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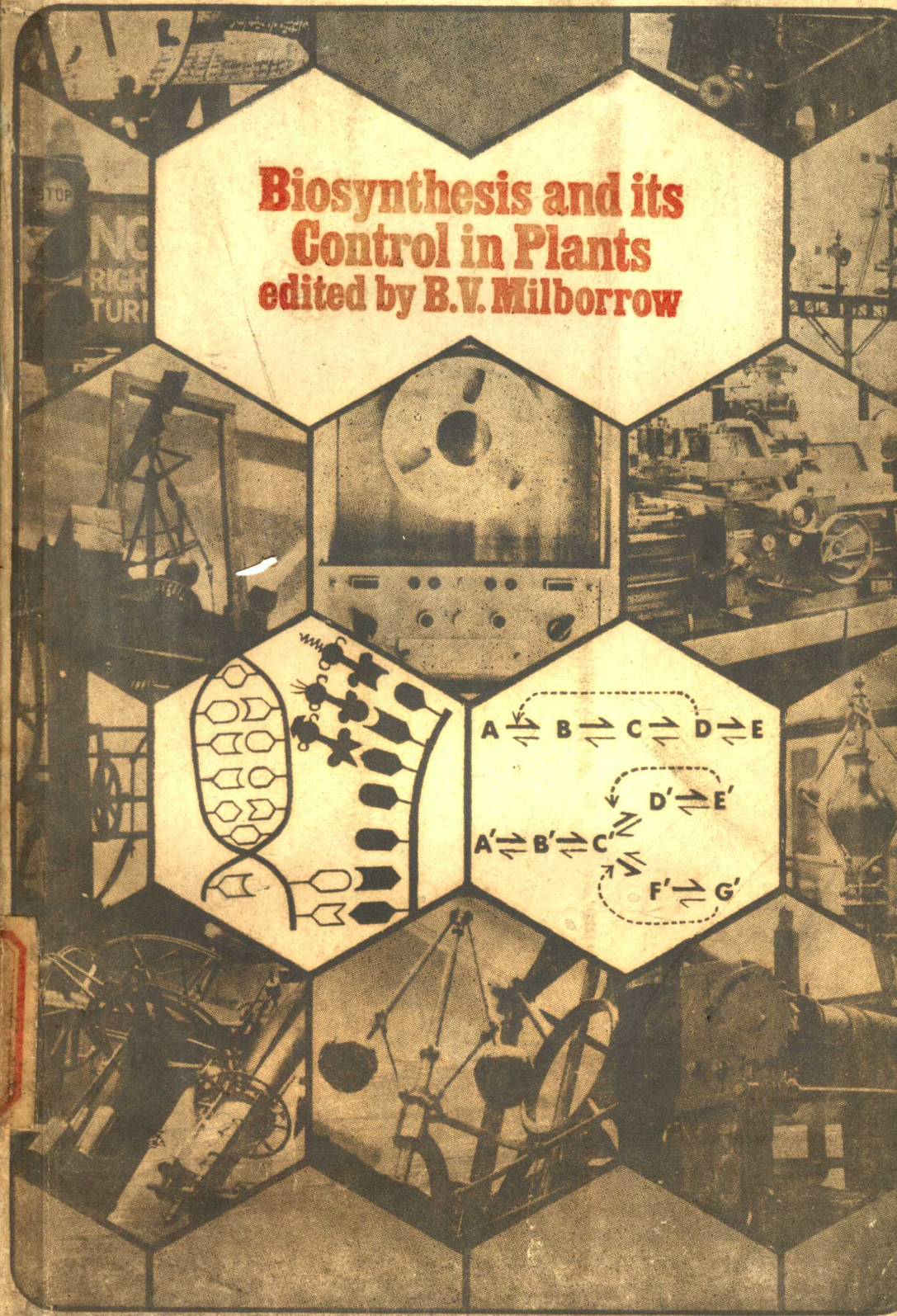
# Biosynthesis and its Control in Plants

edited by B.V. Milborrow



ANNUAL PROCEEDINGS OF THE PHYTOCHEMICAL SOCIETY NUMBER 9

# Biosynthesis and its Control in Plants

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# Preface

Living organisms resist the changes imposed on them by the environment—in the words of geneticists "they exhibit homeostasis". They so maintain themselves against outside influences that this property can be used as a criterion to separate living from non-living matter, and yet, paradoxically, the triumph of biochemistry has been, and continues to be, the analysis of organisms and their metabolism in terms of the same chemical and physical processes that operate on inanimate matter. One may justifiably ask how then can life be separated from non-living things, at what level of complexity or organization does life begin? Alternatively, what are the characteristics of the reactions carried out by living organisms which inanimate reactions lack? The reader is advised that clear answers to these questions are not to be found in this book. What he will find is a survey of the current state of our knowledge of the diverse mechanisms used by plants to regulate their biosynthetic metabolism and growth.

