Plant Physiol. (1974) 54, 437–442

# Conceptual Developments in Metabolic Control, 1924–1974[1]

Received for publication June 10, 1974

HARRY BEEVERS
*Thimann Laboratories, University of California, Santa Cruz, California 95064*

## ABSTRACT

**A brief sketch is given of the development of the understanding of the respiratory mechanism in plants over the past 50 years. Against this background the following aspects of control are discussed: (*a*) nonreversibility of catabolic sequences; (*b*) compartmentation of reactions and reactants; (*c*) control by amount of enzyme; (*d*) control by NAD and NADP; (*e*) control by ADP supply; (*f*) pacemaker reactions in glycolysis; and (*g*) control at branch points: further examples of allostery.**

The past 50 years have seen great changes in the field of plant physiology and none more remarkable than those in the area of metabolism. The development of concepts of regulation, or at least their experimental investigation, had, of course, to await the elucidation of the basic metabolic pathways themselves. For many of the pathways, the establishing of their roles as components of the metabolism of plants usually lagged for several years behind the initial elucidation in the hands of more numerous investigators of microbial and animal cells.

That is not to deny that a great deal was known before 1924 about the wide range of compounds present in higher plants; chemical analysis by classical methods had led to the identification of many of the components that we now know to play central roles in metabolism, and some important interconversions had been deduced to occur. But, the construction of detailed metabolic maps and their substantiation in plant tissues has been achieved only during the past 25 years. Of course, there is still a lot to be learned about metabolic sequences, particularly those leading to the host of so-called secondary products in plants. But, as the details of major sequences have been filled in, attention has naturally become increasingly focused on questions of how regulation is imposed; how the sequences are compartmented among the various organelles, how individual pathways are shut off or brought into play at appropriate times, how traffic at important branch points is controlled, and how the pace of central sequences is regulated and coordinated with other events in the cell. Answers to such questions emphasize the distinction between metabolism on the one hand and a mere road map of the reactions on the other. Moreover, an understanding of these general problems at the cellular level would seem to be a prerequisite to approaching the more formidable problems of control at the higher level of hormonal and environmental influences on development.

I will concentrate primarily in this review on one aspect of regulation, that of respiratory metabolism and some of its ramifications. One reason for this choice is that by the 1920's a good deal already had been learned about respiration. From classical experiments using the input-output approach, the external manifestations of respiration of whole organs and the response to changing external conditions had been established. In a particularly penetrating series of papers on the respiration of apples, begun in the 1920's, Blackman of Cambridge brought to bear an analytical approach which has had a lasting effect (9). His detailed investigations of the effect of $O_2$ tension on respiration and his analysis of the Pasteur effect as a regulatory phenomenon is a monument to this approach. [See Forward (22) for an appreciation of this work and a retrospective discussion of the concept of oxidative anabolism.] In the present context, his deduction about the mechanism underlying the climacteric, the spontaneous respiratory rise during fruit ripening, is of particular importance. For 50 years ago, well before the mechanism and compartmentation of the respiratory reactions were established, he proposed with great prescience that "organization resistance," defined as restraints imposed by cell structure on accessibility of enzymes and substrates, were important in the regulation of respiratory metabolism, and that changes in this limitation could lead to large changes in the respiratory rate (9, 22, 56).

## ELUCIDATION OF THE RESPIRATORY MECHANISM IN PLANTS

Following leads from the work on yeast and muscle, the investigation of the enzymology of plant respiration proceeded, slowly at first, and even by the early 1950's when James, a prominent contributor, published his important book on plant respiration (30), the details of the glycolytic pathway to pyruvate (EMP[2] sequence) were still not established firmly and the aerobic fate of pyruvate was unknown. Shortly thereafter, Stumpf (50) and Hatch and Turner (29) investigated the EMP sequence by newer methods and put the individual reactions on a firm and quantitative basis.

Much of the early work on the biochemistry of plant respiration focused on the very active soluble oxidases, phenol oxidase, peroxidase, and ascorbate oxidase. It seemed that these distinctively plant enzymes must have a major role in their respiration, and enzyme sequences whereby the oxidation of respiratory intermediates might plausibly be linked through these enzymes to oxygen were proposed (7, 30). Even at the stage where virtually nothing was known about the complexity of metabolic compartmentation, and the view of the cell as a bag of enzymes was tacitly in vogue, it was recognized that substrates might be sequestered in vacuoles and thus, that cell

[1] The research in my laboratory has been supported by National Science Foundation Grant GB 35376 and Atomic Energy Commission Contract AT(04-3)34.

