

RESPIRATORY METABOLISM IN PLANTS

Harry Beevers

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Harry Beevers

*Professor of Plant Physiology
Department of Biological Sciences
Purdue University*

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Preface

In this, the second monograph of the series, the present status of our knowledge of respiratory metabolism in plants is reviewed, with the needs of honors and graduate students in mind. No attempt has been made to cover all aspects of respiration in plants. Instead, a factual presentation of the biochemical events which have been shown to occur in higher plants and which are conceivably of importance in respiration is given. An assessment is then made of the experimental evidence for their participation and importance *in vivo* and for the interplay with other metabolic events going on in plant cells.

Harry Beevers

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Introduction

In plants, as in other organisms, respiration involves the oxidative breakdown of certain organic substrates normally present in the cells. When these cells have access to air, as they usually do, O_2 uptake and CO_2 output are the external manifestations of this process, and so long as the study of plant respiration was limited to following gas exchanges without regard to the internal events, the process could be simply defined. A great deal of data has been collected about the gas exchanges of various plant parts under a wide range of external conditions. At the same time, analytical work and feeding experiments have established that sucrose or its component hexoses—the major free sugars in plants—are the usual substrates for respiration in most plant cells. This core of information representing the beginning and end of the respiration process poses the problem with which this review is primarily concerned, namely, the mechanisms by which sugars are respired to CO_2 and H_2O in plants and the interrelationships which have become apparent between this process and various others going on at the same time in plant cells.

Over the past thirty years, and particularly during the past decade, an increasing amount of information has been forthcoming on these dynamic biochemical events underlying respiration in plants. To be sure, the gathering of this information has in large part lagged a few years behind, and has frequently followed the directions established by, similar activity of workers engaged with cells of animals and some microorganisms. Nevertheless, the outcome is not unimpressive. Although formidable problems remain, experimental evidence is now at hand to construct a coherent picture of intermediary respiratory metabolism in plant cells without depending seriously on unproven analogies. An important result of this kind

of research has been the definition of many points of contact and interchange with other kinds of metabolic events going on in cells—so much so that it may seriously be questioned whether it is justifiable to dissect out a part of the total machinery, to assign to it a respiratory role, and to consider it in isolation from the rest.

Certainly the ramifications of hexose dissimilation which have become apparent make it impossible to regard the paths of breakdown as leading inevitably to CO_2 . These breakdown sequences lead not only to the production of energy in a utilizable form but at the same time to precursors of cellular materials which in younger cells may account for the diversion of a large fraction of the carbon of the starting substrate (Chapter 10). These inextricable connections with other metabolic processes and the different usages which have been adopted in the past (for a summary see James, 1953, Chapter I) make it difficult and perhaps pointless to attempt a hard and fast definition of respiration which would be acceptable to all plant physiologists. O_2 uptake which occurs as the normal accompaniment of breakdown of hexoses and other materials in higher plants is the aspect of the process which is most frequently used as an operational definition. It has long been realized of course that the important functional aspect of the process is the provision of energy in a utilizable form during the breakdown of more complex to simpler molecules. To this we must now add that the intermediates which are produced may also be used for synthetic events going on at the same time. If we use the term so widely as to include "all those material changes undergone by complex cellular substances . . . which lead to a diminution in free energy" (James, 1953), we stand in danger of including all metabolic changes in the scope of the definition.

It is now customary to use "fermentation" in place of the older term "anaerobic respiration," which may be considered self-contradictory. By fermentation we mean the breakdown of substrates which leads, *inter alia*, to the accumulation of products such as alcohol which are not immediately utilizable by the tissue. We may note that this kind of breakdown can

be induced by conditions other than anaerobiosis (Chapter 2) and leads to the production of small amounts of utilizable energy.

Experimental data about rates of respiration in different plant parts, responses to moisture, temperature, inorganic nutrients, excision, starvation, and other treatments are to be found in a very large and scattered literature. No attempt is made here to review this vast amount of information; the reader is referred to a recent handbook (Dittmer and Grebe, 1958) in which a valuable compilation and a bibliography are presented by a group of plant physiologists. Appraisals of pertinent papers on some of these aspects of the process are also presented by Stiles and Leach (1952), in standard texts, and more recently in W. O. James's important book on plant respiration (1953), which should be consulted for an exhaustive treatment of physiological matters. It should be recognized that a complete understanding of respiratory metabolism at the cellular level would provide a sufficient basis for accounting for the large variations in respiratory rate which have been observed and for the responses to external variables.

We may note that in the past decade there have been some innovations in the actual measurement of respiratory rates. The use of infrared gas analysis for CO_2 and automatically recording O_2 -measuring devices have greatly simplified the collection of respiratory data from large samples of tissue. Measurements of changes in conductivity or pH of an alkaline solution through which an air stream is passed from the respiratory chamber have allowed precise and continuous recording of respiration drifts. Polarographic methods for O_2 have been used in determinations on very small amounts of material, for which Cartesian divers and other sensitive devices have also been exploited.