During the last fifty years the major metabolic pathways have been largely worked out in sufficient detail to show that the overall reactions do not proceed exactly as predicted from measurements of the kinetics of the individual reactions. Even quite small sequences of reactions that produce cellular products show an unexpected degree of regulation and the more complex growth processes are obviously subject to intricate control.

Although some of the individual steps of many reaction sequences and the enzymes that catalyse them are still undiscovered, a new insight has been obtained into the behaviour of the overall processes during the last fifteen to twenty, and particularly during the last five, years. This new knowledge has begun to give an understanding of how the reactions are controlled within the cell. An earlier volume in this series ("Biosynthetic Pathways in Higher Plants", J. B. Pridham and T. Swain, eds) dealt with the biosynthetic pathways used by plants; in 1972, at the meeting in Canterbury, we returned to the same topic but our discussions had a different emphasis. Obviously, knowledge of this subject is still far from complete but enough is now known of the different kinds of mechanism that regulate growth and metabolism for one to be able to review the subject of control mechanisms which operate in plants.

In the first lecture of the symposium Professor Davies brought out an exciting new concept of metabolic control, arguing that the pH of cells is regulated by the mutually antagonistic activities of carboxylating and decarboxylating enzymes being adjusted as the pH of the cellular environment moved towards or away from their respective optima. He also discusses the other broad concepts of metabolic regulation "energy charge", the NAD/NADH ratio, the pyradoxamine phosphate/pyridoxal phosphate ratio and the

balance and co-ordination of the enzymes of nitrogen and carbohydrate metabolism.

Bacteria, honorary plants for the meeting, regulate their enzymatic activities principally by making new enzyme molecules and the intricacies of the process are described with clarity by Mr Tristram so that the methods of control, described by Dr Miflin, which occur in higher plants (barley roots) are shown in counterpoint. The biosynthesis of amino acids is perhaps better defined than that of any other comparable cellular constituent; consequently, this basic knowledge provides the best framework for investigating regulatory mechanisms. Mr Tristram's review is illustrated by his work using naturally-occurring amino acid analogues (isolated from various higher plants by Professor Fowden and co-workers) as novel inhibitors to probe the capacities of the bacterial biosynthetic system. Similarly, Dr Miflin describes some of his own work to illustrate the interrelationships between the separate pathways by which the amino acids are formed.

The contrasts between the methods of regulation employed by the two kinds of organism are profound: the control of enzyme production used by bacteria can hardly be demonstrated in barley roots where allosteric effects and feedback inhibition are all important and allow the cells to respond more rapidly than if they depended on synthesis and destruction of enzyme protein.

Dr Ingle describes the intricate factors concerned in the production of large quantities of ribosomal RNA in cells, the devices used by plants to make sufficient copies of it and the variety of biochemical, genetic, cytological and analytical techniques that are used to investigate the problem.

Professor Street describes the behaviour of plant cells in tissue culture and shows the potential of the technique for investigating cellular metabolism without the complications of permeation into a cell mass and variations in cell types that bedevil experiments with isolated organs, or even separated tissues. The manipulation of free cells in a chemostat promises to provide a useful addition to the techniques available for biosynthetic and genetic investigations.

In the paper by Dr Daphne Osborne, the molecular events are followed through to their visible, morphological conclusions. She records the effects of ethylene on the favoured test object—the plumular hook of pea seedlings, and describes some of the results. Ethylene is a regulator of plant growth that has some of the attributes of an insect pheromone but its complex action is intimately concerned with the cell wall and its constituent hydroxyproline-rich proteins.

The other paper on the subject of plant hormones was given by Professor West, who reviews gibberellin biosynthesis and what is known of the regulatory processes that operate on it.

In his review Professor Cornforth discusses the influence the isotope effect has on the choice of hydrogen atom removed from a methyl group by iso-

pentenylpyrophosphate isomerase and relates the selection to the strict stereospecificity of enzyme reactions. The stereospecificity can be considered as a "labour-saving device"—in this case it is simpler to be highly specific. However, the same picture of integration and control is described in other reviews at higher and higher levels of complexity; at the atomic, molecular, enzymatic, pathway, organelle, cell and organ levels of organization there is precision and regulation.

