PLANT PHYSIOLOGY

658


THE EFFECT OF pH AND TEMPERATURE ON THE ABSORPTION OF POTASSIUM AND BROMIDE BY BARLEY ROOTS1,2

LOUIS JACOBSON, ROY OVERSTREET, ROBERT M. CARLSON
AND JAMES A. CHÄSTAIN

DEPARTMENT OF SOILS AND PLANT NUTRITION, COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

It has long been recognized that the process of ion absorption has optimum ranges of pH and temperature, i.e., the physiological pH and temperature range. In general, there has been a tendency to assume that the factors of pH and temperature are not important provided they fall within the physiological range. However, marked changes in the pH of the culture media can result due to unequal absorption of cations and anions. This suggests that H+ and OH− are involved in the absorption process and accordingly they have been given a key role in a generalized expression for the absorption of cations and anions (9). This expression involves a metabolically produced ion carrier whose rate of production is dependent upon those factors which effect metabolism such as temperature, O2, substrate, etc.

For these reasons and because previous experience has led us to believe that pH and temperature, even within the so-called physiological range may exert a much higher degree of influence than generally suspected, absorption experiments have been carried out with KBr in which pH and temperature were rigidly controlled.

1 Received June 18, 1957.
This paper is based on work performed under contract No. AT-(11-1)-34, project 5, with the Atomic Energy Commission.

MATERIALS AND METHODS

Excised root material from Tennessee Winter barley 1954 crop, prepared as previously described (9) was used for these experiments. In all experiments, 7 grams of root material in 7 liters of solution was used. An absorption period of 3 hours was adopted throughout. During the course of the experiments, the solution was aerated with CO2-free air. The concentration of KBr in all culture solutions was nominally 0.005 M. During the absorption period, the pH of the solutions was frequently checked and maintained by the addition of KOH or HBr. The maximum deviation from a given pH throughout an experiment was never more than 0.1 pH unit. The small changes in concentration caused by the addition of KOH or HBr were negligible and were found, by supplementary experiments, to have no detectable effect on the rate of absorption. The absorption vessels were immersed in a water bath in which the temperature was controlled within 0.1°C.

At the conclusion of an experiment, the roots were separated from the culture media by means of a nylon mesh filter and washed for 10 seconds in running distilled water. After this period of washing, the roots were found to possess an apparent free space of 18% as determined by leaching experiments. Potassium was determined by a flame photometric proce-
dure and bromide by oxidation to bromate (12). All data are expressed in milliequivalents per kilogram fresh weight of roots and have been corrected for apparent free space.

**Results**

The absorption of K⁺ and Br⁻ as functions of pH were determined at fourteen different temperatures from 0° C to 50° C. The essential character of the interaction of temperature and pH on K⁺ absorption is shown by the five curves of figure 1. In all cases K⁺ was lost from the roots at the lower pH values. At any given pH, the loss was marked by temperature. In general, the lower the temperature, the lower the pH at which K⁺ was neither lost nor gained by the roots. This is in essential agreement with previous results (9). Of particular interest is the fact that at most temperatures, absorption is not reduced even at pH values of 10. Only when the temperature exceeded 37.5° C was the absorption observed to drop markedly at the high pH values.

The picture for the temperature and pH relationships for Br⁻ absorption is presented in figure 2. As in the case of K⁺ absorption, less Br⁻ is taken up at low pH values at all temperatures and, at a given pH, the uptake of Br⁻ is modified by temperature. The major difference between K⁺ and Br⁻ absorption is the greater sensitivity of the latter to high pH values.

Absorption curves for K⁺ and Br⁻ at 35° C are presented in figure 3. At this temperature, the maximum uptake of the two ions are very similar in magnitude and the absorption curves can be most readily compared. It may be clearly seen that Br⁻ uptake falls off rapidly at pH values above 5 whereas K⁺ uptake is but little affected. Below pH 5, the absorption of both ions falls off rapidly but that of K⁺ shows a sharper decrease; that is, K⁺ absorption appears to be somewhat more sensitive to lowered pH. Essentially similar conclusions can be drawn by comparing the absorptions at other temperatures.

In figures 4 and 5, the effect of temperature on the absorption of K⁺ and Br⁻ at four selected pH values is presented. In constructing these curves, the data for the fourteen different temperatures were used. It is evident from figure 4 that the temperature optimum, at least for a 3-hour absorption period, of K⁺ uptake shifts with increasing pH. At pH 4, the optimum is about 22° C, at pH 6, the optimum is 30° to 35° C. Other data, not presented in these curves, indicate that the optimum tends to move back to lower temperatures as the pH increases above 6. The curves in figure 4 show a considerable amount of ir-
The absorption of K⁺ from solutions of 0.005 N KBr as a function of temperature at different pH values. Three-hr absorption period.

regularities which are most probably real since a given irregularity may be repeatedly obtained.

The Br⁻ data, presented in figure 5, also indicate a shift of optimum temperature with pH, the optimum temperatures being a little higher than in case of K⁺ uptake. At pH 3, the optimum is about 23°C and at pH 5, the optimum is about 37°C. Again at pH values above 5, the optimum shifts back to lower temperatures.

