V_s	value of v when an enzyme is saturated with substrate
Z	sensitivity coefficient

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3. Regulation of ligand binding

4. Regulation by reversible covalent modification

5. Signals, transducers and modifiers

6. Regulation of enzymes in plants

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1

Amplification, time scales and feedback

'Was this the face that launch'd a thousand ships?'
Marlowe

1. Introduction

For reasons of space this book does not deal with irreversible cascades, such as blood clotting or complement formation, which have the nature of a rapid activation followed by a 'damage limitation' exercise. Only reversible systems are considered, and there are basically two types: systems with, or without, amplification. Systems with no amplification are those in which an end-product or allosteric modifier accumulates or disappears, and changes the activity of an enzyme. The response may be non-linear (e.g. a sigmoidal curve of v against [S], Chapter 3) but the *gain* is unity. Systems with amplification typically have a cascade mechanism, and a gain which can be much greater than unity. The properties of such cascades are discussed in the next section.

2. Features of amplification mechanisms

Stadtman and co-workers (1) have investigated the regulatory properties of reversible cyclic cascades, and have classified them under three main headings, in terms of a notional monocyclic cascade (*Figure 1.1*), using reversible phosphorylation of an enzyme as an example.

2.1 Signal amplification

This is given by the following quotient: the concentration of modulator ligand L_1 required to give 50% activation of a converter enzyme C to C_a, divided by the concentration of L_1 required to give 50% of the interconvertible enzyme I in its modified form. Typically this quotient is greater than unity, that is unless

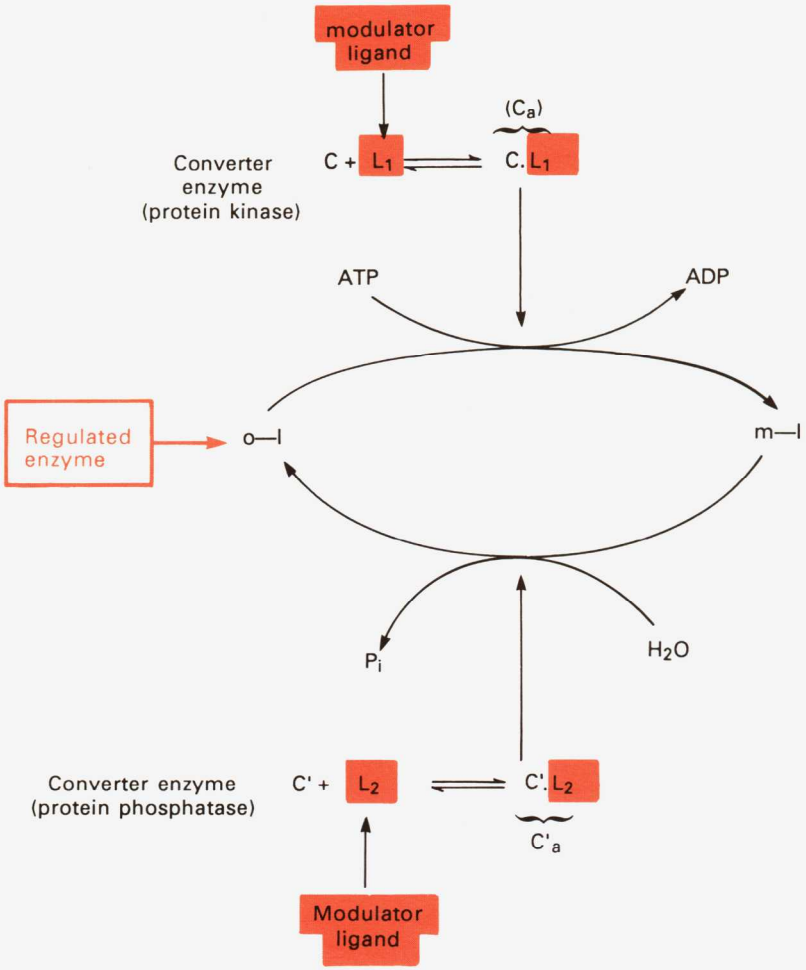

Figure 1.1. An open monocyclic reversible enzyme cascade. When the regulated enzyme has been modified (m-I), it may be either in the activated or inactivated form.

