light, the light acting as the agent only in the ultimate step in chlorophyll formation. Sugar and other organic and inorganic compounds are presumably needed for the initial and continuous formation of protochlorophyll. A short time after chlorophyll has been formed, the plant becomes capable of photosynthesis and new sugars are thus supplied for further pigment synthesis. When such a seedling is deprived of light, chlorophyll formation is halted by the direct removal of the agent in the protochlorophyll-chlorophyll transformation; secondarily, sugar production ceases, and this affects the plant's ability to synthesize protochlorophyll, and other simpler precursors of the chlorophyll molecule.

We have seen here, however, that removing the plant from the light not only halts further pigment synthesis but affects the plant's ability to maintain the pigments it already has.

The seedling returned to the dark fails to maintain both chlorophyll and carotenoids. In earlier work with oat seedlings (6) we had shown that when chlorophyll was formed, the carotenoids fall in concentration, suggesting that the carotenoid molecule might be involved in the synthesis of the chlorophyll molecule, perhaps by supplying the phytol group. In the light-independent destruction of the chlorophyll molecule, if the backward reaction followed the same course as the forward reaction in reverse, one might expect to get an accumulation of carotenoids as the chlorophyll content decreased. However, the study reported here shows clearly that when chlorophyll is destroyed, carotenoids are also destroyed. So no reciprocal relationship exists between the two pigments with respect to their light-independent destruction.

SUMMARY

One-week-old corn seedlings containing known amounts of chlorophyll and carotenoids were placed in the dark, and the time course of the destruction of the pigments followed. Both chlorophyll and carotenoids were destroyed rather rapidly at 27° C in the dark. All the chlorophyll disappeared completely after 120 hours in the dark, while the carotenoids fell to 40 % of their original value.

It was found that, even though the initial pigment concentration varied as much as 150-fold, the time required for the destruction of a given percentage of pigment was independent of the initial concentration. This suggests that the light-independent pigment destruction follows the kinetics of a first-order reaction.

Adding sucrose to the environment of the seedlings when placed in the dark effectively protected for a time against depletion of both chlorophyll and carotenoids. Concentrations of 3 % sucrose protected the carotenoids completely from destruction in the dark, whereas the chlorophyll concentration decreased 30 % in 3 % sucrose as compared with 72 % destruction in the absence of sucrose.

The protective effect of exogenous sucrose on pigment destruction seemed to be to delay the onset of destruction rather than to prevent it from occurring.

LITERATURE CITED

1. Koski, V. M. and Smith, J. H. C. Chlorophyll formation in a mutant, white seedling—3. Arch. Biochem. Biophys. 34: 189–195. 1951.
2. Rabinowitch, E. Photosynthesis, Vol. I. Pp. 339–558. Interscience Press, New York. 1945.
3. Bonner, J. Plant Biochemistry. Pp. 470–478. Academic Press, Inc., New York. 1950.
4. Rudolph, H. Über die Einwirkung des Farbigen Lichtes auf die Entstehung der Chloroplastenfarbstoffe. Planta 21: 104–115. 1933.
5. Seybold, A. and Egle, K. Leichtfeld und Blattfarbstoffe II. Planta 28: 87–123. 1938.
6. Frank, S. The relation between carotenoid and chlorophyll pigments in Avena coleoptiles. Arch. Biochem. 30: 52–61. 1951.
7. Garton, G. A., Goodwin, T. W., and Lijinsky, W. Studies in carotenogenesis. I. General conditions governing β-carotene synthesis by the fungus *Phycomyces Blakesleanus* Burgeff. Biochem. Jour. 48: 154–163. 1951.
8. Chichester, C. O., Wong, P. S., and Mackinney, G. On the biosynthesis of carotenoids. Plant Physiol. 29: 238–241. 1954.
9. Goodwin, T. W. The Comparative Biochemistry of the Carotenoids. Pp. 19–23, 91–93. Chapman and Hall, London. 1952.
10. Strain, H. H. Leaf Xanthophylls. Carnegie Institute Publ. 490: 1–147. 1938.

RESPIRATION AND SALT ABSORPTION BY EXCISED BARLEY ROOTS[1,2]

RAYMOND HANDLEY AND ROY OVERSTREET

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

The fact that absorption of salt by plant tissue is usually accompanied by stimulation of respiration has been recognized for some twenty years.

Salt effects upon respiration have been studied in various types of plant tissue: wheat roots (8 to 10),

carrot slices (15 to 20), beet slices (18), potato discs (23 to 25) and barley roots (11) among others. The phenomenon may then reasonably be believed to be of general occurrence.

In the early thirties Lundegårdh and Burström began a series of researches which culminated in an ingenious theory to account for the simultaneous absorption of salt and increase in the respiratory rate. The point of view of Lundegårdh and Burström has

been espoused with modification by Robertson and his co-workers.

The bases of this theory are well known and need not be presented here. In brief, it is proposed that oxidized cytochrome is capable of attracting anions from the external medium and that accumulation occurs either as a result of the inward movement of anions along a chain of stationary cytochrome enzymes or under Robertson's modification, of the inward diffusion of particles (mitochondria?) bearing the oxidized cytochrome-anion complex through a region in the tissue which is impermeable to free anions. At the inner boundary of this barrier, anions are released as reduction of the cytochrome occurs and outward diffusion of the particles begins. This is depicted schematically in figure 1.