**DISCUSSION**

Before discussing the data, it is perhaps pertinent to consider the question of "apparent free space" as it applies to absorption data. It has been found that a fraction of the ions taken up may be readily removed by subsequent treatment with distilled water. Recently a number of investigators have reported that roots have an apparent free space for solutes and a fraction of the solutes entering a root does so by non-metabolic means (2, 7). The amounts of K⁺ and Br⁻ observed in this research to be easily removed by water treatment agree approximately with values reported by the above investigators. We have corrected the absorption data for these loosely held ions with-

out, for the time being, committing ourselves as to the interpretation of this fraction of the uptake. However, it should be emphasized that these corrections are quite minor and have a negligible effect on the character of the absorption curves except under conditions of relatively low uptake. In general, when the concentration of the absorbed ions becomes large relative to that of the culture medium, the apparent free space correction is of little importance in so far as the absorption data are concerned. It is only when the concentration of the absorbed ions in the tissues is of the same magnitude as or is less than that of the external solution that apparent free space assumes important proportions.

Attempts have been made to utilize Q₁₀ values of absorption as an aid in establishing the mechanism of the absorption process (10, 11). An examination of the data reported here reveal a marked dependence of Q₁₀ on pH as well as on temperature range. Moreover, it is likely that this dependence will vary with different cations and anions. For these reasons, the comparison of Q₁₀ values for an anion and a cation at a given pH and temperature range probably is of little general significance.

Of particular interest is the relationship between the amounts of cation and anion uptake as a function of pH and temperature. By the appropriate selection of pH and temperature, it is possible to have excess K⁺ absorption, excess Br⁻ absorption or equal absorption of K⁺ and Br⁻. These separate conditions would result in quite different chemical changes in the root (8). Thus it appears that the pH and temperature of the external solution have a major influence on the biochemistry of the root.

The data presented in the curves have interesting implications concerning the mechanism of absorption. In a previous publication (9), it was suggested that absorption could best be described by the following expressions.

\[ \text{M}^+ + \text{HR} = \text{MR} + \text{H}^+ \] for cations

and

\[ \text{A}^- + \text{R'}\text{OH} = \text{R'A} + \text{OH}^- \] for anions

Our present view is that these equations should be considered as generalized formulations of the absorption process. It is quite possible that they do not represent specific chemical reactions. More than one reaction and more than one compound may be involved for each ion. The value of the above expressions is that they conveniently summarize the overall features of mineral absorption. That is, they indicate the necessity of a carrier produced by the plant and the requirement for electrical balance both within the plant and the culture media. Furthermore, they emphasize the importance of H⁺ and OH⁻ and they allow for a degree of independence of cation and anion absorption.

The data for the absorption of K⁺ and Br⁻ are consistent with this scheme. Br⁻ absorption is markedly reduced as OH⁻ concentration increases whereas K⁺ absorption is little affected. At low pH values, the

---

**Fig. 4.** The absorption of K⁺ from solutions of 0.005 N KBr as a function of temperature at different pH values. Three-hr absorption period.

**Fig. 5.** The absorption of Br⁻ from solutions of 0.005 N KBr as a function of temperature at different pH values. Three-hr absorption period.
absorptions of both K⁺ and Br⁻ are markedly reduced. However at the very low pH values, the situation is complicated by tissue injury (9). In spite of this, K⁺ absorption is more affected by increased H⁺ concentration than Br⁻ absorption. Two explanations of this present themselves; 1) the cation absorbing system is more subject to H⁺ injury than the anion absorbing system, or 2) both systems are equally subject to H⁺ injury but the more rapid decrease of K⁺ absorption at the lower pH values represents a competing effect of H⁺ for K⁺. If the latter is true, then the losses of K⁺ which occur under conditions where Br⁻ is still being absorbed, i.e., figure 3, may represent, in part, a reversal of absorption although not necessarily through the same pathway that uptake occurs (1).

The concept of reversibility of accumulation has recently been challenged by Epstein on the basis of short term translocation experiments with radioactive isotopes (3). However, there appears to be substantial evidence that the accumulation process is reversible. That is, it has been well established that roots may be depleted of previously accumulated ions by translocation to the shoots. Also it has been commonly observed that older leaves are depleted of certain accumulated ions by new growth. A good example of such evidence is that of Woolley et al (13) showing that Cl⁻ which has been accumulated by the root is subsequently lost from the root by translocation. Since this occurred at a time when the culture media was essentially free from Cl⁻, it would appear to be conclusive evidence that root cells may lose previously accumulated ions in the normal course of growth.

The results of this research show that such factors as temperature and pH have a profound influence on the absorption process. Furthermore past experiments have shown a marked interaction between the ion under study and other cations and anions which may be present (5). This interaction may take the form of a stimulation or depression of absorption which is pH dependent. All of these influences apply to roots grown from a given sample of seed.

It has been found that each sample of seed gives roots having a somewhat different absorption behavior. Even using the same variety will not insure consistent behavior. For example, roots obtained from a sample of Sacramento barley seed 1951 crop showed a considerably greater reduction of K⁺ uptake at low pH values than those obtained from the 1948 crop of the same strain grown at the same place. Such factors as pH, temperature, concentration, etc. affect the absorption process in different varieties or even in different samples of the same variety in only roughly a similar manner. Large quantitative as well as qualitative differences are frequently encountered.