[2] Abbreviations: EMP: Embden-Meyerhof-Parnas; FDP: fructose 1,6-diphosphate; F6P: fructose 6-phosphate; DNP: 2,4-dinitrophenol; G6P: glucose 6-phosphate; and PFK: phosphofructokinase.

organization was nevertheless important in regulating the action, *e.g.* of the phenol oxidases, whose unrestrained action upon cell breakage frequently bedeviled the work of those searching for enzymes in plant extracts.

Goddard's isolation of Cyt *c* from wheat germ in 1944 (25) was followed by the growing recognition of the pre-eminence of the Cyt system and the particulate nature of the pyruvate oxidation complex. The importance of the organic acids, so long known as major constituents of plants, was coming to a focus at this time, *e.g.* Laties (34). It was in 1951 that the first isolation of mitochondria from mung bean with tricarboxylic acid cycle activity was announced by Bonner *et al.* at Caltech (42) and this was followed shortly thereafter by similar demonstrations from a variety of plast tissues (14, 35, 36). It is difficult for younger members, brought up on elegant beginning biology tests with potted versions of the tricarboxylic acid cycle and the electron transport system in glorious technicolor, to realize that there was a time, as recently as 25 years ago, when every schoolboy did not know that these are universal systems in higher aerobic organisms. It was several years after the formulation of the tricarboxylic acid cycle by Krebs that even the most eminent investigators of plant metabolism, *e.g.* Chibnall in his Sillman lectures at Yale (13), felt justified in making tentative proposals about the existence of the tricarboxylic acid cycle in plants.

By the mid-50's, a wealth of data supporting the role of the mitochondria and the tricarboxylic acid cycle in pyruvate utilization in plant cells was in hand; the equally important role of the respiratory sequences as a source of intermediates used in the biosynthesis of cell constituents was documented (7, 8). The dependence of all of those synthetic events contributing to growth on energy provided during respiration could now be understood in biochemical terms. The specific respiratory reactions producing $NADH_2$ and ATP on the one hand were linked through these ingredients to synthetic reactions consuming them on the other. It was in this period that it was shown that the pentose phosphate pathway, a specific source of $NADPH_2$ and intermediates such as tetrose phosphate, plays a significant role in the respiration of plant tissues (3, 7).

In all of these developments, the increasing availability of new techniques such as chromatography and isotopic procedures, instruments, and fine biochemicals played an indispensible role. At the risk of appearing tedious and old-fashioned, I will point out that 20 years ago pure NAD, NADP, ATP, and all those other goodies now supplied by Calbiochem, Sigma, and their competitors were simply not available over the counter, one prepared one's own from yeast or muscle.

Against this very sketchy background, we can discuss the development of the concepts of regulation. It is difficult to trace the specific sources of concepts of metabolic control in plants or to state precisely when they became generally accepted or discarded, and in any event, most of the concepts are not unique to plant physiologists but are adopted or adapted from what has been established elsewhere. In what follows, I will discuss various aspects of control with illustrative examples rather than try to trace the various ideas to their original sources. For more detailed information, the reviews by Laties (37, 38), Preiss and Kosuge (45), and Thomas *et al.* (51) should be consulted.