The reaction occurring at the inside boundary (i.e., presumably at the tonoplast, if the model is assumed to represent a single cells) is as follows:

$$FH_2 + 2\,Cyt\,Fe^{+++} \longrightarrow 2\,Cyt\,Fe^{++} + 2\,H^+ + F$$
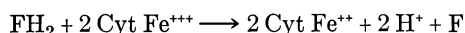
where F and FH_2 represent oxidized and reduced forms respectively of the flavoprotein enzyme. At the outer boundary the reoxidation of cytochrome occurs:

$$2\,H^+ + \tfrac{1}{2}\,O_2 + 2\,Cyt\,Fe^{++} \longrightarrow 2\,Cyt\,Fe^{+++} + H_2O$$
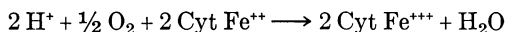
It should be noted that this scheme contains an assumption not readily susceptible of proof, namely that the loss of an electron consequent upon the oxidation of the iron of reduced cytochrome must be balanced by the acquisition of an anion. Of the components of the cytochrome system only cytochrome c has been isolated and characterized. Calvin and Martell (3) state that ferrocytochrome c is diamagnetic while the ferric form is paramagnetic, having one unpaired electron. The bonds of both forms are covalent and the only change taking place in the course of its biological action is the gain or loss of an electron. If, as the theory assumes oxidation of ferrocytochrome results in an unbalanced positive charge,

the charge so produced may be balanced either by the absorption of an anion as shown in figure 1, or by the disappearance of H^+ produced in the substrate oxidation. In the former case, the H^+ utilized in the reoxidation of reduced cytochrome must come from another source, possibly the external medium as shown.

According to the Lundegårdh view, increased respiration results from the stimulating effect of anions in general upon the activity of the cytochrome system. When anions are not provided in the external medium as in distilled water, the terminal oxidase becomes dependent upon endogenous anions presumed to be largely malate and is only very sluggishly operative.

Cations are viewed as being inert in this regard. They are presumed to pass readily through the salt barrier by a process of repeated exchange for H^+ derived from organic constituents of acid dissociation. Measurements of root surface potentials (9) indicate that the protoplasm is predominantly electronegative. The driving force for cation accumulation (fig 1) lies evidently in the production and utilization of H^+ at the inner and outer boundaries respectively.

Steward and his collaborators working in the thirties and early forties opposed the Lundegårdh hypothesis and presented evidence that, in potato slices at least, the cation plays a dominant role in the production of salt respiration as well as in other salt effects upon metabolism. In their work potassium ion was found to stimulate a number of metabolic processes: protein formation, water absorption, phenolase activity and hydrolysis of starch as well as respiration. Calcium ion was found to exert a depressing effect upon these same activities. The extent of these phenomena are clearly seen in the data of Steward et al (24).

Although postulating that the function of aerobic respiration in the salt absorption process lies in the provision of energy necessary for accumulation of ions against their diffusion gradients rather than in mere production of HCO_3^- and H^+ for exchange with other ions in the external medium, Steward and his co-workers did not speculate upon actual absorption mechanisms.

The present work was designed to investigate the phenomenon of salt respiration as it occurs in the material currently being used at Berkeley in ion absorption studies. In particular it was desired to learn whether or not the behavior of this material would support the postulation of a direct and causative link between the stimulation of respiration and the absorption process per se.

MATERIALS AND METHODS

All experiments in this study were carried out with the excised roots of Atlas barley grown from seed of the 1946 crop by the method of Jacobson and Overstreet (6). This method was modified slightly in that the preliminary treatment of the seed with H_2O_2 was eliminated. Respiration was determined as
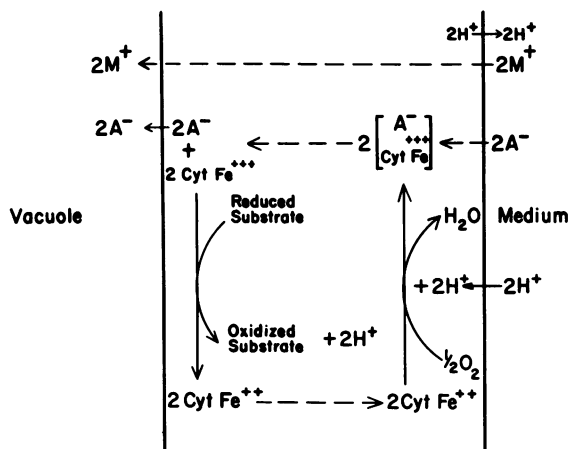

FIG. 1. Schematic representation of the Lundegårdh-Robertson hypothesis. M^+ = inorganic cation, A^- = inorganic anion.

O_2 absorbed. During the experimental period the excised roots were confined in glass stoppered, bubble-free reagent bottles completely filled with the appropriate solutions. The solutions to be used for any experiment were aerated for a period of 48 hours at 26° C prior to the start of the experiment.

Except where otherwise noted, a root to solution ratio of 3 gm/l was used, the weight of roots being calculated for each bottle according to its total capacity.

The bubble-free stoppered bottles containing roots were kept at 26° C ± 0.1° C during the experiment by means of a water bath. During this period the solutions were "stirred" by inverting the bottles several times every 5 minutes.

As the experimental period for each sample ended, the bottle was removed from the bath and duplicate samples were taken for analysis. These were collected in 250-ml reagent bottles, glass stoppered and calibrated for total volume. Collection was made using a siphon, the delivery end of which was kept below the level of liquid in the reagent bottle to avoid introduction of oxygen by splashing. The collection bottles were completely filled and stoppered immediately.

The difference in concentration of oxygen between the initial sample and that taken at the end of the experimental period was used to determine the O_2 absorbed; this was expressed as millimoles/kg fresh weight. Oxygen was determined by Winkler's iodometric method (22).

In this way very reliable results were obtained, the difference between duplicate samples being usually less than 0.2 %.

This procedure for measurement of respiration was adopted instead of the conventional Warburg method because it permits use of root solution ratios comparable to those heretofore used at Berkeley in ion absorption studies. Also it permits measurement of both absorption and respiration by the same root sample thus eliminating one source of variability.