Dr Sedgwick describes what is known of the biosynthesis of fatty acids in plants and how the processes are controlled. The reactions have been more thoroughly studied in animals and several of the regulatory mechanisms are different although recent results obtained by Dr Sedgwick, using chirally-labelled acetate, have shown that the stereochemistry of the reactions is the same.

The papers by Dr Briggs, who discusses the changes which occur during the germination of barley, and Professor Bradbeer, who describes the synthesis of enzymes and other materials which occurs during chloroplast formation, both show that these complex reactions are under precise control. As yet the way the control is operated remains largely unknown.

Professor Smith deals with current research on the regulation of flavonoid synthesis and concentrates in particular on the role of phenylalanine ammonia lyase (PAL). It is a branch-point enzyme for several different families of secondary products and its activity in extracts is affected by the previous illumination of the plant material from which it is extracted. The present evidence is insufficient to allow any definitive conclusions to be drawn concerning either the enzyme involved with the light-operated switch or even whether the change in overall activity depends on the synthesis of more enzyme protein or on adjustment of the activity of pre-existing molecules.

The final contribution of the Symposium, by Professor Fowden, provides a paradoxical contrast to the foregoing papers which all show evidence for the rigorous control of all aspects of biosynthesis. The non-protein amino acid, azetidine-2-carboxylic acid, occurs widely in the Liliaceae and can be used as a taxonomic marker as it occurs in a very few other species. Recently, as the result of a fortuitous set of circumstances, azetidine-2-carboxylic acid has been identified in sugarbeet where it occurs at very low concentrations. So low, in fact, that it would be undetectable were it not for the special processing which the juice undergoes.

The intriguing possibility suggested by this discovery is that azetidine-2-carboxylic acid occurs widely in plants as a metabolic mistake—as the result of imprecise selection of a substrate by an enzyme. It might be that azetidine-2-carboxylic acid is an example of "metabolic noise".

Physiological sciences have advanced to their present state of knowledge, largely by the use of a simple mechanistic outlook as expressed by the law of mass action. The "law of the minimum" or "limiting factor hypothesis" was

the first, and until recently, the major exception to this way of regarding complex processes but the self-regulating and interlocking pathways of metabolism are now coming to provide an even more serious exception. The investigation of self-regulating systems in living organisms by the "cause and proportional effect" design of experiment is inadequate because the integrated system may not respond at all to a stimulus, or respond in the reverse manner to that expected on the basis of a simple logic.

From the relative simplicity of the feedback loop, through the repressor/inducer/operator system of bacterial protein synthesis to the incompletely understood complexity of hormonal action, life processes show the capacity for self-regulation. By gathering together in one volume examples of the different kinds of control mechanism we can appreciate more fully the intricacy of metabolism and perhaps become more aware of the dangers of trusting a hypothesis when it accommodates the facts. As Whitehead said "Seek simplicity, then distrust it". Perhaps this book will help to make us more cautious in our interpretation of physiological data.

The review papers in this book were read at the Phytochemical Society Symposium held at Rutherford College of the University of Kent at Canterbury, and at the Sittingbourne Laboratories of Shell Research Limited. Thanks are due to many people in these two organizations for their hospitality and help with the arrangements for the meetings and to Shell Research Limited for financial support. The Phytochemical Society is grateful to the speakers for providing such up-to-date reviews of the topics and for their prompt co-operation during the editing.

Finally, the editor wishes to record his thanks to Mrs Margaret Ogle and Mrs Marilyn Jury for efficient secretarial help, both in the organization of the meeting and with the processing of the manuscripts, and to the staff of Academic Press for expert assistance in preparing the book for publication.