## NONREVERSIBILITY OF CATABOLIC SEQUENCES

Partly because of their stability and the relative simplicity of the reactions they catalyze, enzymes concerned with the hydrolysis of substrates received early attention. Among the many substrates that have long been known to be hydrolyzed by enzymes from plants are urea, various simple glycosides, and phosphate esters as well as more complex molecules such as proteins, lipids, and starch. At one time it was thought that the same enzymes that brought about hydrolysis were also responsible for synthesis. Now it is clear that syntheses are not generally achieved in this way; the role of CoA, ATP, and other nucleotides in producing activated intermediates in specific pathways leading to synthesis is well established. The distinction between catabolic and synthetic pathways is seen also in the interconversion of acetyl units and long chain fatty acids. Gluconeogenesis in photosynthetic tissues and fatty seedlings includes several reactions catalyzed by enzymes (but possibly isozymes differently compartmented) which participate in glycoysis by the EMP pathway. But, the step between FDP and F6P is not reversible and in both instances a specific fructose-diphosphatase produces F6P. Sucrose is produced as the first free sugar by enzymes utilizing the nucleotide derivatives of hexose, and free glucose and fructose arise secondarily by hydrolysis of sucrose. At one time it appeared from Hanes' work on the phosphorylase from plants (27) which catalyzes the interconvention of glucose 1-phosphate and amylose that this enzyme was responsible for starch synthesis. Stress was laid on the fact that the equilibrium position was a function of pH in theories seeking to explain the osmotic changes in guard cells which lead to the opening and closing of stomata. Alternative and better substantiated models involving $K^+$ transport are now generally agreed to account for stomatal movements, and the discovery of synthetases using glucose nucleotides as substrates for unidirectional production of starch has put the role of phosphorylase as a synthetic enzyme in some doubt.

In each of the examples above then, synthetic pathways can be distinguished from pathways of breakdown, and the possibility of independent controls of these pathways affords a superior regulatory mechanism to one based simply on equilibrium shifting and mass action. In addition, of course, the physical separation within the cell of pathways of synthesis and breakdown further enhances the possibilities of control.

## COMPARTMENTATION OF METABOLIC REACTIONS AND REACTANTS

By far the largest intracellular compartment in most plant cells is the vacuole in which a variety of compounds are confined more or less permanently. In addition to the obvious examples of anthocyanins in certain cells and the phenolic derivatives referred to earlier, it can be reasonably deduced from pH measurements and total acid content (8) that organic acids are frequently preferentially located in this region of the cell. Evidence, showing that only a fraction of the total cellular contents of sugars, amino acids, organic acids, and salts such as nitrate and phosphate is temporally in contact with enzyme systems utilizing them, has come from a variety of labeling experiments [see Oaks and Bidwell (44) for a thoughtful review]. This has led to the concept that, within cells, metabolites are segregated into distinct compartments or pools in some of which rapid turnover is occurring, while in others, such as that in the vacuole, the metabolite is not being utilized. There is an increasing tendency to refer to the total cell content of a particular metabolite as a "pool." It seems to me that this term should be used only to define discrete intracellular compartments of the metabolite.

Definitive evidence of metabolic compartmentation comes from the demonstration that particular enzymes or sequences are confined to particular organelles. Mitochondria, chloroplasts, and nucleii each have their own distinctive enzyme components and within them reaction sequences are accomplished efficiently without direct competition with enzymes and metabolites elsewhere in the cell. In the past 15 years,

other kinds of organelles have been isolated successfully from homogenates by differential and sucrose gradient centrifugation. Thus, dictyosomes of the Golgi apparatus, spherosomes, ribosomes and polyribosomes, microbodies (including leaf peroxisomes and glyoxysomes), proplastids, and membranes of the endoplasmic reticulum and of the plasmalemma have been isolated in some degree of purity from particular tissues and the function of each class of organelle is indicated by the marker enzymes they contain. A result of this work has been the recognition that several enzymes once thought to exist freely in the cytosol are in fact confined to particular organelles. It seems inevitable that with improved techniques of grinding and separation, other more fragile enzyme associations will be revealed either as discrete organelles or as microcompartments (44). Certainly, the complexity of cellular organization as revealed by electron microscopy indicates that our present knowledge of enzyme compartmentation is incomplete.

This division of labor within the cells makes for the orderly progress of reaction sequences in compartments where the enzymes are highly concentrated and within which the topographical distribution of the enzymes of a sequence may further facilitate the channeling in isolation from the rest of the cell. A corollary is, of course, that since there must be interaction between the organelles and with enzymes in the cytosol in the over-all accomplishment of metabolism, transfer of specific metabolites must occur. Some progress has been made in showing that the chloroplast membranes are quite selective in regard to the permeation of metabolites (53). In plant mitochondria, more is known about the transport of inorganic ions (28) than that of organic metabolites, and virtually nothing is known specifically about the intrinsic permeation properties of other organelles in their native environment, although it may be deduced from other considerations what compounds are likely to move between compartments. The more limited the access of metabolites, the tighter the compartment will be.

