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GENERAL NATURE OF THE PROCESS OF SALT ACCUMULATION BY ROOTS WITH DESCRIPTION OF EXPERIMENTAL METHODS[1]

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(WITH NINE FIGURES)

Introduction

The discussion in this paper is based on the assumption that the reader is familiar with earlier work on *Valonia, Nitella,* and *Chara* cells (**3, 5, 15–17, 23–25, 42**), and with the series of recent reports by STEWARD (**32–36**) and his co-workers (**2, 37–40**) on accumulation of salts by storage tissues. These latter researches yielded clear evidence that salt accumulation (movement of cations and anions into the vacuole against concentration gradients) by storage tissues is dependent upon the metabolic activities of living cells, reflected in aerobic respiration. It was concluded that capacity for maintained salt accumulation by these tissues is associated with a state of intense cell metabolism characteristic of cell growth and cell division. The problem now to be discussed is that of salt accumulation by roots with particular reference to metabolic processes. This problem has special significance for students of soil and plant interrelations, and at the same time has broad ramifications in the field of general physiology. One of the most fundamental of cell functions is involved.

Materials and methods

Consistent and significant conclusions on salt absorption by root cells must rest on experiments in which control is exercised over certain variables, some of which have failed to receive adequate attention in previous investigations. For this reason, it is deemed advisable to describe in detail

[1] Preliminary reports of some of the most essential features of this investigation, conducted during the past several years, have been presented before the American Society of Plant Physiologists (1932–33).

the methods employed in the present investigation. Experiments were made on entire plants of various species and on excised root systems of the barley plant (*Hordeum vulgare*). This paper will be restricted primarily to the latter. Many of the fundamental questions of salt absorption by root cells can be studied most effectively by eliminating, during a brief experimental period, the complications of root and shoot relationships. The method was to produce for each experiment a large number of uniform absorbing root systems with certain desired characteristics resulting from the conditions imposed during the preliminary growth period, and then to study salt absorption by these systems over short periods. The nature of the process of salt accumulation was the objective, not the cumulative effects of nutritional factors on plant development and their secondary effects on salt absorption.

Root tissues in an appropriate nutritional and developmental state seem to form the most active salt-accumulating system so far investigated. It will be shown that root tissues of this kind under conditions favorable to active aerobic metabolism have the capacity to accumulate large amounts of K, Br, or NO_3 within a 10-hour period. In fact, significant salt accumulation by highly active root systems can occur within 2 hours. Young growing roots are especially adapted to the study of a number of general problems of salt accumulation by living plant cells. So far as the writers are aware, experiments on excised roots similar to those described in this paper have not been reported heretofore. PIRSCHLE and MENGDEHL (**29**), in their studies on excised root systems, did not provide the conditions required for active salt accumulation.

The "Sacramento" variety of barley was used in all experiments except those performed during the preliminary stages of the investigation. This detail is of some importance for the reason that this variety of barley is almost immune to mildew attack under our greenhouse conditions. With other varieties we have found serious difficulty in producing disease-free plants.

FACTORS ESSENTIAL TO DEVELOPMENT OF ROOT SYSTEMS

Our first attempts to induce an active accumulation of potassium salts by excised root systems were not very successful, even when suitable external conditions for salt absorption were provided. Usually there occurred some net accumulation of Br from dilute KBr solutions, but little or no net accumulation of K. Sometimes there was a loss of K from the root system, as a whole, during the experimental period. Without entering into the details of these experiments, we shall present in brief outline certain factors essential to the development of root systems which have a high

capacity for salt absorption, as they have been disclosed in this investigation.

AGE AND DEVELOPMENTAL STATE.—An excised root system as a whole will exhibit maximum potentialities for salt accumulation only when actively metabolizing cells predominate in the system. If they do not, the accumulation of salts by active cells may be obscured by the loss of salts from inactive or moribund cells, and entirely misleading conclusions reached regarding the relations of temperature, aeration, and other factors to salt accumulation. When grown in a favorable climatic and nutritional environment, barley plants 6 weeks or more old can furnish root systems suitable for the study of certain phases of the problem of salt accumulation and several experiments on root systems of this age will be discussed. In general, however, it is highly advantageous to employ very young root systems. If these are developed under properly selected cultural conditions they consistently provide tissues with a high capacity for salt absorption. In the standard technique of this investigation, plants were grown in a special culture solution for a period of approximately 3 weeks preceding the excision of the root systems.

NUTRITIONAL HISTORY OF ROOT TISSUES.—The capacity of root cells to accumulate salt during a brief experimental period is profoundly influenced by the nutritional status of the plant at the time the experiment is begun. It is, therefore, very important to control the concentration, composition, and volume (per unit number of plants) of the nutrient solution in which the plants are grown in preparation for absorption experiments. Healthy root tissues may be caused to vary extremely in their salt content. When the volume and concentration of the nutrient solution are sufficiently limited, the supplying power of the external solution does not keep pace with the translocation of salt from root to growing shoot, and, as a consequence, the root suffers partial depletion of its salt content. An initial low-salt condition is a requirement for the most rapid accumulation of salt by excised roots in a subsequent absorption period. It is, of course, necessary that the supply of nutrients shall not be so restricted during the preliminary growth period that root growth and metabolic activity become impaired through starvation effects. Low-salt root tissues developed under our experimental conditions also have a high sugar content, a point to be discussed presently.

SEASONAL EFFECTS ON DEVELOPMENT.—Plants were grown at all seasons of the year in the greenhouse, with a standardized technique, and the observation was made that yields of shoot varied comparatively little (being limited by nitrate supply) but the root systems grown during several winter months had much lower fresh weights than those of the best summer root

systems. The latter had an extensive development of fine lateral roots, which were decidedly less abundant on winter root systems. Further data must be acquired before positive general statements can be made concerning effects of seasonal conditions on capacity for salt accumulation per unit weight of tissue, but, on this basis, certain sets of roots grown during highly unfavorable seasons had a relatively low capacity for salt absorption.

The influence of season on root growth, under the given greenhouse conditions, must be ascribed rather to the intensity or quality of illumination than to temperature. During one season, the incidental observation was made that thorough cleansing of the glass of the greenhouse roof was followed by a large increase in the ratio of root to shoot. Extending the period of illumination with Mazda lights of considerable intensity had no significant influence on the weight and nature of the root system; neither did an increase of greenhouse temperature. Brief exposures to a mercury arc lamp caused slight injury and retardation of elongation in shoots, but produced no important modification of the root system. The proportion of root to shoot could not be materially increased in the winter season by the various modifications of the nutrient solutions tested. Some specific data on seasonal effects on root-shoot relations are presented elsewhere (**14**).

Culture technique

In earlier experiments, the containers for the culture solutions were glass jars of 2-liter capacity, of the type commonly employed in water culture experiments. Later, 20-liter earthenware jars or large 115-liter tanks made of pure iron served as culture vessels. The tanks and other iron containers were thoroughly coated with an asphalt paint, which has not shown the slightest toxicity to plants. In the experiments with young plants, which comprised the major part of the investigation, shallow trays of approximately 4-liter capacity were used successfully. Over the top of the tray[2] was placed a cover made of black iron, coated with asphalt paint. This cover was perforated with holes 5.7 cm. in diameter, for receiving corks, each of which was bored with 7 holes, in which the seedlings were fixed with non-absorbent cotton in the usual manner. Each cover accommodated 24 corks, so there were 168 plants in each set (fig. 1).