the re-conversion of modified I (m-I) back to its original form (o-I) is very rapid; 50% conversion of the total I present to m-I can be accomplished with much less than 50% of C in the active (C_a) form. The ratio increases exponentially with the number of cascades. In practice, the concentration of L_1 can be very small indeed. How big the amplification is will depend on the speed of the dephosphorylation, and this will depend on the concentration of L_2, among other factors. The numerical value of signal amplification depends on the parameter values, but a computer simulation with arbitrary parameter values gave values of $\simeq 300^n$, where n is the number of stages in the cascade. In practice, the value may be significant, but a good deal less than this; for glutamine synthase, for

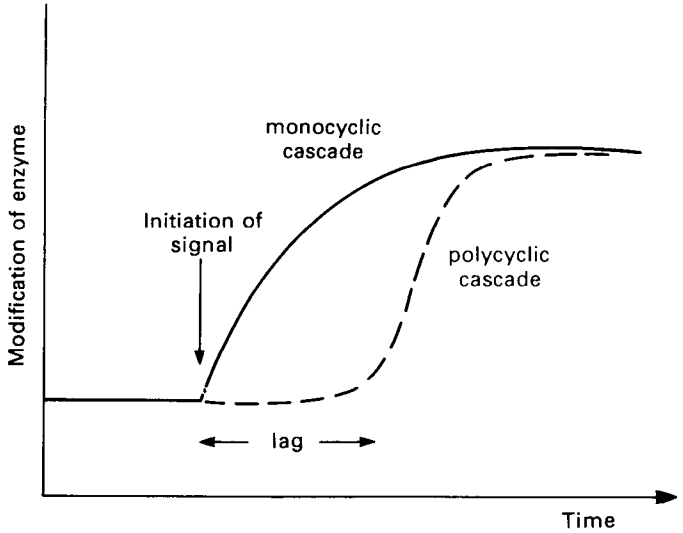

Figure 1.2. Time relationships for enzyme modification by monocyclic and polycyclic cascade systems.

example, a value of 67 was found experimentally for a single stage, and 250–1500, depending on conditions, for a bicyclic cascade (2).

The catalytic amplification, which is simply put as

$$[\text{m-I}]_{max}/[C_a]_{max} \qquad [1.1]$$

is a quite different ratio, and not of great interest. ([m-I] can be replaced by [o-I], if the latter is the active form of I.)

2.2 Amplitude

This is the maximum (fractional) value for the activation of I that is possible, if the concentration of L_1 $[L_1]$ is raised to a saturating level. The maximum value, perhaps surprisingly, need not be unity, as for glycogen synthase, which has several sites that can be phosphorylated, perhaps by different kinases, but is in any event a smooth function of $[L_1]$, not a step function (trigger mechanism).

2.3 Changes of rate

For a monocyclic cascade (*Figure 1.2*), the rate of conversion from one steady state to another is governed (3) by an exponential function of the form

$$I = I_{new}(1 - e^{-kt}) \qquad [1.2]$$

(where k is a rate constant).

For a polycyclic cascade there is always a lag period as the amplifying enzymes become activated (exaggerated in *Figure 1.2*), followed by a change which can be very rapid. For example, the half reaction time for conversion of phosphorylase *b* to phosphorylase *a* in frog sartorius muscle is estimated at 700 ms.

2.4 Sensitivity

This is used in a different sense than in Chapter 2, because it arises from a different cause. If an allosteric ligand—for example α-oxoglutarate in *Figure 4.7b*—has a positive effect on more than one step in the cascade, the fractional modification versus [ligand] curve will be sigmoidal, whereas if a ligand—for example, glutamine in *Figure 4.7b*—has a negative effect, the effector curve will be 'over-square'.These changes do not arise because of cooperativity between multiple binding sites on the same protein, but because these variables appear in more than one of the equations describing the cascade. The definition is not reproduced here, but is so arranged that a sigmoidal curve will give a maximum 'sensitivity' of 3.3 for a monocyclic cascade and 7.9 and 12.5 for bi- and tricyclic cascades, with values of less than one for antagonistic effectors.

Readers will no doubt be familiar with multicyclic cascades—the adrenergic activation of glycogen phosphorylase, for example, has four cycles. The analysis of expected behaviour becomes correspondingly complex (1), but experimental verification, using a synthetic mixture of purified components from the glutamine synthase cascade (see Section 2.2 of Chapter 4) has shown very good agreement with predicted behaviour (4). It is worth mentioning in passing that multistage cascades can be closed, in which the activating and inhibiting catalyst reside on the same protein—as is the case in the regulation of glutamine synthase—or open, when the activating catalyst, for example a protein kinase, and the inhibiting catalyst, for example a phosphatase, are different entities. This is more common.