The compartmentation of metabolic reactions not only increases their over-all efficiency but renders them more susceptible to selective control. That is to say, a particular sequence will be active only if the appropriate metabolite enters the organelle and the pace of the overall sequence will be controlled by the concentration of regulatory molecules in that compartment. Selective changes in the accessibility of metabolites or activating ions could lead to profound changes in the balance between pathways.

Several enzymes occur in more than one cellular compartment, and at least in some instances, *e.g.* the malate dehydrogenase of mitochondria and microbodies, they are distinct isoenzymes and furthermore different from the enzyme(s) catalyzing the same reaction in the cytosol (46). This further extends the possibilities of control.

## CONTROL BY AMOUNT OF ENZYME

The assumption is frequently made that when external factors are adjusted to optimal levels then the pace of a reaction sequence is governed by the catalytic capacity of a particular limiting enzyme in the sequence, and that increasing the amount of that enzyme protein would increase the over-all rate. Indeed, the argument is not always as clear as this; it is sometimes inferred that increasing the amount of any enzyme in a sequence will lead to increased metabolic flux through the pathway. To be sure, there are instances where the changing level of a particular enzyme determines directly the rate of utilization. For example, during the induced development of nitrate reductase or phenylalanine ammonia-lyase activity, the levels of those enzymes are probably overriding in governing the flux. In the early stages of cell growth and in senescence, there may well be limitations imposed by the incomplete coordination of enzyme development and breakdown.

But for the most part this is not so; it can be deduced that the enzymes are present in considerable excess, and that some restriction is placed on their activity so that the overall sequence is operating at less than full capacity. It is this concept of regulation of existing enzyme activities that has contributed most to present day views of metabolic control in plants. The general utility of such an arrangement is obvious, because it provides a flexible system which can respond immediately to changing demands.

For the respiratory sequences in plants, it can be shown readily that the various enzyme capacities are present in excess. Firstly, measurements of the catalytic capacities of the extracted enzymes support this view. Secondly, and unambiguously, the over-all respiration rate can be immediately, and, frequently substantially increased by addition of an appropriate concentration of an uncoupling agent, such as DNP (6). The immediate increase in the glycolytic rate observed when most plant tissues are placed in anaerobic conditions and the spontaneous climacteric rise are other clear indications that limitations are imposed normally on the action of existing enzymes.

## CONTROL BY NAD AND NADP

An early suggestion (26) was that the glycolytic rate may be regulated by the rate at which NAD, reduced in the triosephosphate dehydrogenase stage, was reoxidized, and that this rate might be greater in $N_2$ than in air. It is now clear that the oxidation of $NADH_2$ is not normally a factor limiting glycolytic flux; plant cells have the capacity to handle extra $NADH_2$ and measurement of the levels of $NADH_2$ and NAD show that this coenzyme normally exists preponderantly in the oxidized form (55).

The same is not true for NADP and $NADPH_2$ (55), and it appears that the rate of reoxidation of this coenzyme is important in determining what fraction of G6P is directed through the pentose phosphate pathway. When oxidants for $NADPH_2$ are added to tissue in which flow through the pentose phosphate pathway is very limited *e.g.* corn root tip, G6P is directed preferentially through this pathway (12). Because $NADPH_2$ production is the major function of this pathway, the most economic regulation would appear to be one wherein the pace of useful reductive events such as fatty acid synthesis from acetyl CoA alone determined NADP regeneration. This indeed appears to be the situation in mammalian cells generally. In all but the youngest plant tissues, however, traffic through the pentose phosphate pathway considerably exceeds that expected on this basis, and it appears that wasteful reoxidation of $NADPH_2$ by the distinctive soluble oxidase systems in plants may account for this (7).

## CONTROL BY ADP SUPPLY

At two specific stages in glycolysis, those catalyzed by phosphoglycerate kinase and pyruvate kinase, ADP is a cosubstrate and is converted to ATP. Thus, the level of ADP can govern the rate at which phosphoglycerate is converted to pyruvate, and because there is a net synthesis of ATP during glycolysis by the EMP pathway this process would come to a halt if there were no accompanying reactions whereby ADP was regenerated by ATP utilization.