In some experiments use was made of coated iron trays with dimensions similar to those of the trays described above, but provided with broad flanges, the covers having corresponding flanges. By means of small bolts, the cover could be fastened tightly to the tray when it was desired to pass gases other than air through the solution, but in the majority of experiments 4-liter stoppered bottles were employed for this purpose.

[2] Granite-ware pans served the purpose adequately but pans with broken enamel should not be used. It may be desirable to coat the inside of the pan with asphalt paint.

FIG. 1. Illustration of method of growing uniform sets of barley plants to obtain excised roots for absorption studies. 168 plants in each set.

Ordinarily the roots were not given forced aeration during the preliminary period of growth. The shallow trays with loosely fitting covers allowed sufficient exchange of gases to permit excellent root development. The type of root system can be modified by forced aeration, but the roots so aerated for a long period become partially depleted of sugar, and this may sometimes constitute a disadvantage for the later study of salt absorption. When plants are grown in stoppered jars or bottles, without passing a stream of air through the solution, lack of aeration may prevent the development of root systems highly adapted to absorption experiments, especially if the barley plants are grown under unfavorable seasonal conditions. The discussions of ZIMMERMAN (**43**), and of CANNON (**4**) regarding the movement of oxygen from shoot to root are of interest at this point. According to their evidence, conditions for photosynthesis would influence

the oxygen supply of root cells. The aeration received by roots also varies with transpiration, which determines the amounts and frequency of the additions of water to the culture vessels, with the accompanying introduction of new supplies of oxygen. From our recent experience we conclude that tanks, trays, or very large jars with loosely fitting covers are, in general, greatly preferable to smaller, stoppered bottles or jars, in growing plants in preparation for salt absorption studies, not only because they assure better aeration, but also because the mechanical manipulations become very laborious when many small containers must be separately handled; and, as will appear later, we have found it necessary during the course of our investigations to utilize an extremely large number of plants.

The equipment for the germination of barley seeds is shown in figure 2. The procedure for the germination of seeds was as follows:

1. Soak seed in tap water about 18 hours (overnight is convenient) at 25° C.
2. Assemble thoroughly cleaned equipment (fig. 2).
3. Arrange seed carefully upon the netting (*E*).
4. Adjust screen (*D*) and water-soaked cloth (*F*).
5. Place in the dark, in a warm constant environment (about 25° C.).
6. When shoots begin to pierce through the cheesecloth (*F*) about the third day after soaking, remove this cloth and screen (*D*), leaving the remaining assembly until the fourth day from soaking as specified in 5.
7. On the fourth day, transfer to direct light after a few hours under shaded light.
8. On approximately the seventh day, when shoots have attained a height of about 9 or 10 cm., the seedlings are carefully withdrawn, one by one, selected for uniform development, and fixed in the holes in the supporting corks with non-absorbent cotton.
9. The corks containing the seedlings are then set in place in the covers of the shallow trays described above.

For certain species of plants, germination in pure silica sand may be advantageous, the seedlings being transplanted to corks when size and vigor permit.

In the present investigation all seedlings were germinated in tap water (of very low salt content), limiting salt nutrition primarily to that furnished by the seed up to the time of the transplanting into the culture solution employed for the preliminary growth period.

Increasing the supply of oxygen by forcing air through sintered glass aerators placed in tray *B* increased slightly the percentage of germination,

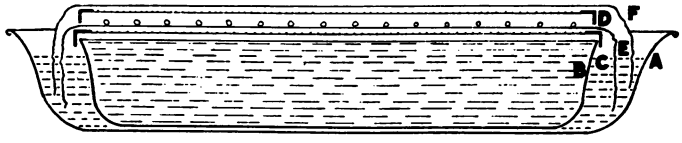

FIG. 2. Diagrammatic sketch of equipment for germination of barley seeds.

A. Royal enamelware oblong stove pan, 40.6 × 28 × 6.3 cm. containing tap water.

B. Pyrex utility or baking dish 32.1 × 21.5 × 5 cm. containing tap water or nutrient solution in which the seedling roots develop. (This tray should be maintained at nearly full capacity of water during the germination period.)

C. Double-tinned, brass wire screen, 8 meshes per inch (varying mesh with type of seed); height of metal side 1.2 cm.; length and width to permit screen to fit loosely over tray *F*.

D. Screen as under *C*, with length and width 0.6 cm. less than *C*.

E. Washed, single thickness of bleached mosquito netting, stretched over *C*, and dipping into water contained in *A*.

F. Washed, double thickness of bleached cheesecloth, stretched over *D* and dipping into water contained in *A*.

but this procedure has not been adopted in the experiments now reported. Aeration of this kind would modify, to some extent, the metabolic state of seedling roots.

NUTRIENT SOLUTION

The standard nutrient solution for the preliminary 3-week growth period had the following composition:

KNO_3	0.0025 M
$Ca(NO_3)_2$	0.0025 M
$MgSO_4$	0.001 M
KH_2PO_4	0.0005 M

For each set of 168 plants 3800 cc. of this solution were used. A solution of double this strength was substituted in some of the earlier experiments of longer preliminary growth periods. Also special low-potassium solutions were employed in certain instances.

Iron, as iron tartrate, 0.5 per cent. solution, was added to the nutrient solution in the proportion of 1 cc. per liter. Additions of the iron tartrate solution were repeated as necessary (usually twice each week) to prevent chlorosis. Impurities in the nutrient solution, and the supply of boron or other supplementary essential elements in the seed, made it unnecessary to introduce these elements during the brief period of growth; but this point requires attention in growing plants of certain other species. Possibly it will also become important in the study of special problems of metabolism in the barley root.

To produce low-salt plants, the nutrient solution was not changed in the 3-week period. High-salt plants were produced by changing the solution every day or on alternate days, after the first week. If the solution is not changed, the original supply of potassium and nitrate becomes exhausted in the nutrient solution, followed by partial depletion of the salt content of the root cells, through translocation of salt from root to shoot, as already explained.

Preparation of root systems for study

The root systems were excised just below the seed residue, and then thoroughly washed twice with distilled water. Afterward they were centrifuged by the technique described below, following which, sets of roots, each representing 168 plants, were immersed in the experimental solution, contained either in the shallow trays already described, or in 4-liter, wide-mouthed bottles provided with stoppers, through which tubes for aeration were passed. At the end of the absorption period, the roots were removed from the solutions, re-washed, and re-centrifuged. Fresh weights were recorded, and the roots were then placed in closed jars which were kept at −15° C. for 48 hours or more, according to convenience. The volumes of residual culture solutions were measured and these solutions kept for analysis when this was desired.

In a number of preliminary experiments with older root systems, attempts were made to obtain a uniform mass of root tissues by cutting root systems into short sections (2 to 5 cm.) and thoroughly mixing the segments in distilled water. Random samples of 75 to 125 gm., centrifuged fresh weight, were then withdrawn for the absorption experiments. This procedure yielded some useful results, but it soon became evident that it was desirable to employ for each treatment a very large number of entire root systems excised from young plants, in order to reduce errors of variability, to provide tissues of higher average metabolic activity, and to limit injury from cutting to a minimum. However, a later experiment showed no significant effect of cutting on respiration.