Even so, standard manometric measurements have provided the great bulk of the data on plant respiration in the past ten years. When this method is used with tissue amenable to accurate sampling, the results are considerably more clear cut and satisfying than many in the older literature. For example, some results on the effects of O_2 tension and tempera-

ture on the respiration of onion root tips are shown in Figure 1. As the temperature is lowered, O_2 saturation is achieved at lower partial pressures; we note that under these conditions maximum rates of O_2 uptake are not attained at $30^\circ C$ with air as the gas phase. At any given O_2 level the familiar effect of changing temperature on respiration rate is shown, and when adequate O_2 is present, the Q_{10} in the range 20° – 30° is greater than 2. At each temperature, as the O_2 uptake becomes curtailed by lowering the O_2 tension, the R.Q. values rise above unity. The O_2 uptake values observed at 35° indicate that this temperature is above the optimal one for aerobic respiration. We may note, however, that CO_2 output is not adversely affected, and this might indicate a partially selective effect of the relatively high temperature. Other indications that glycolysis is less susceptible than subsequent events comes from the work of Oota *et al.* (1956) on *Vigna sesquipedalis*. Here again, at higher temperatures, high R.Q. values (and alcohol production) were a feature of the metabolism. Moreover, in this seedling additional effects of temperature were noted, since at the lower temperatures a higher proportion of fat than of starch was used as respiratory substrate. As the temperature was increased above 25° this situation was reversed.

It will be recalled that striking responses to temperature of the levels of soluble sugars, particularly sucrose, were a feature of Barker's (1933) classical investigations on the respiration of the potato tuber. It became clear from this work that the pace of reactions converting starch, the storage form of carbohydrate, into readily utilizable sugars may under some conditions limit the respiratory rate. The early depletion of free sugars and polysaccharides in many starvation experiments and the fact that declining respiratory rates can frequently be restored by the addition of glucose, fructose, or sucrose (see James, 1953) point unmistakably to the participation of these compounds as major respiratory fuels.

Recent work on enzymes which can interconvert various sugars by way of their phosphorylated derivatives and experiments with various labeled sugars have emphasized the ready

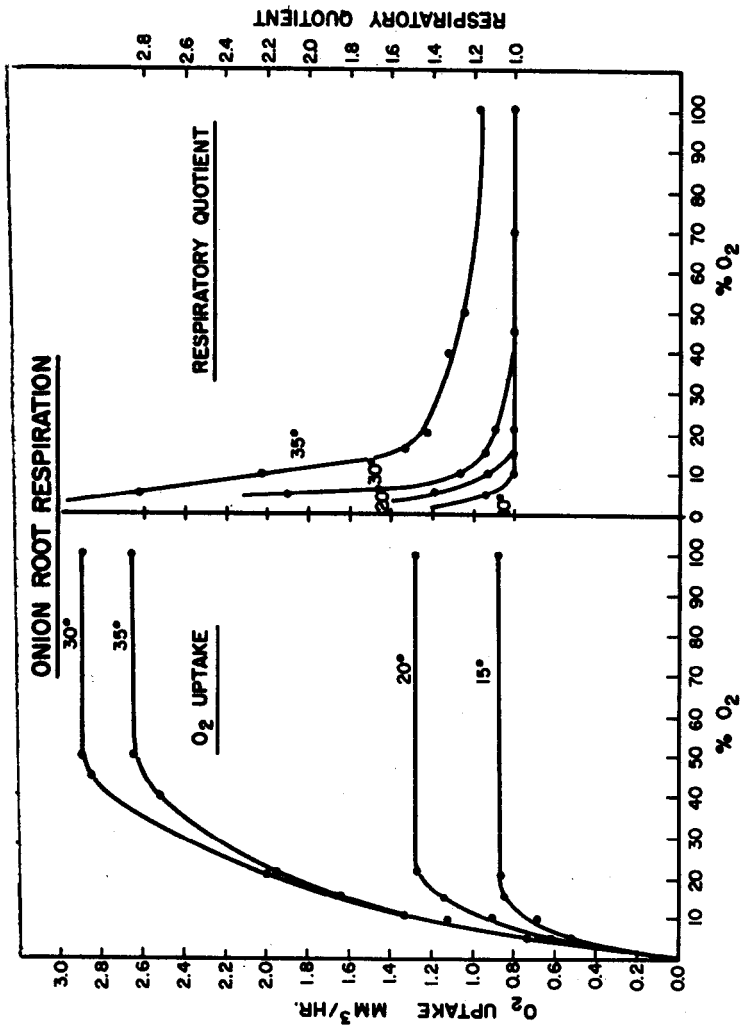


FIGURE 1.—Effects of temperature and O₂ tension on the respiration of onion root tips. (Drawn from data of Berry and Norris, 1949.)

interconversion which may occur, although it is also clear that separate pools may exist (e.g., Porter and May, 1955), so that all of the soluble sugars may not be immediately and equally available as substrates for respiration.