In each case, where the effects of salts upon the respiration rate were being determined, a control determination of respiration in distilled water was made simultaneously in order to eliminate errors arising from differences between batches of roots. The average deviation from the mean respiration rate during a 13-week period amounted to 3.0 %.

After samples of solution had been withdrawn for measurement of O_2, the roots were immediately transferred to a nylon screen, thoroughly washed with distilled water and then transferred again to containers for drying preparatory to analysis for absorbed ions. In every case an initial sample of roots was also analyzed, the amount of a given ion absorbed being determined as the difference between the contents of initial and treated samples. Results of the analyses were expressed as meq absorbed/kg fresh weight.

EXPERIMENTAL RESULTS

PRELIMINARY EXPERIMENTS: The method of measuring respiration and absorption in closed bottles out-lined in the preceding section has not heretofore been widely employed. While it has the advantages previously mentioned, this procedure is obviously suitable only for measurements over a curtailed experimental period, since the tissue respires and absorbs salt in a medium of waning O_2 and waxing CO_2 concentration. It must therefore be expected that at some period after introduction of the roots into the bottle, the influence of one or both of these factors will cause a significant lowering of the absorption and respiration rates, which in an open system might remain constant for a much longer period.

In preliminary experiments using distilled water and solutions giving rise to a pronounced stimulation of respiration it was found that the respiratory rate became constant within 10 to 15 minutes after the root samples had been introduced into the bottles.

These experiments revealed also a significant slackening of the previously stable respiration rate after 3 hours. While this is most probably due to depletion of O_2 and accumulation of CO_2, the possibility exists that other factors such as depletion of respiratory substrate or even production of inhibiting substances (27) may have been partly responsible. To avoid complications arising from differential effects of these factors upon the two processes to be studied and possibly correlated, no measurements of respiration were made after periods longer than 3 hours.

DEPENDENCE OF SALT RESPIRATION UPON CONCENTRATION: Three experiments were devoted to an investigation of the relationship between respiration, absorption and concentration of various salts in the external solution. The data obtained are shown in figures 2, 3 and 4. The experimental period was 3 hours and the root to solution ratio, 5 gm/l.

The data depicted in figures 2 and 3 indicate that the effect of salt upon respiration becomes maximal at relatively low concentrations, no increase in respiration resulting from addition of salt above 0.01 N. Above this level, however, absorption of both cation and anion continues to be a function of concentration. This is in agreement with results obtained by Robertson and Wilkins (19) working with carrot discs.

The theory of Lundegårdh described in the introduction, according to which electrons carried outward by the cytochrome system are exchanged for anions when oxidation of cytochrome occurs, postulates a maximum value of 4 for the ratio, anions accumulated/O_2 consumed in salt respiration. When the accumulation of inorganic anions from the medium is proceeding at the maximum possible rate, i.e., when the oxidized cytochrome molecules are "saturated" with such ions and are not transporting any internally produced anions, the value of this ratio must be maximal at 4. This postulate is derived simply from the fact that 4 electrons are accepted in the reduction of each molecule of oxygen utilized in the reoxidation of cytochrome oxidase.

It is evident in the data presented in figures 2, 3 and 4 that constancy of the ratio was not shown by this material. The ratio tended to increase with con-
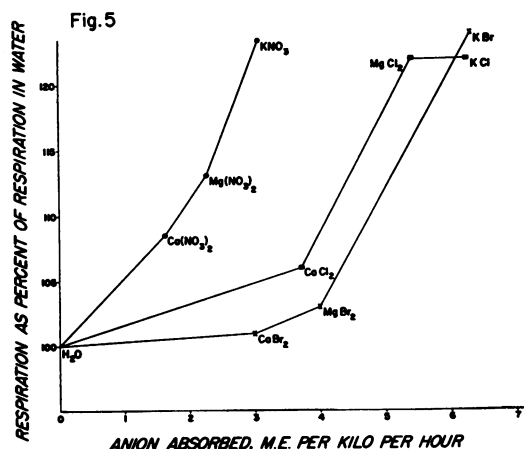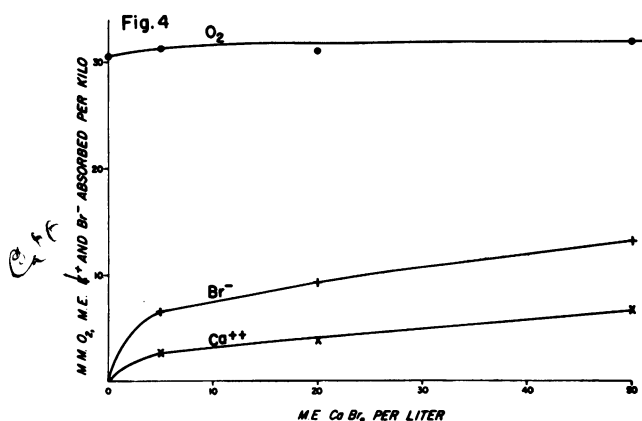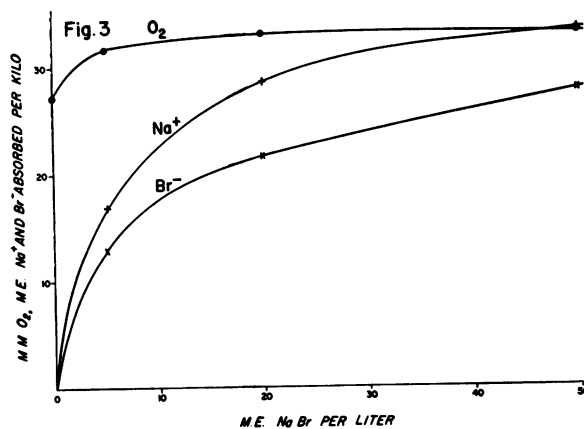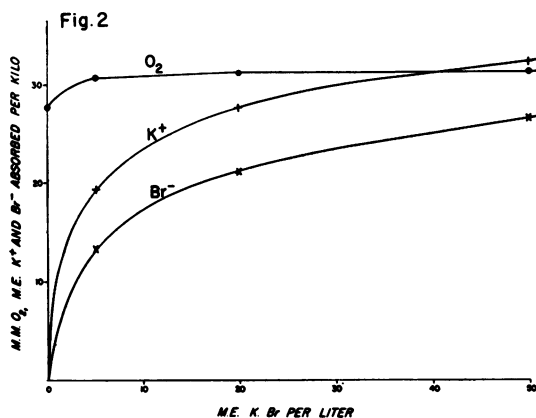
FIG. 2. Respiration and absorption vs conc of KBr.
FIG. 3. Respiration and absorption vs conc of NaBr.
FIG. 4. Respiration and absorption vs conc of CaBr₂.
FIG. 5. Respiration vs anion absorption in 0.005 N solutions of nitrates, chlorides and bromides.