3. Time scales of enzyme regulatory mechanisms

In a cascade system the duration of an 'on' switch is one means of providing amplification, providing that the messenger which is synthesized during the 'on' period is not rapidly removed. The activation of transducin by activated rhodopsin (see Section 1.1 of Chapter 5) is a very good example of such a phenomenon. A separate consideration is *response time*; that is the time interval before the response reaches a steady state. The prize in the slow bicycle race appears at present to be held by the inactivation of the branched chain oxoacid dehydrogenase complex (*Figure 1.3*), which takes two days to reach completion when measured *in vivo*, and is not well correlated with the disappearance of an 'activator protein' (5). *In vitro*, on the other hand, the half reaction time is at most 25 min (6), which illustrates the difficulties sometimes encountered in translating *in vitro* studies to behaviour in the intact animal. The pyruvate dehydrogenase complex is notoriously slow, taking about 20–30 min to move

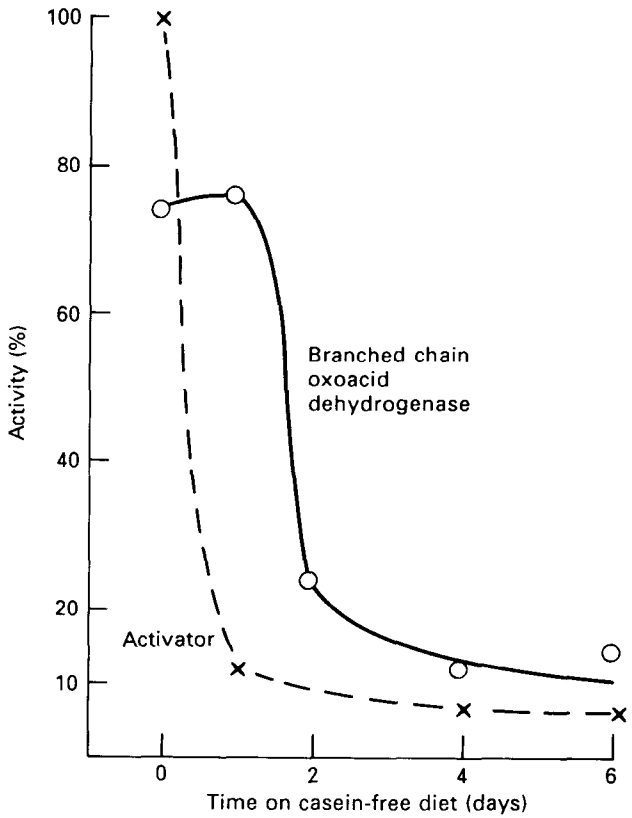

Figure 1.3. Change in activity of branched chain oxoacid dehydrogenase activity in rat-liver mitochondria after the animals have been placed on a low-protein diet.

from complete activation to complete inactivation, or vice versa (7). At the other end of the time sale, activations which may involve covalent modifications are rarely complete in less than a second; the activation of phosphorylase is an example and here we can note the advantage, in metabolic control, of differences in time scale. The activation of glycogen synthase is notably slower than that of phosphorylase (8) (*Figure 1.4*), which makes considerable sense in avoiding futile cycling of glucose residues. There are, of course, even faster responses; the response of a protein to ligand binding can occur in milliseconds, but it may take an elaborate apparatus to procure a reversible change of any magnitude in the concentration of ligand within the time scale (see *Figure 5.5*).

These enormous differences in time scales have to be borne in mind constantly. Not only do they give great flexibility in control, but it does not by any means follow that when two or more enzymes are regulated in a pathway, their response times will be similar. In a metabolic pathway, diagram arrows carrying '+' and '−' signs with no information about response times can be seriously misleading.