Aeration technique

The various devices employed for passing air or other gases through the solutions in which the roots were immersed during the absorption period were: manifolds of pure rubber tubing pierced with needle holes, block tin tubing similarly pierced, and Folin bulbs (for use with bottles). The most satisfactory aerators were those made of sintered glass, according to a technique described by Furnstal and Johnson (8). The small sintered glass aerators can be linked together in manifolds of diversified form and size suitable for jars or bottles, shallow trays, or large tanks. The

important point is that the solution shall be rapidly and uniformly aerated with very small gas bubbles. The air, supplied from a large compressor connected with a reservoir, was passed through water and filtered through cotton. Gases other than air were obtained from tanks under pressure, and in certain experiments the gas was specially purified. The rate of aeration was approximately 1.5 cu. ft. per hour for each set of roots. This rate was shown to be adequate, since it could be decreased considerably without affecting the results significantly.

The masses of root tissues were carefully separated so that air or other gases could readily reach all root surfaces. The roots normally remain suspended in the solution, and the stream of gas obviates the necessity for mechanical stirring. Stirring by the gas stream renews the salt solution at root surfaces and this doubtless is a secondary factor which influences the rate of salt absorption, but stirring alone without supplying oxygen is ineffective, as shown by experiments in which nitrogen gas, or gas mixtures very low in oxygen, were substituted for air.

Technique of centrifuging roots and of expressing sap

The root tissues were loosely tied in cheesecloth and submerged for a few minutes in distilled water. The cloth bags were then suspended (properly counterpoised) from the arms of a centrifuge head and rotated at the relatively slow speed of 400 r.p.m. for 5 minutes. This treatment caused no apparent injury and close reproductions of fresh weight could be obtained, within less than 1 per cent. variations. The moisture content of the centrifuged roots varied from 92 to 95 per cent. for sets grown at different times. It was altered very little during the absorption period, although slight increases occurred in certain experiments in which a large amount of salt was absorbed. With regard to osmotic relations, it is to be noted that while salt is being accumulated, sugar is being utilized.

The frozen root tissues were rapidly thawed in the closed jars in which they were originally placed and the sap quickly expressed in a heavy steel mechanical press by a reproducible technique (**6**). The sap so expressed was clear. The volume of sap recovered was nearly always equivalent to approximately 80 per cent. of the total moisture present in the tissues. Press cakes were dried at 80° C. and dry weights taken. Estimates of approximate total volumes of sap were calculated from these data when necessary. All determinations, except those for stable salts, were started within a few hours after expression of the sap. Sap retained for further study of salt content was preserved with a layer of toluene. This investigation is not concerned with those enzymatic changes of certain organic constituents which may occur in frozen tissue, or during freezing and thawing.

Clearly, expressed sap is a composite fluid derived from cells which belong to different tissues. Despite the differences in salt content which may prevail even in a single isolated root (PREVOT and STEWARD, **28**), or between roots at different developmental stages, this composite fluid represents a mean composition of sap upon which the salt accumulation by these accurately sampled root systems may be determined and compared. While the writers do not contend that the expressed sap is identical with the vacuolar sap as it existed in any particular living cells, they believe that in the light of present knowledge, the composition of expressed sap is an adequate basis for drawing conclusions concerning the general nature of the process of accumulation of mineral solutes by roots, with which they are alone concerned at present. The consistent relations which have emerged between salt accumulation and the different variables (both internal and external) are sufficient justification for this procedure.

When the amounts of K and Br withdrawn from solution are compared with the total amounts accumulated in the sap, computed on the basis of the estimated total volumes of sap, it is evident that normally more than 80 per cent., and sometimes more than 90 per cent., of the total amounts of these elements absorbed by the roots are present in the sap. An increase in the total salt concentration of the sap is accompanied by a parallel increase in electrical conductivity. The values cited may, of course, be too low if the unrecovered sap has a higher concentration than that of the expressed sap. The amount of nitrate absorbed from solution was nearly always much in excess of the amount present in the sap, because of the reduction of nitrate in the tissues. When bases enter the cell in association with nitrate and the latter is subsequently reduced, direct evidence shows that the basic residue is neutralized by organic acids.

Experimentation

REPRODUCTION OF RESULTS

By proper control of all factors, it is feasible to obtain a very close agreement of results on replicate sets of roots, each of which represents a large number (168) of selected seedlings. Evidence of this is found in table I, in which results are reported for roots examined after a period of absorption from a KBr solution. The plants for the experiment supplying the data for table I were grown from December 1 to December 22 in the standard nutrient solution. Each set of root systems, consisting of 168 plants each, was then placed in a shallow, covered tray filled with 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained in the solution for an absorption period of 27 hours. In this

TABLE I

EXAMPLE OF REPLICATION OF RESULTS IN SALT ACCUMULATION EXPERIMENTS WITH EXCISED ROOTS*

CONDITION	SET	CONCENTRATION OF K IN EXPRESSED SAP IN MILLIEQUIVALENTS PER LITER	K ACCUMULATED IN SAP IN MILLIEQUIVALENTS PER LITER
Initial	A	23.7	
	B	22.9	
At end of absorption period	A	85.0	
		85.5	62.0
	B	83.5	
		85.0	61.0
	C	87.5	
		88.0	64.4
	D	85.0	
		85.0	61.7
			Average..... 62.3

* Results of duplicate analyses on samples of sap are also shown.

experiment, the period of absorption was sufficiently long to permit the cells to reach a concentration of salt approaching the maximum attainable under the favorable conditions provided. In some experiments, agreement, although entirely adequate for present purposes, may not be quite so good as that just indicated, when conditions are unfavorable for salt accumulation, or when intermediate periods are involved. Most of the experiments were carried out in duplicate, (several examples are given in tables) and important deductions were checked by repetition of entire experiments at different times and in different ways. Positive conclusions are derived only from consistent effects of such large magnitude that their statistical significance is not open to doubt. Quantitative comparisons are always made within a single experiment, between sets of roots derived from the same uniform population of seedlings.

ANALYTICAL TECHNIQUE

At various times and in accordance with the purpose of the experiment, the following analytical methods have been utilized in the general investigation:

1. Bromide according to general procedure of HIBBARD (9). Ignition was carried out with NaOH at 600° C. Thiosulphate was checked against standardized solutions of dichromate, or in recent experiments by a modi-

fied technique in which the reagents were standardized with a KBr solution, and all titrations limited to volumes of 2 to 5 cc., maintaining a slow and uniform rate of aeration.

2. Total halide by Mohr's method.

3. Potassium by volumetric cobalti-nitrite procedure of HIBBARD and STOUT (**10**).

4. Calcium and magnesium by the McCrudden method.

5. Sodium by uranium acetate method (**1**).

6. Conductivity by Kohlrausch bridge method with ear-phones and amplifier system; freezing-point depression by use of a Beckmann thermometer.

7. Total and nitrate nitrogen by modified methods of PUCHER *et al.* (**30**) and VICKERY and PUCHER (**41**).

8. Sugar (total) by Munson-Walker method.

9. Organic acids by electrometric titration of sap or by separation of non-volatile ether-soluble acids followed by titration (method to be published elsewhere by A. ULRICH).

10. Carbon dioxide evolved by absorption in NaOH and double titration, using Winkler method with use of $BaCl_2$.

11. pH and buffer curves of sap, using the hydrogen electrode. Recent experiments have shown the value of the glass electrode for future work. pH values of external solutions estimated by standard indicator methods.

12. Bicarbonate by direct titration, also by driving off CO_2 from acidified solution into NaOH solution.

13. Inorganic SO_4 and PO_4 by direct precipitation and with standard methods of analysis.

The errors of duplicate analyses on sap or culture solutions never approached the orders of magnitude to which significance is attached.