centration of salt in the medium. Values in excess of the postulated maximum were obtained with all three salts, ranging from 4.3 to 7.9 in KBr, from 2.8 to 4.6 in NaBr and from 9.3 to 18.7 in CaBr₂.

The results obtained with CaBr₂ are especially striking since only a very slight and possibly not significant increase in respiration was obtained although absorption of bromide from this salt at 0.005 N is approximately half that occurring in a solution of KBr at the same concentration. The very small increase in respiration indicated may possibly be due to the presence of small amounts of alkali metals as impurities or may be wholly fortuitous. Repeated trials with CaBr₂ failed to show any signficant stimulation by this salt.

To be consistent with the Lundegårdh-Robertson hypothesis the passivity of CaBr₂ could not be explained as due to a specific depressant effect of Ca⁺⁺ upon the activity of the cytochrome system since, in this case, bromide absorption would, of course, be suppressed along with the salt respiration. There remains, however, the possibility that Ca⁺⁺ exerts such an effect upon some process associated with the non-cytochrome mediated basal respiration. Conceivably, in the latter case, a bromide induced stimulation of the cytochrome system might be masked when total respiration in CaBr₂ is measured. In an attempt to clarify this point the following experiment was carried out. Respiration and bromide absorption were determined in solutions of KBr, CaSO₄, KBr + CaSO₄ and in water in order to obtain a reference value. Measurements of respiration and absorption in this experiment were made at ½ and 1½ hours after the excised roots were placed into bottles. In view of the linearity of the time curve in this interval the differences between O₂ and Br⁻ absorbed measured at ½ hour and at 1½ hours represent rates of respiration and absorption. A root solution ratio of 3 gm/l was used. The results are shown in table I.

The lack of any depressant effect of CaSO₄ upon total respiration makes it unlikely that Ca⁺⁺, at this level at least, exerts any such effect upon the basal

TABLE I

EFFECT OF CaSO₄ UPON SALT RESPIRATION AND
ABSORPTION IN KBr SOLUTION

MEDIUM	RESP RATE	SALT RESP RATE	Br⁻ ABSORP	INCREASE IN SALT RESP	INCREASE IN Br⁻ ABSORP
	millimoles O_2/kg fresh $wt \times hr$		meq/kg fresh $wt \times hr$	%	%
H_2O	10.1	..	..	...	...
$CaSO_4$	10.1	0	..	...	...
KBr, 0.005 N	11.7	1.6	6.5	...	...
KBr+CaSO₄, 0.001 N ..	12.3	2.2	7.9	37.4	21.6

respiration; the salt respiration due to $SO_4^=$ would presumably be negligible since it is only very slowly absorbed by this material.

The stimulation of bromide absorption by Ca^{++} in certain concentration ranges has been known for some time; it is sometimes called the " Viets effect " being named for its discoverer (14, 26). The mechanism of this effect is still a matter of speculation. It is seen in table I that this stimulation of bromide absorption by $CaSO_4$ was accompanied by a corresponding increase in the salt respiration. A specific depressant effect of Ca^{++} upon either the basal or salt respiration was then not found. The results are consistent with the supposition that absorption of bromide entails no stimulation of respiration, the effect noted with KBr and NaBr being due perhaps to the cations absorbed; the effect of Ca^{++} upon absorption of K^+ is similar to that of Br^- (26).

ROLE OF CATIONS IN SALT RESPIRATION: It was earlier noted that the hypothesis orignated by Lundegårdh places special emphasis upon the role of anions in the production of salt respiration. According to this view cations move into the tissue by a process of passive exchange for hydrogen ions and hence are assigned no vital part in the observed stimulation. The hypothesis further suggests that all monovalent inorganic anions are of the same effectiveness; e.g., absorption of a given amount of bromide should be accompanied by the same respiratory response as would the absorption of a like amount of nitrate or chloride.

However, the results of the experiments described in the preceding section suggest that certain cations, either in the absorption process or because of some part they play in metabolism after absorption, may affect the respiration rate.

To further investigate the possible role of cations in the stimulation of respiration, experiments were carried out in which respiration and absorption were measured in solutions of 1) K, Mg and Ca nitrate, 2) K, Mg and Ca bromide and 3) K, Mg and Ca chloride.

The effect of slowly absorbed cations in reducing the absorption rates of their associated anions in single salt solutions is well known. It was to be expected therefore that the amount of nitrate, bromide or chloride absorbed from solutions of the same anionic concentration would depend upon the cation associated.