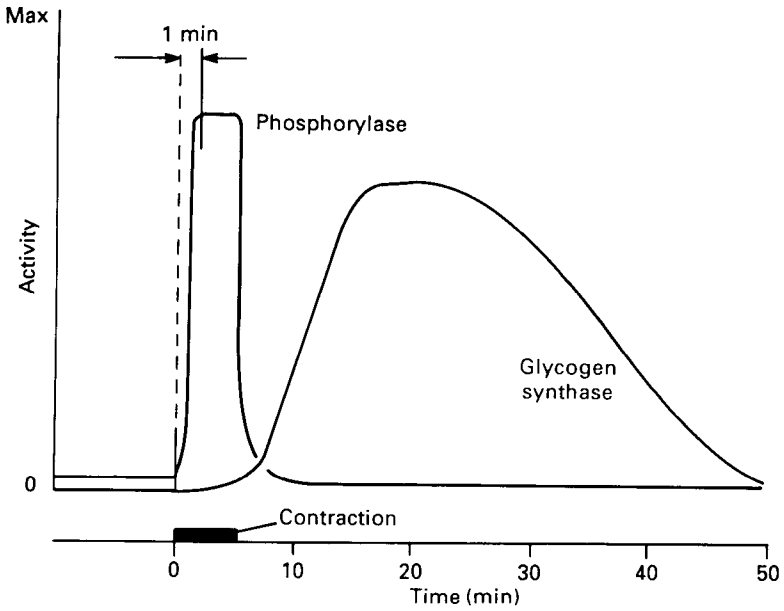

Figure 1.4. Time scale of activation of phosphorylase and glycogen synthase during and after a 5 min contraction of skeletal muscle (derived from ref. 8).

4. Feedback control

Feedback, for electronic and control engineers, is a very serious topic with wide ramifications into such matters as oscillations (9), stability, and proportional gain. Here we shall avoid such advanced topics and discuss only positive and negative feedback, and positive feedforward mechanisms.

It is not essential for a regulatory enzyme to be controlled by a feedback mechanism at all. Hormone-sensitive triglyceride lipase of adipose tissue cells, for example, does not have any end-product control, and it is possible for very steep rises in plasma non-esterified fatty acid levels to occur as a result. There is a recovery system within the fat cells which resynthesizes triacylglycerols from monoacylglycerol and free fatty acids, but this is in no way a feedback.

The point to remember is that if feedback control exists, it must act in such a way as to bring the flux through the enzyme back to a 'normal' level. In the ordinary way, one thinks in this context of an accumulation of end-products which allosterically inhibit the activity of an enzyme near the beginning of a metabolic pathway; a very complex example is the regulation in bacteria of the synthesis of amino acids from aspartate (10). However, the inhibition of pyruvate dehydrogenase kinase by coenzyme A (CoA) (11), which has the effect of increasing the rate of production of acetyl units, is an example of a release of inhibition of flux by a product; it is one of the products of the disposal of acetyl-