HYDROGEN ION EFFECTS

The interrelations between hydrogen ion concentration of the external solution and salt accumulation require a separate later discussion. The general aspects of salt accumulation can be considered now without particular reference to the hydrogen ion factor. We need only say, at this point, that our data emphasize that the effects of the metabolic activities of root cells on the hydrogen ion concentration of the external solution may be of greater moment than the effects of hydrogen ion concentration of the solution, *per se,* on the accumulation of salt, within a wide range of pH.

EFFECTS OF AERATING ROOT TISSUES ON ACCUMULATION OF SALT

It has been proved by STEWARD (**35**) that accumulation of potassium salts by discs of storage tissues occurs only when oxygen is supplied to the

tissues at a suitable tension and rate of flow of the gas stream. Active aerobic respiration was found to be indispensable. ROSENFELS (**31**) has shown that rapid accumulation of Br by *Elodea*, in the dark, is likewise dependent upon aeration. In the light, the necessary oxygen is provided by photosynthetic processes and this is also true of *Nitella*.

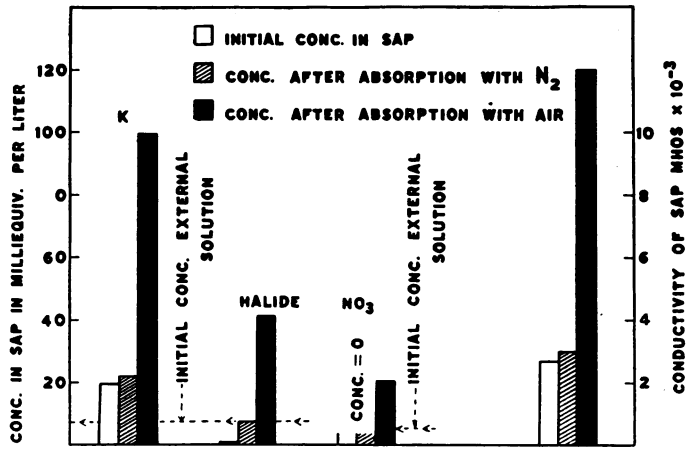

FIG. 3. Effect of aeration on accumulation of salt by excised roots of barley plants.

A general demonstration of the effects of aeration on salt accumulation by root tissues was made in a very direct and simple manner (fig. 3).

A description of the experiment supplying the data for figure 3 follows. The plants (168) of each set were grown in a complete culture solution from May 31 to June 26. Each set of the excised roots was then placed in 3 liters of an external experimental solution consisting of 0.0075 M KBr, and 0.0025 M $Ca(NO_3)_2$, and remained in this solution for an absorption period of 10 hours. The temperature of the solution was kept at 24° C.[2] During the absorption period, a rapid stream of air was passed through one solution and purified nitrogen gas through the other. The nitrogen gas, however, retained traces of oxygen. Some oxygen was also present in the tissues and solution at the beginning of the absorption period. The pH of the initial culture solution was 6; of the final, with air passing through it, 5.8, and with N_2 passing through it, 6.3.

Note should be made of the fact that much of the NO_3 absorbed was reduced, and that K absorbed in association with NO_3 remained in the sap in equilibrium with organic acid anions formed in the course of metabolism.

When a stream of air was passed through the external salt solution, K, Br, and NO_3 accumulated rapidly in the sap, against steep concentration

[2] Except where otherwise noted, temperatures were maintained in bath within ± 0.2° C.

gradients. Conductivity of the expressed sap increased about 500 per cent. On the other hand, when nitrogen gas (containing as an impurity approximately 0.2 per cent. oxygen) was substituted for air, no NO_3 was found in the sap, and K was increased in concentration but very slightly. Br gained entrance to the sap, but only to an approximate equality of concentration with the external solution. Very little change in conductivity occurred in this case.

The plants for experiment A (table II) were first grown from January 11 to March 21 in 20-liter jars in a low-potassium culture solution. Approximately 125 gm. of roots, clipped in 5 cm. lengths, were used for each set of roots which was then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained in the solution for an absorption period of 24 hours. The temperature of the solution was 20° C. The absorption experiment was run in quadruplicate. The bottles containing the roots were tightly stoppered

TABLE II

ACCUMULATION OF SALT BY EXCISED BARLEY ROOTS WITH AND WITHOUT OXYGEN

EXPERIMENT A

CONDITION	COMPOSITION OF SAP		ACCUMULATED IN SAP	
	K	HALIDE	K	HALIDE
	MILLIEQUIVALENTS PER LITER*		MILLIEQUIVALENTS PER LITER*	
Initial	15.4	0.7		
After absorption period with stream of air	72.5	57.2	57.1	56.5
After absorption period without aeration	17.2	10.2	1.8	9.5

EXPERIMENT B

CONDITION	SET	COMPOSITION OF SAP			ACCUMULATED IN SAP		
		K	Br + Cl	NO_3	K	Br + Cl	NO_3†
		MILLIEQUIVALENTS PER LITER			MILLIEQUIVALENTS PER LITER		
Initial		28.7	1.6	0.1			
After absorption period with N_2	A	27.9	7.0	0.1	−0.8	5.4	0.0
	B	27.7	6.4	0.0	−1.0	4.8	−0.1
After absorption period with air	A	89.3	30.4	21.6	60.6	28.8	21.5
	B	81.3	30.8	20.5	62.6	29.2	20.4

* All values were within 10% of the averages given.

† Some NO_3 was absorbed and reduced. Values given are for NO_3 ion.

during the absorption period, but the solution and tissues were not initially free of oxygen. Four other sets were treated in the same way except that a stream of air was passed through the solutions. Again the effect of aeration is evident. The oxygen initially present in the solution or in the tissues maintained the cells in apparently healthy condition for the period in question and permitted a small net accumulation of Br and a very slight net accumulation of K, which, however, may not be significant. In marked contrast, the aerated tissues accumulated large amounts of K and Br. The concentrations of these ions in the sap attained values four or five times higher than the corresponding concentrations for the unaerated tissues. Prolonging the experiment would doubtless have resulted in a loss of salt from the unaerated tissues.

Still another experiment (table II, experiment B), conducted under different conditions, has a similarly clear significance. Each set of 168 plants for experiment B was grown in the standard nutrient solution from April 22 to May 13. Each set of the root systems was then placed in 3800 cc. of an external experimental solution having an initial composition (in milliequivalents per liter) of 10 K, 5 Br, 9 NO_3, and 4 Ca, and remained there for an absorption period of 9 hours. The temperature of the solution was 23.6° C. A gas stream was passed through the solution. The pH of the external solution varied from the initial of 5.6 to a final of 6.5 for air treatment; and from 5.6 to 7.8 for N_2 treatment. The roots in the solution through which nitrogen gas was passed did not accumulate any K or NO_3 (although considerable NO_3 was absorbed from solution and rapidly re-

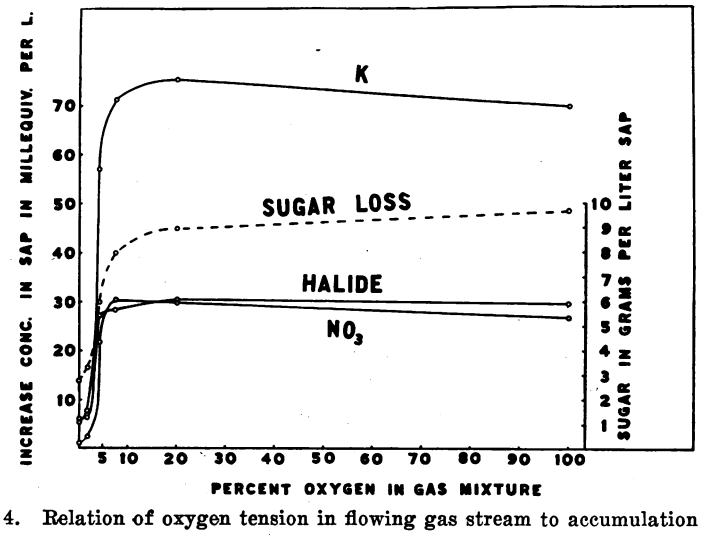

FIG. 4. Relation of oxygen tension in flowing gas stream to accumulation of salt by excised barley root systems.

duced) and halide concentration in the sap rose to a value only slightly in excess of that of the external solution, while the aerated tissues, in the 9-hour period, accumulated large amounts of all three ions, K, Br, and NO_3. The internal concentrations of the latter ions far exceeded the external at the end of the period of salt absorption.