The earlier investigations showed that respiration may be supported by a variety of compounds (e.g., Spoehr and McGee, 1923), but these observations do not of course imply that several entirely different pathways exist. To the extent that a given compound is itself, or can be readily converted by the tissue into, an intermediate of the sequences outlined in subsequent chapters, it will be respired along with the intermediate arising from the true substrate—always provided, of course, that it gains access to the respiratory centers. Recent work with C^{14} -labeled materials has made it clear that a variety of compounds related to respiratory intermediates are actually metabolized and do not act merely as stimulants of the respiratory sequence without themselves being used up in the process (see Thomas *et al.*, 1957). Thus, for example, glycerol, pyruvate, and acids of the TCA cycle have been shown to be utilized (Chapter 3), and the ability of plant tissues to deal oxidatively with gluconate and some pentoses (Chapter 2) has been established. The oxidative breakdown of compounds such as acetate, glutamate, and alanine is brought about because these compounds are readily converted into known respiratory intermediates. These experiments show that even when food reserves such as proteins and fats are being utilized the pathways of dissimulation may be ones which are also utilized—in part—during the respiration of sugars.

Considerable importance has been attached to the respiratory quotient (R.Q.) in earlier work on respiratory substrates, and there is no doubt that the nature of the substrate usually determines what the value of the quotients is. The frequency with which values close to unity have been observed (James, 1953, Chapter IV) attests to the part played by sugars as the respiratory substrate in plant tissues. In recent years, however, reactions linking respiration with other events in the cell have been established, and it has become clear that these may

have repercussions on the R.Q. Appreciable changes from unity might result even though sugars continue to furnish the substrate. Some of these considerations are:

1. Incomplete oxidation of the sugar due to a diversion in synthetic events of respiratory intermediates with oxidation levels different from the original substrate (Chapter 10).
2. Reductive events such as sulphate and nitrate reduction, reductive syntheses (Chapter 9).
3. Oxidations and decarboxylations unrelated to respiration proper.
4. The retention of respired CO_2 in tissue fluids.
5. Nonphotosynthetic carboxylation reactions leading to acid accumulation. The acid fluctuations in succulents have, because of their magnitude, attracted most attention, but the elucidation of these reactions (see, e.g., Ranson and Thomas, 1960) and the general distribution of carboxylating enzymes in plants make the pickup of CO_2 a general possibility to be reckoned with. Experiments with C^{14}O_2 show in fact that such carboxylation reactions are of wide occurrence, and they achieve sizable proportions in some tissues other than succulents (e.g., germinating fatty seedlings and roots in certain salt solutions; see Chapter 8).

In addition to the above, the persistence of detectable fermentation in some tissues, e.g., senescent fruits, even when these are in air, has been recognized as a cause of high R.Q. values. It is frequently assumed that in bulky tissues, such as potato tubers, O_2 levels are sufficiently subnormal to induce some fermentation, but actual measurements (Burton, 1950) indicate that O_2 levels are not in fact limiting (see Laties, 1957, for a valuable discussion). Temporary high R.Q.'s due to alcoholic fermentation are a feature of the germination of some seeds in which the access of O_2 is limited by the tissues surrounding the embryo. In addition, even in actively growing tissues there may be alcohol accumulation. Betz (1955, 1957, 1958) has shown this to be so in corn root tips. An al-

ternative explanation, suggested by Kandler (1958), that reductive events may be responsible for the high R.Q. values was shown not to apply (Betz, 1958). In general, if the occurrence of a high R.Q. is due to persisting aerobic fermentation, it may be prevented by supplying additional O_2 (Ramshorn, 1957), and in manometric experiments the effect of the diffusion barrier maintained by the liquid bathing the tissue should not be overlooked (see Figure 1 and a very recent paper by Ohmura and Howell [1960]).

In the chapters that follow on the mechanism of plant respiration no attempt is made to trace the historical development of early theories; most of the work discussed has in fact appeared in the past twenty years. Of course, central and all-important concepts of plant respiration were developed well before this time, and it will be assumed that the reader has become familiar with the experimental and theoretical contributions of Pfeffer, Kostychev, and Blackman (see Thomas *et al.*, 1957; James, 1953; and Blackman, 1954) which have formed the basis for the subsequent work on intermediary respiratory metabolism.

The main trends of the recent work are the following:

- a. Intensive investigation of the respiratory enzymes which has been made possible by the introduction of new and improved methods, and the availability of intermediates and coenzymes. The recognition of the spatial localization of some of the respiratory enzymes in cells was an important factor in the development of our knowledge of pyruvate oxidation, about which very little was known ten years ago.
- b. The investigation by sensitive procedures, particularly chromatographic ones, which have allowed the separation, detection, and measurement of intermediates and cofactors which had hitherto escaped notice.
- c. The utilization of radioactive isotopes, particularly compounds labeled with C^{14} . These have helped in the elucidation of reactions *in vitro*, but, what is more important in the present context, they have allowed the testing of