If the cation were inactive in the stimulation of respiration, a plot of anion absorbed (NO_3^-, Br^- or Cl^-) versus respiration should result in a straight line regardless of the nature of the cation associated with a given value of anion absorption.

The results of these experiments are shown in figure 5. The plot obtained for nitrate salts was approximately linear indicating possibly that in the case of these the principal causative agent in the respiratory stimulation is the anion absorbed. However, some stimulation due to potassium is suggested.

The reduction of nitrate to other forms of nitrogen in the plant might be expected to stimulate respiration whether or not such stimulation is attendant upon the absorption process per se. Nitrate then is perhaps a particularly unsuitable anion for studies of this kind. Hamner (5) reports that in intact tomato plants, the reduction of NO_3^- and the appearance of NO_2^- is accompanied by a parallel depletion of stored carbohydrate and by striking increases in the respiratory rate amounting to 100 to 300 %. The mechanism of nitrate reduction in plants is not completely known. Nitrite is presumably the first intermediate in the stepwise reduction to amino nitrogen since this appears very rapidly in the tissues of plants supplied with nitrate (5). Stimulation of respiration by nitrate may be related to the utilization of alpha keto acids by some other intermediate of the reduction process (possibly hydroxylamine) in the eventual formation of amino acids.

The data obtained in pretreatment experiments to be described subsequently indicate significant declines in the nitrate content of these roots when they are allowed to stand in distilled water. In one such experiment, roots with an intial nitrate content of 4.21 meq/kg were found to have 3.00 meq/kg after 1½ hours in water while after 3½ hours this had declined to 1.68 meq/kg. While these changes are probably due to the reduction of absorbed nitrate, the possibility remains that nitrate may have "leaked" from the tissue. The nitrate curve of figure 5 in any case is doubtful since part of the nitrate absorbed was undoubtedly reduced or was lost to the medium after absorption. The extent of the discrepancy between the nitrate absorbed and that found in the roots upon analysis is unknown.

In the case of bromide salts a vastly different picture is revealed. Bromides of calcium and magnesium alike produce little stimulation whereas KBr is again very effective. This again suggests that absorption of bromide does not affect the respiratory rate.

The experiments with chloride salts produced unexpected results in that the stimulation caused by $MgCl_2$ equaled that of KCl although the amount of chloride ion absorbed from its magnesium salt was about 25 % less. This was surprising in view of the almost negligible response obtained with $MgBr_2$. The experiment was repeated with similar results.

DEPENDENCE OF SALT RESPIRATION UPON THE AC-
CUMULATION PROCESS: In view of the results obtained
in the previous experiments which suggest that ab-
sorption of certain ions by this material is not accom-
panied by a rise in the respiratory rate, the question
arises whether the salt stimulation observed with
many but not all salts occurs as a result of the opera-
tion of the absorption mechanism or is due to specific
effects of ions upon metabolism after absorption has
occurred.

An attempt to answer this question was made by
pretreating the excised roots in various solutions and
subsequently measuring their respiration in distilled
water. Pretreatment was carried out in aerated solu-
tions and in distilled water.

In two experiments (fig 6) the roots were pre-
treated for 2 hours in $0.005 N$ and $0.010 N$ solutions
of KNO_3 and $CaBr_2$, respectively. After pretreat-
ment they were washed and transferred quantitatively
to the closed bottles containing distilled water. Res-
piration was determined in the one-hour interval of
$\frac{1}{2}$ to $1\frac{1}{2}$ hours after start. Analyses were made for
nitrate and bromide, respectively, and the mean con-
tent of these ions during the experimental period cal-
culated as $\frac{1}{2}$ the sum of the contents at the begin-
ning and end of the hour.

It is evident from the data presented in figure 6
that the level of nitrate in the tissue has a decided
effect upon respiration in distilled water when pre-
sumably the absorption process is inoperative.
Robertson and Thorn (16) working with carrot discs
which were exposed successively to dilute KCl solu-
tions and distilled water found that a period of 20
hours was required to overcome the effects of the
absorbed salt when the electrolyte was replaced by
distilled water. Their results are then in agreement
with those obtained here.

The data of figure 6 show again the impotency of
$CaBr_2$ to affect respiration although considerable
bromide is absorbed.
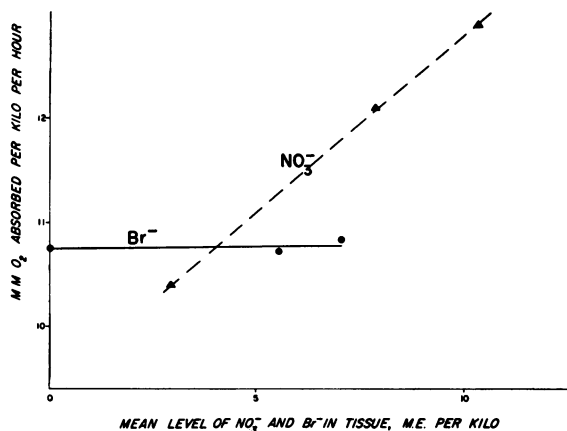
In a third experiment of this type the pretreat-

FIG. 6. Respiration in distilled H_2O after pretreat-
ment in $CaBr_2$ and KNO_3 solutions.

TABLE II
EFFECT OF PRETREATMENT UPON RESPIRATION IN H_2O

PRE-TREATMENT	SUBSEQUENT RESP RATE IN H_2O	MEAN LEVEL OF Cl^-	INCREASE IN RESP RATE DUE TO PRE-TREATMENT
	millimoles O_2/kg fresh $wt \times hr$	meq/kg fresh $wt \times hr$	%
None	10.1	2.9	...
Dist H_2O	10.6	2.8	...
KCl, $0.01 N$	13.2	17.9	24.6
$MgCl_2$, $0.01 N$ ..	13.8	11.9	30.1

ment solutions consisted of water, $0.01 N$ KCl and
$0.01 N MgCl_2$. Again a stimulated respiration was
observed when roots pretreated in these solutions
were placed in distilled water after thorough washing.
The data obtained are shown in table II.