*November* 1972 B. V. MILBORROW

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CHAPTER 1

# Metabolic Control in Higher Plants

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## I. Introduction

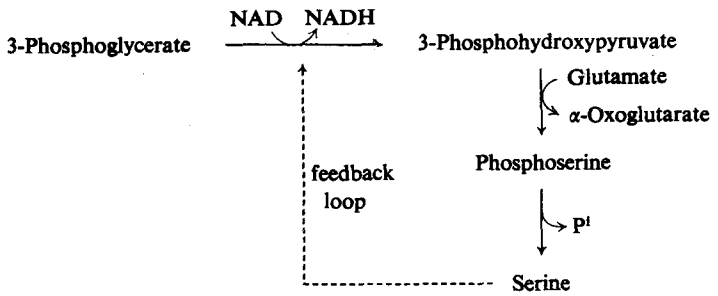
The common inheritance of living organisms is reflected in their biochemistry. Thus the study of intermediary metabolism has shown that the major metabolic pathways are common to all living organisms. In the mid-1950s, the study of metabolic control took a major step forward with the discovery that many metabolic sequences are controlled by their end products in a manner analogous to the negative feedback circuits of electronics (Umbarger, 1956; Yates and Pardee, 1956). Detailed study of feedback systems has shown considerable variation from species to species and it is now apparent that whilst we can reasonably anticipate the existence of, say the Krebs cycle in a given species, we cannot assume the presence of particular control mechanisms.

Fortunately we can define basic patterns of control, and the principles involved in the control of single metabolic pathways, branched pathways and cyclical process have been recognized (see Stadtman, 1970 for review). Other contributors to this symposium will be concerned with these principles and with specific examples of allosteric enzymes.

## II. Metabolic Interlock

My intention is to discuss the integration of metabolic control. One aspect of this has been termed metabolic interlock (Jensen, 1969) and can be illustrated by reference to the biosynthesis of serine in plants. Slaughter and Davies

(1968) have shown that serine inhibits the first step in the specific metabolic sequence leading to its synthesis. Subsequently Slaughter (1970) has shown that




3-phosphoglycerate dehydrogenase is inhibited by L-methionine and thus we have a metabolic interlock between the sequence leading to serine biosynthesis and the end product (methionine) of another sequence in which the methyl group of methionine is indirectly derived from the hydroxymethyl group of serine.

Our understanding of the interlocking of metabolic processes is only just beginning, but considerable attention has been paid to the energy charge of the adenine nucleotides and to the redox state of the pyridine nucleotides. I will briefly review the limited amount of work which has been done with plants, but will concentrate on two other aspects: (i) the role of pH in control and (ii) the role of pyridoxal phosphate and pyridoxamine phosphate in the control of nitrogen metabolism.

## III. Energy Charge

The role of adenine nucleotides in the regulation of carbohydrate metabolism was discussed by Krebs in 1964. Subsequently Atkinson and Walton (1967) developed the concept of energy charge—a parameter intended to indicate quantitatively the energy state of the cell. The energy charge is defined as $[(ATP) + 0{\cdot}5\ (ADP)]/[(ATP) + (ADP) + (AMP)]$ and the work of Atkinson has shown that the activities of many enzymes are regulated by energy charge as shown in Fig. 1.

Work with plants has not contradicted the generalization proposed by Atkinson, but little evidence has been obtained in support of the concept. Thus in animals and bacteria, phosphofructokinase is inhibited by ATP and citrate and this inhibition is modulated by ADP, AMP and inorganic phosphate. The inhibition of phosphofructokinase by ATP and citrate has been confirmed for the carrot (*Daucus carota*) enzyme (Dennis and Coultate, 1966) for the enzyme of corn (*Zea mays*) scutellum (Garrard and Humphreys, 1968) and for the pea seed enzyme (Kelly and Turner, 1969) which is also inhibited by phos-

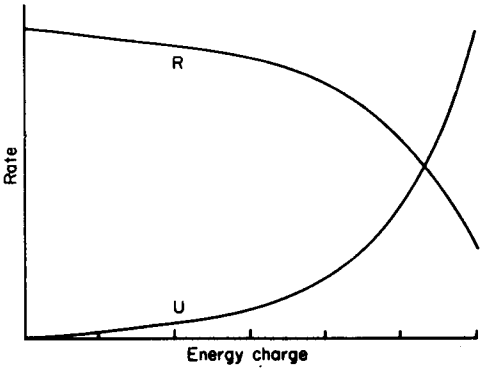



FIG. 1. Generalized response to the energy charge of enzymes involved in regulation of ATP-regenerating (R) and ATP-utilizing (U) sequences (After Alkinson and Walton, 1967).

phoenolpyruvate, though at low concentrations of ATP the enzyme is stimulated by phosphoenolpyruvate. However ADP and AMP were relatively ineffective in modulating the response to ATP.

The activity of NAD specific isocitrate dehydrogenase of bacteria and fungi is modulated by AMP and the activity of the animal enzyme is modulated by ADP. However, detailed studies of plant isocitrate dehydrogenase (Cox and Davies, 1967; Dennis and Coultate, 1967) have shown that the enzyme responds neither to single adenine nucleotides nor to energy charge.