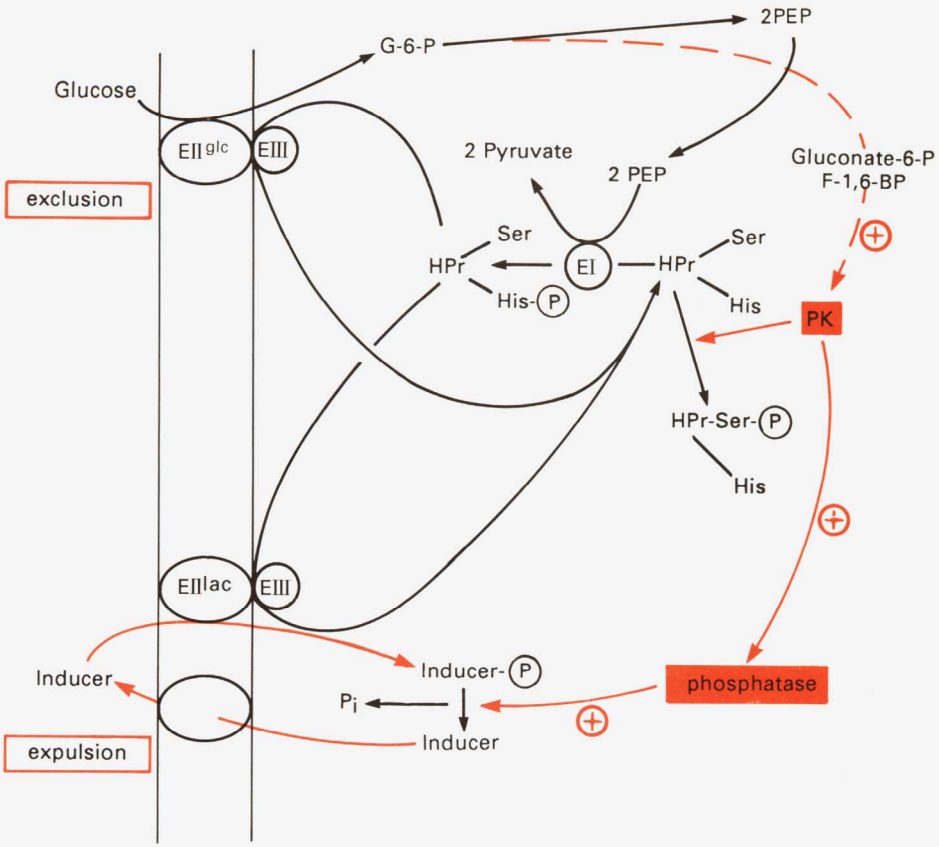

Figure 1.5. Mechanism of sugar uptake in Gram-positive bacteria. The upper half of the figure focuses on the positive feedback inherent in the mechanism. Enzyme EI catalyses the transfer of a phosphoryl group from PEP to the small carrier protein, HPr. EIII catalyses transfer of the phosphoryl residue to one of a series of integral membrane permeases (EII), of which two are shown. These in turn phosphorylate the external sugar molecule, coupling this with transfer through the membrane. The lower half of the figure, and the parts printed in orange, show the regulation of the permease system both by inactivation of HPr by a protein kinase (PK), and by expulsion of inducers of new permease synthesis such as thio-β-methyl galactoside. Ser, serine; His, histidine; Lac, lactose; Glc, glucose.

CoA generated by pyruvate oxidation (citrate being the other) and could be classed as negative feedback.

Positive feedback does exist, but it has to be very tightly regulated if control of a system is to be maintained. For example glucose transport in Gram-positive bacteria is by means of a phosphate-exchange enzyme (12) combined with a membrane-bound transporter (*Figure 1.5*). Since each molecule of glucose-6-phosphate (G-6-P) can give rise to two molecules of phosphoenolpyruvate (PEP),

this system is inherently unstable, and it is controlled by inactivation of an essential component, the phosphocarrier protein HPr, by a protein kinase activated by intermediates of glycolysis or the pentose phosphate pathway (12). A description of details of positive feedback leading to oscillations is given elsewhere (9). Positive *feedforward* is a phenomenon which is not very usual in control engineering, because it can lead to unstable situations, but it is not uncommon in biochemical systems. In its simplest form, it consists of the activation of an enzyme by its substrate, either directly by homotropic cooperativity (Chapter 3), or indirectly through the action of a kinase. For example coenzyme A is a substrate of pyruvate dehydrogenase, and the inhibition of the kinase described above could be described equally well as positive feedforward or negative feedback. Pyruvate, the main substrate of the complex, also inhibits the kinase, giving a more clear-cut example of positive feedforward. A more sophisticated example, also taken from carbohydrate metabolism, is the allosteric activation of yeast and liver pyruvate kinase (13,14) by fructose-1,6-bisphosphate (F-1,6-BP). The effect of this is that an increase in glycolytic flux, with an accompanying increase in activity of phosphofructokinase, and increase of concentration of F-1,6-BP will lead to activation of the terminal enzyme of the sequence *before* the 'slug' of metabolites reaches it. Since pyruvate (or acetaldehyde) is the major acceptor of reducing equivalents in anaerobic glycolysis, this ensures that the central part of the glycolytic chain can also work at maximum throughput, limited only by supply of ADP and inorganic phosphate. The system is stable, because as the concentration of F-1,6-BP falls, so pyruvate kinase loses activity. One can also see that such a system would work very well when there is lack of correspondence between the response times of an early and a late enzyme in a sequence.

5. Further reading

5.1 Further reading for Chapter 1

Riggs,D.S. (1967) *Adv. Enzyme Regul.*, **5**, 357.

Segel,L.A. (1984) *Modeling Dynamic Phenomena in Molecular and Cellular Biology.* Cambridge University Press, Cambridge.

Stadtman,E.R. (1970) In *The Enzymes*, Volume 1. Boyer,P.D. (ed.), Academic Press, New York, 3rd edn. (An invaluable guide to all types of feedback regulation in bacteria and animal cells.)

5.2 Recommended general reading

Cohen,P. (1976) *Control of Enzyme Activity* (Outline Studies in Biology Series). Chapman & Hall, London.

Martin,B.R. (1987) *Metabolic Regulation—a Molecular Approach.* Blackwell Scientific Publishers, Oxford.

Ochs,R.S., Hanson,R.W. and Hall,J. (eds) (1985) *Metabolic Regulation.* Elsevier, Amsterdam.