These data are consistent with the supposition that
an enhanced respiration rate is associated with an
increased concentration of certain ions in the tissue
rather than with the operation of the accumulation
mechanism. However, it must be admitted that other
interpretations are possible. While the data of
Robertson and Thorn (16) indicate that very little
salt is lost from the tissue when it is transferred to
distilled water after accumulating KCl, the retention
of salt may be ascribed to its continuous loss and re-
absorption. The reabsorption process may be effi-
cient enough that the bulk of the medium shows no
measurable increase in salt concentration. The con-
centration of salt in the immediate vicinity of the root
is unknown. The data of Robertson and Turner (17)
indicating that no loss of salt already accumulated
takes place when the accumulation mechanism is
poisoned by cyanide are manifestly in conflict with
this interpretation.

EFFECT OF VARIOUS SALTS UPON RESPIRATION:
During the course of this study respiration was meas-
ured in a rather wide variety of media. The results
are presented in table III. In each case the concen-
tration of salt used was $0.005 N$. Respiration was
measured for the period between $\frac{1}{2}$ and $1\frac{1}{2}$ hours
from start. A control was determined in water in
every instance.

It is of particular interest that a considerable
stimulation was obtained in 2 cases where presumably
no absorption of anions was involved, i.e., in the
$KHCO_3$ solution and in the suspension of K bento-
nite. In the latter case the clay was electrodialyzed
for about 10 days, suspended in distilled water and
adjusted to pH 7 with KOH.

DISCUSSION

In dealing with such a complex, sensitive, and im-
perfectly understood system as living plant tissue, it
must be admitted that conclusions reached on the
basis of measurements of gross effects such as those
of salt absorption upon respiration are subject to

TABLE III

EFFECT OF VARIOUS SALTS UPON RESPIRATION

MEDIUM	RESP RATE IN H_2O	RESP RATE IN SALT SOLUTION	INCREASE IN RESP RATE
0.005 N	millimoles O_2/kg fresh wt × hr		%
KBr	10.1	11.8	16.8
KCl	11.1	13.7	23.4
$KHCO_3$	10.2	12.0	17.6
KNO_3	10.0	12.3	22.6
K_2SO_4	10.3	10.9	5.8
K bentonite ...	10.6	12.6	18.9
NaBr	10.0	12.1	21.0
$CaBr_2$	10.4	10.4	0
$CaSO_4$	10.3	10.3	0
$Ca(NO_3)_2$	10.0	10.8	8.0
$MgBr_2$	9.9	10.2	3.0
$MgCl_2$	11.1	13.5	21.6
$Mg(NO_3)_2$	10.0	11.3	13.0

varied interpretation. In particular it seems clear that the absorption of any particular ion may entail physical and chemical effects upon aspects of metabolism other than the absorption mechanism per se. For example it is probable that potassium plays an essential part in glycolysis (12) and nitrogen metabolism (4) as well as in the hydration of protoplasm. The effect of potassium upon respiration might then, in the absence of more specific information, be better ascribed to the sum total of its varied effects upon metabolism rather than to any one effect. The same thing might be said for any other ion. For this reason the relation of salt respiration to a general nonspecific effect of inorganic anions upon the cytochrome system postulated by the Lundegårdh hypothesis may be open to question.

In an attempt to show such a simple relationship Robertson and Wilkins (19) have made a rather exhaustive study of the ratio, anions absorbed/O_2 consumed in salt respiration. As indicated earlier, the theoretical maximum value for this ratio is 4. Their results indicate the same want of constancy for the value of the ratio as has been shown here. Very low values (less than one) were found when the medium employed was extremely dilute (6×10^{-4} N KCl) and increasing values with more concentrated solutions.

The low efficiency of the salt respiration in very dilute media was, as indicated previously, ascribed to partial satisfaction of the oxidized cytochrome carrier by endogenous anions. This explanation involves a significant departure from the original hypothesis in which the cytochrome system was pictured as being almost inoperative in the absence of inorganic anions. Under this modification the observed stimulations are not simply the result of an inherent necessity for oxidized cytochrome to be electrically satisfied by inorganic rather than organic anions. Rather they are due to some "catalytic" influence exerted by inorganic anions upon the whole cytochrome system, that portion satisfied by organic endogenous anions included. The acceptance of the cytochrome system as the

carrier in salt absorption is made more difficult by the finding of values of the ratio in excess of four since this would mean that more anions are being accepted than electrons released—an impossible situation.

The possibility that only certain cations absorbed may be efficient in production of the salt respiration is evident in the data presented in figure 5. The bromide and chloride data are particularly striking. Calcium and magnesium bromides alike have but little effect upon respiration although considerable amounts of bromide are absorbed from these salts. Potassium bromide on the other hand produces a relatively large stimulation. These data suggest that the absorption of Ca^{++}, Mg^{++} and Br^- has little, if any, effect upon respiration. K^+ on the other hand even when accompanied by an ion metabolically inert, such as Br^- causes a marked stimulation. The supposition of a specific effect upon respiration exerted by K^+ is borne out by the effect of K^+ absorbed from a pure bentonite clay suspension and from the bicarbonate, in which cases the possibility of anion effects is presumably eliminated.