Even when a series of experiments is conducted with a selected batch of seed, it has been found necessary to consider the factor of age of the seeds, particularly when an extended period of time is involved. That is, the absorption characteristics of roots grown from a sample of seed will begin changing after a period of seed storage and moreover the change may manifest itself at certain temperatures and pH values and not at others. For example, a sample of Atlas 46 barley, after storage for 2 years, began to display a markedly enhanced uptake of Br⁻ at pH 5 and at 35.5°C, although at 23°C, its absorption behavior did not differ appreciably from that observed previously. It has therefore been found necessary to check the absorption characteristics at different temperatures and pH values, from time to time, to verify constancy of behavior during the course of a series of experiments.

Thus any satisfactory mechanism of absorption must allow for the interplay of all the various factors mentioned above. It must not be limited to a narrow range of environmental conditions nor to any specific sample of material. As an experimental procedure, it would appear unwise to explain the kinetics of absorption solely or largely by means of mathematical analyses of absorption curves obtained under a given set of conditions as has been attempted recently (4, 6). That is, we must conclude that it is highly unlikely that a mechanistic and quantitative picture of absorption based on apparent similarities to known kinetic models and dependent upon restricted experimental data will be generally applicable.

SUMMARY

The influence of pH and temperature of the culture media on the absorption of K⁺ and Br⁻ by excised barley roots was studied. These two factors were shown to have marked and differing effects on the uptake of the two ions. The significance of apparent free space as a correction factor was briefly discussed. When material possessing a high absorption capacity is used, the correction due to apparent free space appears to be insignificant in most cases.

On the basis of the experimental data and other considerations, it is concluded that the accumulation process is reversible.

The significance of Q₁₀ values and the applicability of kinetic absorption models are discussed in terms of the experimental results.

LITERATURE CITED


METABOLIC PROCESSES IN CYTOPLASMIC PARTICLES OF THE AVOCADO FRUIT. IV. RIPENING AND THE SUPERNATANT FRACTION

ROGER J. ROMANI and JACOB B. BIALE
DEPARTMENT OF SUBTROPICAL HORTICULTURE, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA

The activities of the cytoplasmic particles reported in the first three papers of this series (1, 4, 5) were obtained mostly from firm or pre-climacteric fruit. Little or no attention was paid to the mitochondrial reactions of ripe fruit and of fruit in the course of ripening. In the avocado, a well described pattern of respiration for the intact fruit is associated with the ripening process (2). This pattern is essentially the same as that reported for other fruits and is referred to as the "climacteric" (10). Under appropriate temperature and oxygen tension, a rapid rise in respiratory activity takes place within a short time after harvesting. This so-called "climacteric rise" reaches a peak and is followed by a decline referred to as the post-climacteric phase. It is during this final stage that senescence sets in, resulting in breakdown and death. Fruit physiologists have been concerned with the onset of the climacteric rise since it might be considered as the "beginning of the end," the prelude to senescence and death. Recent hypotheses concerning the onset of the rise revolve around the phenomenon of the coupling of phosphorylation to respiration (13) or the relationship between the levels of ADP and ATP (14). It is the purpose of this paper to examine the biochemical reactions of avocado particles prepared from fruit at different stages of the climacteric.

MATERIALS AND METHODS

The Fuerte and Anaheim varieties of the avocado, Persea americana Mill, were used, but no substantial qualitative or quantitative differences between these varieties were observed. Respiration of the whole fruit was determined by methods described by Biale and Shepherd (3), with the exception that oxygen consumption was measured with the Beckman Oxygen Analyzer (19). The preparative procedure and the determination of oxidative and phosphorylative activities for hard fruit were described previously (4). In brief, the method consisted of blending 150 grams of peeled and grated avocado tissue with 300 ml of 0.5 M sucrose. The homogenate was then strained through cheesecloth and centrifuged at 500 x g for 5 minutes to separate out large fragments, unbroken cells, etc. The resultant supernatant solution was then centrifuged at 17,000 x g for 15 minutes. After this high-speed centrifugation, the supernatant solution was saved for further study and the cytoplasmic particles contained in the pellet were washed by resuspension in 0.5 M sucrose and recentrifuged at 17,000 x g. In the case of soft fruit, the tissue was prepared with a coarse grater. The speed of blending was adjusted so as to prevent the formation of a suction cone in the homogenate.

A major portion of this study was concerned with the effects of the deproteinized supernatant fraction on ripe fruit mitochondria. For this purpose, the supernatant solution resulting from the first high speed centrifugation was placed in conical centrifuge tubes and the proteins were denatured by immersing in boiling water for five minutes. Coagulated proteins were removed by centrifugation at 1000 x g for five minutes.

The following abbreviations are used in this paper: DNP, 2,4-dinitrophenol; DS, deproteinized supernatant fraction; KG, α-ketoglutarate; AMP, adenine monophosphate; ADP, adenine di-phosphate; CoA,