One experiment had for its purpose a study of the effects on salt absorption of varying oxygen tensions, in a rapidly flowing gas stream (fig. 4). The plants, consisting of 168 for each set, were first grown in a complete culture solution from March 29 to April 24. Each set of root systems was then placed in 4-liter bottles containing 3 liters of an external solution having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 10½ hours.

Mixtures of oxygen and nitrogen gases were made in cylinders under compression, and the composition of the mixtures determined by analysis. The gas mixtures used were as follows:

Gas	Oxygen content
	%
1. N_2 purified with pyrogallol	0.2
2. N_2 unpurified, from cylinder	1.7
3. $N_2 + O_2$ mixture	4.1
4. $N_2 + O_2$ mixture	7.5
5. Air from compressor, filtered through water	
6. O_2 from cylinder	

The values recorded in figure 4 are averages of duplicate experiments in which the results agreed within 5 per cent., except for the very low absorption values, where agreement was of the order of 10 per cent.

It is of interest to compare the salt absorption curves for the root tissues with those for discs of potato tuber, reported by STEWARD (35). Under the conditions of the experiments the oxygen percentage required for maximum Br accumulation was higher for potato tuber tissues than for barley root tissues. The bromide (halide) accumulation curve for the latter rises very sharply to a maximum of salt absorption at less than 10 per cent. oxygen, while the corresponding value for potato tissues is approximately 20 per cent. Recent experiments show that potato root tissues resemble in this regard barley root tissues rather than discs of potato tuber. Data are also presented for K and NO_3 accumulation, and sugar loss, by the barley root tissues. All the curves follow a very similar trend. The experiment

was concerned with only one type of root system and it will become of interest to conduct similar experiments on root systems of different initial status with regard to salt and sugar content. Research should also be done with reference to possible effects of manganese, copper, zinc or other elements required in minute quantity.

Respiration and salt accumulation

Respiration data were not obtained in the root experiment on the effects of oxygen tensions, but the experiment of Steward (35) on potato tuber tissues provides evidence that the curves for CO_2 production and for oxygen tension assume the same form. Respiration data from other experiments on root tissues are presented in tables III and IV.

TABLE III

Carbon dioxide production by tissue as influenced by oxygen supply*

Condition	Mg. CO_2 per gm. fresh tissue per hr.	Relative to air as 100
	mg.	
Excised barley roots with air	0.734	100
" " " " N_2†	0.282	38
Discs of potato tuber‡ with air	0.183	100
" " " with N_2 containing 2.9 per cent. O_2	0.086	47

* Compare also table 4.

† The nitrogen gas was compressed in a cylinder and usually contains about 2 per cent. of O_2.

‡ The potato tuber results are from Steward (35), table 5, page 217.

The barley plants used, in the experiment determining CO_2 production by tissues as influenced by oxygen supply (table III), were grown in a standard nutrient solution from April 22 to May 12. Each root system was placed in 3 liters of an external solution, having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 9 hours. The temperature of the solution was 23.5° C.

The relative values for CO_2 production are in general agreement with the results given by Steward (35) and Steward and Broyer (39) on potato tuber tissues. The same broad conclusion seems to apply to every kind of tissue so far studied with reference to the relation between respiration and salt accumulation, namely, that CO_2 production (aerobic) by tissues capable of marked salt accumulation, reflects metabolic activities essential to

TABLE IV

REVERSIBILITY OF EFFECT ON SALT ACCUMULATION OF PASSING NITROGEN THROUGH SOLUTION

CONDITION	COMPOSITION OF SAP			INCREASE OF CONCENTRATION IN SAP			RESPIRATION RATE*
	K	Br	CONDUCTIVITY	K	Br	CONDUCTIVITY	Mg. CO_2 / Gm. tissue × hr.
	MILLIEQUIVALENTS PER LITER		MHOS. × 10^{-3}	MILLIEQUIVALENTS PER LITER		MHOS. × 10^{-3}	
Initial	29.7	0.0	3.97				
After absorption with air 6 hrs.	61.3	26.0	7.31	31.6	26.0	3.34	0.422
After absorption with N_2 6 hrs.	28.9	3.3	3.95	− 0.8	3.3	−0.02	0.213
After absorption with air 6 hrs. following 6 hrs. with N_2	53.2	17.3	6.47	23.5	17.3	2.50	0.320

* The respiration rate is the average for the period involved.

this process, but without a stoichiometric relation between amount of salt accumulated and amount of CO_2 produced.

Is the effect of an inadequate supply of oxygen in preventing salt accumulation irreversible? This question we have sought to answer by subjecting root tissues to a stream of purified nitrogen gas for six hours and then changing the gas from nitrogen to air. The plants used in this experiment (table IV) were grown in the standard nutrient solution from October 30 to November 21. The root tissues were then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 7.5 KBr, and remained there during the absorption period. A stream of gas was passed through the solution. The nitrogen gas was passed through sodium pyrogallate to remove the oxygen. CO_2 was removed from the nitrogen and from the air by passing the gas through sodium hydroxide. The temperature of the solution during the absorption period was 20° ± 0.5° C.

Within the period designated, nitrogen gas completely suppressed salt accumulation (Br was absorbed but the sap concentration was lower than that of the external solution), yet this process was resumed when oxygen was later supplied to the same tissues, although at a somewhat diminished rate. It is significant that the rate of respiration was likewise diminished (table IV).

Incidentally, this experiment also illustrates that the *mere production of* CO_2 by roots does not explain salt accumulation as certain theories would suggest. There are involved rather metabolic activities associated with aerobic respiration (**36**).

All our experiments lead to the conclusion that oxygen must be supplied to barley roots to maintain the cell activities associated with accumulation of salt, or the retention of the salt already accumulated, over an extended period. It is not yet so certain to what extent it is essential to lower the carbon dioxide content of the medium. Several preliminary experiments on root tissues, using a complete nutrient solution, showed no important retardation of salt accumulation when an air-CO_2 mixture containing 10 per cent. of CO_2 by volume was passed through the solution. An admixture of even 20 per cent. carbon dioxide did not produce a very great effect under the conditions of the experiment. This general question, however, demands further research.