Of the ions tested in this study (see table III), K^+, Na^+, NO_3^-, and Cl^- were found to exert stimulating effects upon respiration; Ca^{++}, Mg^{++}, $So_4^=$ and Br^- had little, if any influence. In no case did the presence of salt depress the respiration rate below that obtaining in distilled water. Of the ions whose absorption apparently entails no stimulation, only Br^- is rapidly absorbed by this tissue. Ca^{++}, Mg^{++} and $SO_4^=$ are only very slowly absorbed and the slight effects noted for these ions may be due to this factor. Provisionally it may be postulated that respiration is affected by ions which are both absorbed rapidly and are important in metabolism. In this connection it may be noted that recent work by Broyer et al (1) indicates that chloride may be essential for higher plants.

The results of the pretreatment experiments (fig 6) indicate that stimulations due to salts are not the results of effects exerted upon the absorption mechanism per se. The results shown here are in accord with those obtained by Robertson and Thorn (16) showing stimulation of respiration induced by absorption of salt to be persistent for long periods after removal of the tissue to a salt free medium.

Implicit in the assumption of a direct connection between the salt respiration and the absorption mechanism is the postulate that the plant tissue must do work in order to accumulate salt against a diffusion gradient. The extra energy expenditure is equated with the extra respiration which occurs. However, Robertson (15) has shown that the calculated energy required to effect accumulation from 0.01 N KCl is only about 1 % of that freed in the salt respiration accompanying the accumulation. Conversely, the data presented here indicate that ion accumulation may proceed in the absence of measurable salt respiration; considerable amounts of bromide are absorbed from solutions of $CaBr_2$ without any detectable salt respiration for example. These facts, it would seem, cast considerable doubt upon the

assumption that salt respiration represents work done by the plant in accumulating salt.

Overstreet and Jacobson (13) from a consideration of theoretical ion absorption models devised by Rosenberg (21) have shown that if certain appropriate and reasonable assumptions are made regarding the permeability characteristics of the tissue (or at least that part of it through which accumulation occurs), no work may be required for the accumulation of salt against a diffusion gradient other than that work associated with the production and alteration of an ion binding compound at the outside and inside phases respectively; these processes must indeed occur with a decrease in free energy or be linked to other reactions so occurring but the energy released need bear no particular relationship to the amount of work theoretically required for the actual movement of free ions against their diffusion gradient. In the Rosenberg models no such actual movement of substances against diffusion gradients is visualized; ions are moved inward only as complexes with metabolically produced binding compounds and always in the direction dictated by the diffusion gradients of these complexes. The possibility of the operation of an ion absorption mechanism similar to one of those devised by Rosenberg makes it unnecessary to postulate on theoretical grounds an increased energy production by tissue when salt absorption occurs. The fact that certain ions, notably Br^-, may be absorbed without appreciable effect upon the respiration rate suggests that although aerobic respiration is essential for salt absorption, this latter process involves particular chemical pathways; these are not necessarily associated with an increase in the overall rate of respiration. The dependence of the accumulation process upon the aerobic phase of carbohydrate degradation further suggests that these particular pathways involve component parts of the tricarboxylic acid cycle. One might speculate that alternate pathways in aerobic respiration involve the production of ion binding compounds from acids of the Krebs cycle. Such a process would involve no unique relationship between the amount of cation or anion absorbed and the stimulation of respiration obtained thereby. Further speculation as to the exact nature of the binding compounds is perhaps unwarranted. However, the response of respiration and absorption to the inhibitor 2,4-dinitrophenol may suggest that the alternate pathways involve phosphorylated compounds (20).

SUMMARY

1. A procedure for the determination of respiration and ion absorption by the same sample of tissue using closed bottles is described.

2. The relationship between concentration of salt in the external solution and the salt stimulation of respiration was investigated. Maximum stimulation was found to occur at concentrations of less than $0.005 N$ when the external solution consisted of KBr and NaBr. Negligible stimulation was obtained with solutions of $CaBr_2$ of concentrations ranging from $0.001 N$ to $0.05 N$.

3. The values of the ratio, anions absorbed/O_2 consumed in salt respiration, were calculated for various external concentrations of KBr, NaBr, and $CaBr_2$. These values were found to be variable and in some cases to exceed considerably the maximum postulated by the Lundegårdh hypothesis.

4. The possible depressant effect of Ca^{++} upon the ground respiration was investigated by measurement of salt respiration in KBr solutions to which was added $CaSO_4$. No evidence for such an effect was found.

5. The role of cations in the production of salt respiration was investigated. The data indicate that K^+ and Na^+ have a stimulating effect; Ca^{++} and Mg^{++} seem inert in this regard.

6. Salt respiration was found to persist after the roots were pretreated in KNO_3, KCl and $MgCl_2$ solutions and subsequently washed and transferred to distilled water. Similar pretreatment in $CaBr_2$ solutions and in water had no effect upon the respiration rate.

7. A number of experiments were performed to investigate the effects of a variety of salts upon respiration. Stimulation was obtained with $KHCO_3$ solutions and with a potassium bentonite suspension in which cases anion absorption could not have been appreciable. No stimulation was shown by $CaBr_2$ and $CaSO_4$ and only a very small stimulation by K_2SO_4 solutions.

8. The results obtained in this study permit several conclusions to be drawn concerning the nature of salt respiration. These may be listed as follows: a) Stimulation of respiration may be brought about by absorption of either cations or anions or both; it is not uniquely related to the absorption of either. b) The process of absorption need not occur concomitantly with the enhancement of respiration; the presence of certain ions in the tissue affects respiration after absorption has occurred. c) Absorption of certain ions appears to take place in this tissue without stimulating effect upon respiration. d) No fixed relationship is discernible between the amount of any ion absorbed and the amount of respiration occurring in excess of that taking place in distilled water.

LITERATURE CITED

1. BROYER, T. C., CARLTON, A. B., JOHNSON, C. M., and STOUT, P. R. Chlorine—A micronutrient element for higher plants. Plant Physiol. 29: 526–532. 1954.