The major fraction of ATP synthesis during respiration is that coupled to electron transfer from $NADH_2$ to $O_2$ in the mitochondria. As better preparations of mitochondria from plants became available in the late 1950's, the efficiency of the coupling between ATP synthesis and $O_2$ uptake (as measured

by the P:O ratio) increased towards that expected from the existence of specific coupling sites in the chain (40). Although the precise mechanism of ATP production in this process is still not available, it is clear that ADP is a cosubstrate and that, as in glycolysis, the supply of ADP can itself regulate the rate of the coupled reactions. Thus, in tightly coupled plant mitochondria, supplied with substrate, the rate of $O_2$ uptake is increased strikingly when ADP is added (state 3) and the rate slows to that seen before addition of ADP when the ADP is completely converted to ATP (state 4). The ratio of the rates observed in states 3 and 4, the respiratory control ratio, is a measure of the tightness of coupling and it is inferred reasonably that those preparations showing high ratios (40) are representative of mitochondria *in vivo*.

The beauty of these interactions is, of course, that the overall rate of glucose oxidation in respiration can respond directly to the overall rate of ATP utilization; when this is slow, as in mature or resting cells, the respiratory reactions would be held in check, whereas in actively growing cells or those in which the rate of ATP utilization was temporarily increased, the respiratory rate would increase in synchrony. Thus, a mechanism whereby the rates of synthetic reactions themselves determined the rate of respiration was at hand (37). Demonstration that such limitations are indeed operational *in vivo* came from experiments with DNP, which specifically uncouples ATP synthesis from electron transport in the mitochondria and removes the restraint normally imposed by ADP supply. Tissues such as fresh discs of mature carrot, in which ATP turnover is low, respond strikingly to the addition of DNP and the rate of respiration may be increased as much as 3-fold (6). In slices from young, actively growing carrots (7), corn root tips (6), or buckwheat seedlings (20) in which the endogenous conversion of ATP to ADP during coupled synthetic reactions is high, the stimulation observed on adding the optimum concentration of DNP is at most 20 to 30%. Further, when the discs from mature carrots or potato tubers are aged over a 12-hr period, the respiratory rate rises spontaneously as events leading to a resumption of growth are set in train and the rate of ATP turnover is increased. Correspondingly, the respiration of the slices becomes decreasingly sensitive to DNP stimulation (1, 38).

It should be noted that although the site of uncoupling by DNP is the mitochondria and the substrate level phosphorylations in glycolysis are not affected directly, the changing levels of ADP and ATP resulting from uncoupling in the electron transport system elicit a stimulated rate of glycolysis. Indeed, the adenylates play a major role in coordinating the pace of glycolysis on the one hand with the operation of the tricarboxylic acid cycle on the other. In some tissues, however, the indirect result of mitochondrial uncoupling by DNP is that glycolysis is stimulated to such a degree that the capacity of the pyruvate oxidation system is exceeded with the result that ethanol accumulates and the R.Q. rises accordingly (6, 7).

The other aspect of respiratory metabolism which became more understandable with the realization of the importance of ATP turnover in regulation is the Pasteur Effect. In most plant tissues that have been examined, transfer to anaerobic conditions leads to an acceleration in the rate of sugar breakdown, which may be as high as 3-fold [see Thomas *et al.* (51) for an excellent discussion]. Significantly, tissues showing the smallest Pasteur Effect, *e.g.* buckwheat seedlings, are those in which ATP turnover in air is already rapid in most of the cells. The mechanism suggested is as follows. Because all mitochondrial ATP synthesis is prevented in anaerobiosis more ADP and less ATP will be available than in air. If the rate of glycolysis is limited by ADP supply as argued above, transfer to $N_2$ would then result in an acceleration of glycolysis.

It is now clear that for a variety of plant tissues the imposition of anaerobic conditions does result in a rapid fall in ATP level and an increase in ADP (and AMP) as expected if these were causal in inducing the increased glycolytic flux (4, 5, 20, 24, 33, 47). Associated with these changes are shifts in the balance between some of the glycolytic intermediates. Although the specific responses in all plant tissues are not the same (51), it has been observed frequently that the level of FDP is increased at the expense of hexose monophosphates, and pyruvate increases as P-enolpyruvate declines (4, 5, 24, 33). This latter change is believed to be the first to follow the change in adenylate levels in peas (5) as expected from the increased availability of ADP. However, it is now clear that the repercussions on glycolysis of a changing balance within the adenylates go beyond those expected from simply changing the supply of the ~P acceptor ADP on those glycolytic reactions in which ATP is generated, and this has made possible a more complete understanding of the Pasteur Effect.