However the activities of a number of plant enzymes are modulated by adenine nucleotides, for example the decarboxylation of α-oxoglutarate by preparations from pea (*Pisum sativum*) and cauliflower (*Brassica oleracea*) mitochondria (Davies and Kenworthy, 1970; Wedding and Black, 1971) is stimulated by AMP. Another example is nitrate reductase whose activity is modulated by ADP (Nelson and Ilan, 1969; Eaglesham and Hewitt, 1971). Finally, attention should be drawn to the work of Bomsel and Pradet (1968) and Pradet and Bomsel (1969) who have examined the levels of adenine nucleotides in wheat (*Triticum sativum*) and lettuce (*Lactuca sativa*) in the presence and absence of oxygen. They noted wide variations in the ratios ATP/ADP and ATP/AMP while the energy charge remained relatively constant. These results are considered to be a confirmation of the energy charge hypothesis of Atkinson. It therefore seems necessary to wait further experimental data before evaluating the extent to which the Atkinson hypothesis is applicable to plants.

## IV. CONTROL INVOLVING THE RATIO NAD/NADH

Krebs (1969) has pointed out that equilibrium enzymes may play an important role in regulation since they determine the concentrations of substrates for non-equilibrium regulatory enzymes. Thus lactate dehydrogenase maintains the reactants pyruvate, lactate, NAD and NADH at equilibrium and an

increase in the ratio NAD/NADH will tend to increase gluconeogenesis by increasing the concentration of pyruvate. A related equilibrium situation exists in potato (*Solanum tuberosum*) tubers where under anaerobic conditions lactate accumulates until pyruvate decarboxylase is activated. Eventually ethanol is formed and the lactate concentration declines. In such a situation it is difficult to see the biological significance of product inhibition unless the

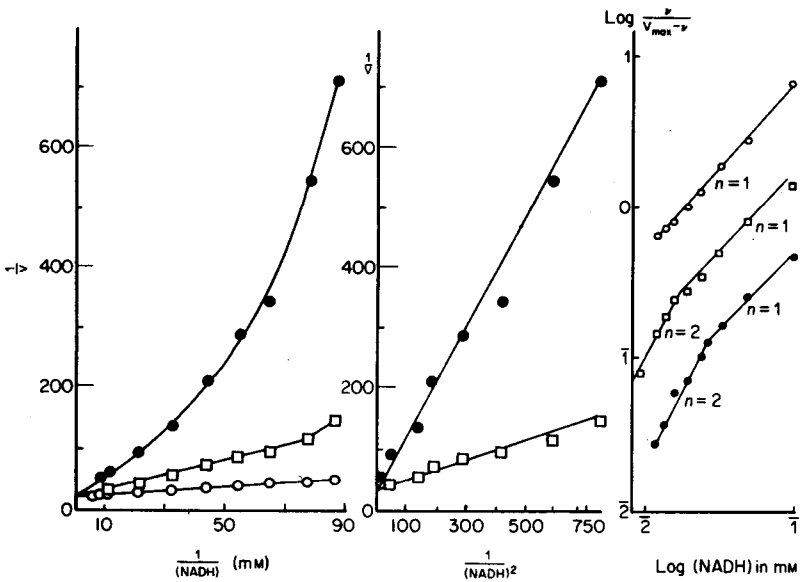



FIG. 2. Effect of NAD on the reduction of pyruvate by NADH at pH 6·1. MES buffer pH 6·1, 33 mM; pyruvate 0·2 mM; ● NAD 3 mM; □ NAD 1 mM; ○ Control (Davies and Davies, 1972).

inhibition is sufficiently great to convert an equilibrium situation into a non-equilibrium situation. The inhibition of lactate dehydrogenase by NAD may or may not have physiological significance, but some evidence for the allosteric binding of NAD (Fig. 2) tends to support the physiological significance of the inhibition.

Isocitrate dehydrogenase has been considered as a control site in plant mitochondria (Laties, 1967). However the enzyme does not respond to energy charge and it has been suggested (Cox and Davies, 1967; Dennis and Coultate, 1967) that the NAD/NADH ratio may be an important regulatory factor. Some evidence for the allosteric binding of NADH has been obtained with crude preparations of isocitrate dehydrogenase (Fig. 3) but purified preparations show competitive inhibitions by NADH. (Cox and Davies, 1967; Duggleby and Dennis, 1970). In considering the physiological significance of such competitive inhibition, Atkinson (1968) has pointed out that surprisingly large changes in enzyme activity can result from small changes in relative