2. BURSTRÖM, H. The mechanism of ion absorption. In: Mineral Nutrition of Plants, E. Truog, ed. Pp. 251–260. Univ. of Wisconsin Press, Madison, Wisconsin. 1950.

3. CALVIN, M. and MARTELL, A. E. Chemistry of the Metal Chelate Compounds. Pp. 1–613. Prentice-Hall Company, New York. 1952.

4. GREGORY, F. G. and SEN, P. K. Physiological studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and

potassium deficiency. Annals Bot. NS 1: 521–561. 1937.

5. HAMNER, K. C. Effects of nitrogen supply on rates of photosynthesis and respiration in plants. Bot. Gaz. 97: 744–764. 1936.

6. JACOBSON, L., OVERSTREET, R., KING, H. M., and HANDLEY, R. A study of potassium absorption by barley roots. Plant Physiol. 25: 639–647. 1950.

7. LOOMIS, W. F. and LIPMANN, F. Reversible inhibition of the coupling between phosphorylation and oxidation. Jour. Biol. Chem. 173: 807–808. 1948.

8. LUNDEGÅRDH, H. Quantitative relations between respiration and salt absorption. Kgl. Lantbruks-Högskol. Annaler 16: 372–403. 1949.

9. LUNDEGÅRDH, H. Spectroscopic evidence of the participation of the cytochrome–cytochrome oxidase system in the active transport of salts. Arkiv Kemi 3: 69–79. 1951.

10. LUNDEGÅRDH, H. Properties of the cytochrome system of wheat roots. Arkiv Kemi 5: 97–146. 1953.

11. MILTHORPE, J. and ROBERTSON, R. N. Studies in the metabolism of plant cells. VI. Salt respiration and accumulation in barley roots. Australian Jour. Exptl. Biol. Med. Sci. 26: 189–197. 1948.

12. MUNTZ, J. The role of potassium and ammonium ions in alcoholic fermentation. Jour. Biol. Chem. 171: 653–665. 1947.

13. OVERSTREET, R. and JACOBSON, L. Mechanisms of ion absorption by roots. Ann. Rev. Plant Physiol. 3: 189–206. 1952.

14. OVERSTREET, R., JACOBSON, L., and HANDLEY, R. The effect of calcium on the absorption of potassium by barley roots. Plant Physiol. 27: 583–590. 1952.

15. ROBERTSON, R. N. Accumulation of chlorides by plant cells and its relation to respiration. Australian Jour. Exptl. Biol. Med. Sci. 19: 265–278. 1941.

16. ROBERTSON, R. N. and THORN, M. Studies in the metabolism of plant cells. 4. The reversibility of the salt respiration. Australian Jour. Exptl. Biol. Med. Sci. 23: 305–309. 1945.

17. ROBERTSON, R. N. and TURNER, J. S. Studies in the metabolism of plant cells. 3. The effects of cyanide on the accumulation of potassium chloride and on respiration; the nature of salt respiration. Australian Jour. Exptl. Biol. Med. Sci. 23: 63–73. 1945.

18. ROBERTSON, R. N., TURNER, J. S., and WILKINS, M. J. Studies in the metabolism of plant cells. 5. Salt respiration and accumulation in red beet tissue. Australian Jour. Exptl. Biol. Med. Sci. 25: 1–8. 1947.

19. ROBERTSON, R. N. and WILKINS, M. J. Studies in the metabolism of plant cells. 7. The quantitative relation between salt accumulation and salt respiration. Australian Jour. Sci. Research B 1: 17–37. 1948.

20. ROBERTSON, R. N., WILKINS, M. J., and WEEKS, D. C. Studies in the metabolism of plant cells. 9. The effects of 2,4-dinitrophenol on salt accumulation and salt respiration. Australian Jour. Sci. Research B 4: 248–264. 1951.

22. SCOTT, W. W. Standard Methods of Chemical Analysis, 5th ed. Vol. 2. Van Nostrand Company, New York. 1939.

23. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma 15: 497–516. 1932.

24. STEWARD, F. C., STOUT, P. R., and PRESTON, C. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. Plant Physiol. 15: 409–447. 1940.

25. STEWARD, F. C. and PRESTON, C. Metabolic processes of potato discs under conditions conducive to salt accumulation. Plant Physiol. 15: 23–61. 1940.

26. VIETS, F. G. Calcium and other polyvalent ions as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466–480. 1944.

27. WANNER, H. and SCHMUKE, S. Hemmung der Wurzelatmung durch Ausscheifungen des Wurzelsystems. Ber. schweiz. bot. Ges. 60: 413–425. 1950.

28. WEEKS, D. C. and ROBERTSON, R. N. Studies in the metabolism of plant cells. 8. Dependence of salt accumulation and salt respiration upon the cytochrome system. Australian Jour. Sci. Research B 3: 487–500. 1950.

THE HISTOCHEMICAL LOCALIZATION OF PEROXIDASE IN ROOTS AND ITS INDUCTION BY INDOLEACETIC ACID [1]

WILLIAM A. JENSEN [2]

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA 4, CALIFORNIA

Recent evidence (2) has indicated that the peroxidase activity of certain plant tissues rises significantly following the administration of indoleacetic acid (IAA). This phenomenon appears to be related to the induced formation of the IAA-oxidase system (4), of which peroxidase is one component (3). Since peroxidase has recently been shown to be involved in the biosynthesis of lignin (1, 6), the induced formation of this enzyme would appear to have morphogenetic significance.

The purpose of the present paper is to localize in the root those cells and tissues that contain peroxidase, both induced by IAA and non-induced, and to investigate the physiological role of the induced peroxidase in the development of the cell.

Two different histochemical methods were employed. The first involved the use of freshly excised sections and the determination of peroxidase activity