## PACEMAKER REACTIONS IN GLYCOLYSIS

The availability of enzymatic methods of analysis has allowed precise determination of the amounts of glycolytic intermediates in plant tissues. These have shown that, for most of the reactions, the relative levels of substrates and products are close to those predicted on thermodynamic grounds, and equilibrium is maintained by the existing enzymes (*e.g.*, 24, 33, 39). However, for the two essentially irreversible reactions, F6P → FDP and P-enolpyruvate → pyruvate, the amounts of reactants and products show that these reactions are far from equilibrium (24, 33, 39). On transfer to $N_2$ as indicated above, there is a marked shift in the levels of these particular intermediates; F6P and P-enolpyruvate decline while FDP and pyruvate increase. This further emphasizes that these are the major pacemaker reactions in glycolysis, as they are in other cells.

Particular attention has focused on the PFK reaction because it was discovered that it is catalyzed by a multivalent allosteric enzyme. This is to say, it shows complex kinetics and the rate of production of FDP is altered drastically by a variety of metabolites, which, by inducing conformational change, affect the binding of substrate.

In mammalian cells and yeast, the reaction is inhibited by high concentrations of ATP (which is also a substrate) and stimulated by ADP, AMP, and $P_i$. In addition, the enzyme is inhibited by citrate and P-enolpyruvate at appropriate concentrations. The complex reactions to positive effectors (those compounds which stimulate the reaction) and negative effectors (those compounds which inhibit) make this a particularly sensitive point at which control can be exerted, and perhaps in most circumstances, it is the major pacemaker of glycolysis.

The PFK from plants has now been examined in several laboratories, and although the detailed responses are not the same as for the mammalian and yeast enzymes, or the enzymes from different higher plants, it is clear that its major properties are such that it qualifies as a major control point (17, 18, 31, 32). Kelly and Turner (32) list 10 metabolites which are effectors of the pea seed PFK. Among the negative effectors are ATP, 6-P-gluconate, citrate, and at very low concentrations, P-enolpyruvate.

In regard to the restraint normally imposed on glycolysis in plants in air, the view has gained weight that the ATP level prevailing under these conditions is such that it inhibits the PFK reaction (*e.g.* 24). Certainly such an inhibition would enhance, and possibly override the limitation imposed on later glycolytic reactions by the correspondingly low levels of ADP

in air. Transfer to anaerobic conditions, resulting in a sharp decrease in ATP level, would be expected to relieve the limitations at the PFK stage, leading to an increased level of FDP and a higher glycolytic flux. Again, the effect would be to augment the response at the steps producing ATP to the reciprocal increase in ADP supply.

The concept of the PFK reaction in plants as the major pacemaker of glycolysis has received considerable support (4, 5, 24, 33), although the reciprocal changes in levels of F6P and FDP are not observed universally on transfer to anoxia (51). Moreover, there are instances, *e.g.* the climacteric in avocado slices (57) and substrate-induced aerobic fermentation in maize scutellum (23, 49) and in developing peas (48) in which large increases in glycolytic flux occur which are not, apparently, the result of relief of PFK inhibition as a result of changing adenylate levels.

Barker *et al.* (5) have used the concept of a "glycolytic granule," an assemblage of glycolytic enzymes with special permeability properties to adenylate, to account for the Pasteur Effect, but this remains speculative (24). However, they have shown clearly that when peas are made anaerobic, the fall in P-enolpyruvate (resulting from increased ADP availability) precedes the increase in FDP, and in this tissue, where the PFK is known to be particularly sensitive to P-enolpyruvate (31), the major limitation on glycolysis may be imposed by the P-enolpyruvate level prevailing in air.

It is not possible at present to generalize about which of the possible limitations is normally overriding in air or to arrive at a single general account of the detailed changes which lead to the establishment of a new rate of glycolysis. The reasons are as follows: (*a*) the complexities of the multiple interactions at different points in glycolysis; (*b*) in different plant tissues the properties of the pacemaker enzymes are different; (*c*) we do not have information on what changes in levels of effectors and substrates occur at the sites of enzyme activity; and (*d*) other respiratory enzymes are also affected by changing levels of adenylates (10, 16, 54).