The absence of aerobic metabolism associated with rapid salt accumulation does not necessarily mean that the cell becomes impermeable to a salt. Bromide may enter the sap to approximate equality of concentration with the external solution under anaerobic conditions. The reason nitrate apparently does not always behave in the same way is that the nitrate can be reduced as it is absorbed. Attention is called to the fact that a completely anaerobic condition was not attained in any of the experiments. There was a slight oxygen impurity in even the purified nitrogen gas and some oxygen must have been initially present in the tissues or solutions. This

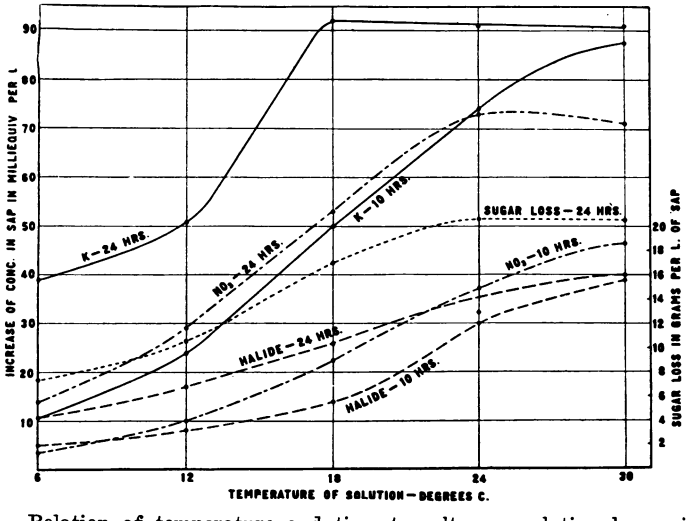

FIG. 5. Relation of temperature and time to salt accumulation by excised young barley root systems.

may explain slight accumulations of salt, or failure of tissues to lose potassium, during a brief period, when nitrogen gas is passed through the culture solution.

The experiments recently reported by PETRIE (**27**) on entire plants have led him to conclude from statistical evaluation of results that aeration of the root medium accelerates absorption of salts although, as PETRIE recognizes, in experiments of this type, conducted over an extended period, it is difficult to relate the effects of oxygen directly to the process of salt absorption. Data are not reported on the extent of salt accumulation in the root sap.

The studies of LUNDEGÅRDH (**18**), and LUNDEGÅRDH and BURSTRÖM (**19–22**) on respiration in relation to salt absorption were undertaken from a point of view different from that of STEWARD or of the present writers.

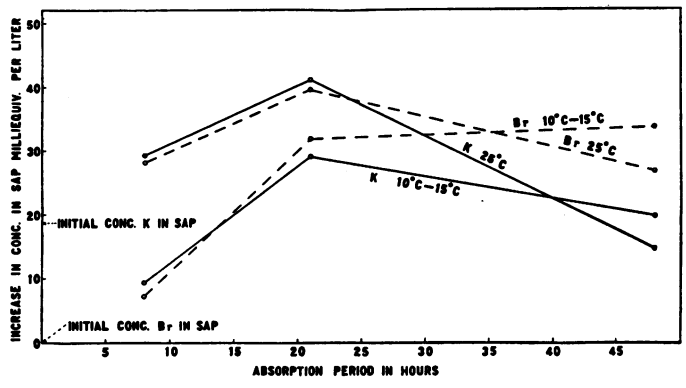

FIG. 6. Relation of temperature and time to accumulation of salt by excised root systems of older barley plants.

The objectives and technique of the two investigations are so dissimilar that a comparison of results is of limited value. LUNDEGÅRDH and BURSTRÖM experimented with the entire plant, a more complex system than that of excised roots. However, our unreported data do not suggest any fundamental differences between excised and attached roots, as far as the general requirements for salt accumulation are concerned. Translocation of salt from root to shoot and possible movement of oxygen from illuminated shoot to root complicate the problem.

LUNDEGÅRDH and BURSTRÖM seem to draw a rather sharp distinction between the absorption of cations and anions, and conclude that respiration is especially concerned with the latter and some adsorption process with the former. They stress the effect of anion absorption on respiration rather than the point we are emphasizing that respiration reflects energy exchanges essential to accumulation by root cells of *both* cations and anions.

The experiments reported by LUNDEGÅRDH and BURSTRÖM were confined to the removal of salt from the culture solution, so that it is uncertain if they were dealing with salt accumulations in the sap of magnitudes comparable to those of the present experiments. It is conceivable that the environmental conditions, in their experiments, especially that of light, did not permit the production of root systems of high capacity for salt accumulation. The question also arises whether the aeration was fully adequate for the most rapid rate of salt absorption, or the attainment of high levels of salt concentration in the root sap. The initial salt status of the roots was not stated and, as has been indicated, this is an important factor determining salt absorption during a limited experimental period.

With regard to the relation of anaerobic conditions to anion absorption and to respiration, we have noted that nitrate may be removed from solution to an appreciable extent under conditions which are not sufficiently aerobic to permit accumulation of nitrate as such in the sap. In this connection it may become necessary to give consideration to bacterial activities when the environment is unfavorable to aerobic root metabolism, and absorption is studied over prolonged periods. We desire to postpone further discussion of the effects of salts on respiration, awaiting additional evidence.

TEMPERATURE EFFECTS

The principle that salt accumulation is dependent upon metabolic activities of cells, suggests that the process of accumulation should have a high temperature coefficient over certain ranges of temperature. This expectation is fulfilled by the experimental evidence on roots, which is in accord with evidence on storage tissues and on *Nitella* and *Elodea* cells (**16, 31**). The Q_{10} values for salt accumulation by excised roots are of a high order (2.5 to 5.0) over a considerable range of temperature (6 to 24° C.), and similarly high temperature coefficients were obtained in comparable studies with entire barley plants. The data from several experiments on excised roots are presented in figures 5 and 6, and tables V and VI.

In the experiment performed to determine the relation of temperature and time to salt accumulation by excised young barley root systems (fig. 5), each set of plants (168) was first grown in a complete culture solution from February 8 to March 2. Each set of the excised roots was then placed in shallow covered trays containing 3800 cc. of an external culture solution having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 10 or 24 hours.

The data pertaining to NO_3, given in the graph (fig. 5), refers to NO_3 accumulation as such in the sap. Additional nitrate was also absorbed and reduced in the tissues.

The plants in the experiment performed to determine the relation of temperature and time to accumulation of salt by excised root systems of older barley plants (fig. 6), were first grown in large tanks containing a low-potassium culture solution from December 21 to March 4. Each sample of root tissues then taken was composed of 75 gm. (fresh weight) of tissues obtained from a large mass, prepared by clipping many uniform root systems into approximately 5-cm. segments. This root material was placed in bottles containing 3800 cc. of external culture solution having an initial

TABLE V

TEMPERATURE EFFECTS ON SALT ABSORPTION BY EXCISED ROOTS OVER PROLONGED PERIODS

TEMPERATURE	PERIOD	REMOVED FROM SOLUTION	
		K	Br
		MILLIEQUIVALENTS PER LITER	
°C.	*hours*		
10	73	4.56	3.94
10	124	3.81	4.19
21	73	1.32	3.04
21	124	−0.36	0.53

TABLE VI

ACCUMULATION RATIOS FOR POTASSIUM, NITRATE, AND HALIDE IN EXCISED BARLEY ROOT SYSTEMS

TEMPERATURE	COMPOSITION OF CULTURE SOLUTION			COMPOSITION OF SAP			ACCUMULATION RATIOS*		
	K	NO_3	HALIDE	K	NO_3	HALIDE	K	NO_3	HALIDE
	MILLIEQUIVALENTS PER LITER			MILLIEQUIVALENTS PER LITER					
°C.									
6	9.62	8.35	4.84	34.7	4.8	5.9	3.6	0.6	1.2
12	9.32	8.27	4.76	47.7	11.1	8.8	5.1	1.3	1.9
18	8.45	7.75	4.64	73.2	23.7	14.6	8.7	3.1	3.1
24	7.98	7.29	4.30	97.8	38.1	30.9	12.3	5.2	7.2
30	7.42	6.70	3.96	112.0	47.8	39.9	15.1	7.1	10.1

* Accumulation ratio is the concentration in sap divided by concentration in external solution (final).

composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained there during the absorption period.