Atkinson (2) has introduced the concept of energy charge, a relationship between the amounts of ATP, ADP, and AMP, rather than the individual levels of each, as the major determinant of the pace of catabolic and anabolic reactions. In a general sense, this has wide appeal, although as pointed out by Davies (15), work with plants is not advanced sufficiently to gauge the general applicability of the concept and exceptions have been noted above. Nevertheless, Bomsel and Pradet (11) developed a similar model to account for the adenylate changes observed when leaves were placed in conditions of different energy balance.

## CONTROL AT BRANCH POINTS: FURTHER EXAMPLES OF ALLOSTERIC REGULATION

In the respiratory sequence and in plant metabolism generally there are points at which an intermediate may enter alternative pathways. The regulation of which pathway is taken is not usually a result simply of the amounts of the competing enzymes. Thus the capacity of the G6P and 6-P-gluconate dehydrogenases and the other enzymes of the pentose phosphate pathway are more than adequate to consume all the G6P produced, and yet most of this substrate enters the EMP sequence. Again, the activities of pyruvate decarboxylase and ethanol dehydrogenase are fully adequate to consume all of the pyruvate produced aerobically yet none is, in fact, so diverted. Nor is the diversion necessarily a matter of Km values of the competing reactions, because in both of the above examples, the major traffic is through the enzyme with the lower affinity for its substrate.

One possible way in which regulation could be imposed would be provided if the competing enzymes were in different cellular compartments and the substrate generated in only one. A second, more important mechanism, is the limitation imposed on one of the pathways by coenzyme regeneration, as in the pentose phosphate pathway alluded to above. Competition between two alternative pathways for a coenzyme common to both may also be important. Thus, aerobic oxidation appears to compete successfully with the ethanol dehydrogenase for $NADH_2$ and prevent ethanol production in air.

A whole new area of regulation at branch points was opened by Umbarger (52) when, working with amino acid synthesis in a microorganism, he discovered the phenomenon of end-product inhibition. His demonstration of the ability of isoleucine to inhibit threonine deaminase, the first enzyme in the unique sequence leading to its production set a pattern which has since been greatly extended to include many other metabolites as well. This mechanism is of great utility in assuring the coordination, *e.g.* of the production of the appropriate assemblage of amino acids required for protein synthesis. An encouraging amount of information is now available from work with plants to show that here also similar allosteric controls are important. For example, threonine, isoleucine, tyrosine, leucine, and valine have been shown to inhibit the enzymes from plants at branch points leading uniquely to their synthesis and more complex coordinated inhibitions have also been demonstrated [see Miflin (41) for a recent review]. The operation of such controls in intact plant cells has been demonstrated by Oaks (43) and others (19, 21).

Another example of allosteric control is the action of phosphoglycerate and P-enolpyruvate as positive effectors for the enzyme ADP-glucose-pyrophosphorylase leading to starch synthesis. High levels of these intermediates would promote the synthesis of starch and this mechanism would be further augmented by the allosteric inhibition of the PFK by P-enolpyruvate. A valuable and extensive account of regulation of these and other plant enzymes, including those operating in photosynthesis, has been published by Preiss and Kosuge (45).

The view of regulation of metabolism in plants has thus expanded enormously in recent years, as a result of the elucidation of allostery. There is, however, a tendency to assume that when a particular metabolite is shown to be a positive or negative effector of an extracted enzyme and that the response appears "sensible" to the investigator, that such regulation is indeed imposed *in vivo*. The second requirement is that the levels of the particular metabolite in the living cells at the site of enzyme action are adequate to sustain the proposed mechanism of regulation. Insofar as the enormously complicated internal structure and the existence of separate pools of metabolites (44) makes this virtually impossible, guesses at precise mechanisms are hazardous at best.

It is clear that a variety of control mechanisms exist and we return to the problem of cellular organization and internal distribution, not only of enzymes and their substrates but of the coenzymes, activating ions, and allosteric modifiers as well. In this sense we have a more concrete realization and expansion of the organization resistance of Blackman (9). In a recent paper, Solomos and Laties (49), in presenting new information on the induction of the climacteric by ethylene, argue persuasively that the underlying mechanism is indeed one in which subtle intracellular permeability changes occur, resulting in "the cellular redistribution of various enzyme modulators, mainly ions."

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