The results of the temperature effects on salt absorption by excised roots over prolonged periods are given in table V. The plants for this experiment were grown in 20-liter jars, containing 25 plants each, from January 28 to March 15. The culture solution in which these plants were grown consisted of 0.005 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4. Excised entire root systems of 25 plants were used for each absorption treatment. Each set of these roots was next placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained there during the absorption period.

The set up of the experiment which provided the data for table VI was the same as that described for figure 5. The absorption period was 10 hours.

It is important to emphasize that the temperature effects apply to both cations and anions. STEWARD has pointed out that certain earlier conclusions from experiments on storage tissues (**26**)—that temperature acceleration of salt absorption may be restricted to anions—is possibly explained on the ground that the tissues were not in a highly active state of metabolism, because of insufficient aeration.

While high temperature coefficients are, in fact, characteristic of salt accumulation by roots like those used in our experiments, to establish this conclusion demands the correct experimental conditions. In preliminary work we were unable to obtain satisfactory evidence of temperature effects, for reasons which subsequently became clear. It is essential to do the experiments with tissues in which active cells of high potentiality for salt accumulation predominate. As has already been suggested, the losses of salts by inactive or senescent cells in older root systems may partially or wholly obscure the accumulation by active cells, since the whole mass of tissues, or the composite culture solution, is under examination. The cells should have an initially low salt content, and especially in the absence of rate studies, the effect of temperature upon their salt absorption should be determined only during periods when the cells remain capable of continued increase in salt concentration. Furthermore, in a long period available carbohydrates may be depleted or other changes occur in the tissues unfavorable to salt accumulation or retention. These changes are accelerated with increasing temperatures and when a sufficient interval of time has elapsed, the accumulation of salt may be depressed by the secondary effects of temperature, or some cells may begin to lose salt (table V and fig. 6). In long experiments the apparent paradox may be presented that a greater salt content is obtained at the lower temperature. Unless properly interpreted such results confuse the effect of temperature upon salt accumula-

tion with other effects of temperature. The young low-salt, high-sugar tissues, developed according to the technique already described, are well suited to the study of temperature acceleration of salt accumulation, but time effects and sugar depletion must be taken into consideration.

The temperature effects in question concern primarily K, Br, and NO_3 ions. Temperature data have been obtained on the removal of Ca, Mg, SO_4, and HPO_4, or H_2PO_4 from culture solutions and these suggest that high temperature coefficients for absorption apply also to these ions. However, special difficulties are found in the interpretation of the results. The amounts of Ca, Mg, or SO_4 absorbed in a brief period are usually very small, and questions of precipitation of calcium and magnesium salts on root surfaces arise, especially when secondary hydrogen ion changes occur in the solution.

Whether temperature coefficients are the same for the accumulation of all ions cannot be decided without greater knowledge of the mechanism of salt accumulation. Adequate rate studies remain to be carried out. Also ionic exchanges versus simultaneous uptake of cation and anion and, in a mixed salt solution, complex interionic effects are involved. Reduction of NO_3 in the tissues is another complicating factor. Temperature effects on salt accumulation are, of course, indirect in the sense that it is the metabolism of the plant which is being affected, one of the results of accelerated metabolism being accelerated salt absorption, provided that other conditions favor this process.[3]

ACCUMULATION RATIOS.—One of the temperature experiments also affords information concerning the magnitude of accumulation ratios for potassium, nitrate, and halide (ratio obtained by dividing concentration in expressed sap by concentration in external solution). These ratios rise to approximately 15 for potassium, 7 for nitrate, and 10 for halide (table VI). The values apply to culture solutions containing over 7 milliequivalents per liter of potassium salt. Far higher accumulation ratios are easily obtainable by utilizing more dilute solutions. They may reach values in excess of 1000. In fact, in some tests the root tissues accumulated potassium to a high concentration, and left only a trace in the culture medium.

SALT STATUS OF TISSUES

It has been pointed out that the capacity of root tissues to accumulate salt over a given period is dependent upon the salt supply previously

[3] Further study should be made of the translocation of solutes from root to shoot. In our experiments with excised roots the conditions were so chosen that temperature effects on salt accumulation could be easily demonstrated. We feel that these effects are inherently characteristic of the process of accumulation, but appreciate how many complicating factors enter into the study of salt absorption by entire plants grown under diverse conditions.

accumulated by the tissues. By frequent changes of external culture solution in the preliminary growth period, the supply of nutrients is renewed and this leads to the production of root tissues with a very high salt content, since uptake of salt is in excess of translocation to the shoot. The excised high-salt root tissues had a very limited capacity for absorbing additional salt and in some cases no K was removed from solution or even a slight loss from the tissues as a whole occurred, although a small amount of anion, NO_3 or Br, could be absorbed by a process in effect equivalent to an exchange of these anions for bicarbonate or organic acid anions derived from the tissues (table VII, experiment A). The actual mechanism of salt accumulation of this type has not yet been elucidated.

TABLE VII

RELATION OF INITIAL SALT STATUS OF ROOT SYSTEM TO ABSORPTION OF SALT IN SUBSEQUENT PERIOD

EXPERIMENT A

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	Br
	MILLIEQUIVALENTS PER LITER	
High-salt, excised roots	−0.20	0.50
Low-salt, " "	2.66	1.90

EXPERIMENT B

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	NO_3
	MILLIEQUIVALENTS PER LITER	
Entire plants	2.64	2.56
Excised root systems	2.53	2.43

EXPERIMENT C

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	NO_3
	MILLIEQUIVALENTS PER LITER	
High-salt, entire plants	2.05	2.40
" " , excised root systems	0.67	0.89
Low-salt, entire plants	3.66	3.49
" " , excised root systems	3.02	2.93

The sets of plants, consisting of 168 plants each, for experiment A (table VII) were grown in the standard culture solution from May 23 to June 15. For the low-salt sets, the culture solution was not changed during the growth period; for the high-salt sets, the culture solution was renewed daily from May 29 to June 14 inclusive. The roots of each set were then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 7.5 K, and 7.5 Br, and remained there for an absorption period of 20 hours. The solution also contained small amounts of carbonates and chlorides of Ca and Mg. The pH of the culture solution was modified during the experimental period from 7.5 initial to 8.1 for the high-salt, and from 7.5 to 7.3 for the low-salt condition. The temperature of the solution was 25° C.

The very different behavior of low- and high-salt tissues is illustrated in table VII. During the given periods of salt accumulation, far more salt was absorbed by the low-salt roots than by the high-salt roots and the relation between cation and anion absorption was also altered to some extent.

It is not the intention of the writers to discuss at this time the problem of translocation of salt in relation to accumulation, but a comparison of the absorption of salt by excised root systems and by entire plants, for low- and high-salt plants, is of immediate interest. (table VII, experiments B and C).

The sets of plants, consisting of 168 plants each, for experiment B (table VII) were grown in shallow trays containing the standard nutrient solution from April 28 to May 17. Each set of roots was then placed in 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained there during an absorption period of 7 hours. The temperature of the solution was was 23.5° C.

The sets of plants, consisting of 168 plants each, of experiment C (table VII) were first grown in standard nutrient solution from June 10 to June 29 as in experiment A. The culture solution of the low-salt sets was not changed during the growing period; that of the high-salt sets was renewed daily from June 18 to June 28 inclusive. Each set of the excised roots was then placed in 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained there during the absorption period of 7 hours.

Over the limited period of the experiments, the amount of salt absorbed by the roots excised from the low-salt plants was almost as large as the amount absorbed by the entire plants, while the entire high-salt plants had a much higher absorbing capacity than did the corresponding excised root systems. The removal of salt from root to shoot permitted more salt to enter the

root system. Despite this influence of the shoot, more salt was absorbed by the excised root systems of the low-salt plants than by the entire high-salt plants, even though the latter were considerably larger than the low-salt plants. One cannot doubt the importance of knowing the salt status at a given time, of either excised or attached roots. Incidentally, these experiments, supported by many others, illustrate that the process of accumulation of salt by root cells has no direct dependence on transpiration, but the continuance of salt absorption is influenced by transpiration to the extent that the latter may be a factor in determining the upward movement of salt, a point to be discussed in another article.

A clearer understanding can now be gained of the effects of illumination on the absorption of salt. If, during the day period, the translocation of salt out of the root system is rapid enough partially to deplete the root, then, in a subsequent dark period, large absorption of salt can occur under conditions favorable for the metabolic activities of root cells.

Relations between cation and anion accumulation

Time effects are an important consideration in the study of salt accumulation by excised roots (fig. 7). In the experiment, for making this study, the plants were first grown in large jars containing a large reserve of culture solution per plant from November 5 to January 6. The method used, of cutting root systems into segments, was the same as that described for the experiment for figure 6. The absorption experiment was carried out in bottles containing 3800 cc. of culture solution, and 75 gm. of root tissue. The initial composition of the external solution for the absorption period consisted of 0.002 M KBr, and 0.0002 M $CaBr_2$. The temperature of the solution was 20° C.

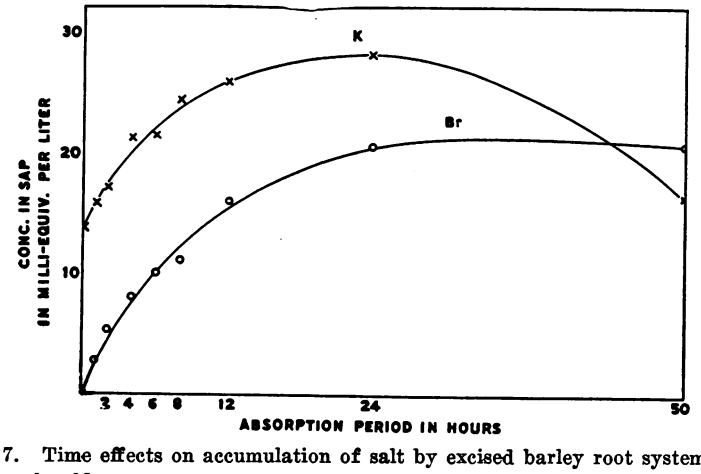

Fig. 7. Time effects on accumulation of salt by excised barley root systems approximately 9 weeks old.

In a number of the experiments on root tissues possessing a high potentiality for salt absorption, K and Br were accumulated in the sap, or withdrawn from solution, in nearly equivalent quantities (figs. 6, 7, early period, and unpublished results). This type of absorption has been designated by STEWARD as "primary" absorption and in some respects forms the basic problem of accumulation. However, various secondary effects complicate the study of this relation and it is apparent that actual equivalent absorption by root tissues of cation and anion of the salt under study can be demonstrated only under special conditions, some of which are still obscure. Nearly equivalent absorption of K and Br may be found over one period of time, while, over a longer period, the same tissues may give results which seem to indicate a larger absorption of Br than K (figs. 6, 7). The explanation probably is that when the activity of the tissues declines, some cells lose K, accompanied by bicarbonate or organic acid anions in excess of the loss of Br. The excess accumulation of K over Br found in some experiments to be reported later requires a further study of the possibility that HCO_3 ions may be involved in this process.

Discs of potato tissues during an initial period lose K, Ca, and Mg, together with organic acid anions, followed by a period of reabsorption. In this later period, K and Br may be absorbed in approximately equivalent quantities (**32**). Young root tissues show no appreciable initial loss of potassium ions but small amounts of calcium and magnesium ions enter the culture solution, accompanied by bicarbonate, phosphate, or organic acid anions. Some evidence suggests that the calcium and magnesium salts may be derived in part from surface deposits on roots. It is possible that potassium may be subsequently absorbed by active cells together with these released anions. Also, earlier work by various investigators supports the view that exchange of cations between cell wall or protoplasm and the culture solution can take place, and it is not unreasonable to postulate that hydrogen ions may participate in such exchanges under certain conditions, resulting in an increase of hydrogen ion concentration in the unbuffered culture solution. An exchange of potassium for other cations would be particularly expected in an initial period, when the tissues are extremely low in potassium content and relatively high in other cations. But, in general this type of exchange process, with respect to cations, is a minor phenomenon compared with the active accumulation of potassium salts in the vacuole, and the two processes should not be confused. Direct cation exchange between solution and vacuolar sap remains uncertain in the case of root tissues, but since the losses of sodium, calcium, or magnesium from the expressed sap are very small in comparison with the increases of potassium, exchange with these cations cannot explain the behavior of these actively absorbing tissues.

From solutions of $CaBr_2$ or $Ca(NO_3)_2$, or mixed solutions of potassium and calcium salts, anions can sometimes be accumulated by root systems in

much greater equivalent quantity than cations, and NO_3 may be absorbed by roots initially high in K in greater equivalent quantity than K from KNO_3 solutions. As already suggested, the effect is that of an exchange of HCO_3 ions for Br or NO_3 ions. This process is also dependent upon aerobic metabolism of the cell according to our experimental data. Under anaerobic conditions little or no accumulation of anions in the sap occurred even though considerable amounts of CO_2 were evolved (fig. 3 and table II). The absorption of NO_3 from solution with concurrent reduction presents another phase of the general problem. A detailed study of cation-anion accumulation with a series of salts is now being undertaken.

Available carbohydrate and accumulation of salt

Inasmuch as a high rate of respiration is associated with rapid accumulation of salt by root tissues, the available carbohydrate supply is of importance. A rapid loss of sugar occurs when root tissues are aerated as in the absorption procedure, although generally more sugar is lost than is accounted for by carbon dioxide liberated, and no simple relation is found between sugar concentration and CO_2 evolved. A number of experiments have been carried out in which the excised roots were largely depleted of sugar by vigorous aeration, while immersed in tap water or a dilute calcium sulphate solution, preceding the period during which the absorption of potassium salts was studied. It is desired to extend these experiments before drawing final conclusions, but the results now available indicate that root tissues sufficiently reduced in sugar content suffer a large decrease in their capacity to accumulate salts, which can be restored, at least in part, by supplying sugar to the roots through the culture solution. Apparently it is necessary to reduce the sugar concentration to a low value before salt absorption declines significantly. In an article following this one, Prevot and Steward (**28**) have presented data on segments of primary roots of barley which also indicate that accumulation of Br by root cells low in sugar is accelerated by providing a supply of sugar in the external solution during the period of salt absorption.

The high-salt roots have a markedly lower initial sugar content than the low-salt roots, and this is significant in certain experiments, especially those carried on for a number of days. However, various lines of evidence, including the time effects described in the next section, suggest that the limitation of salt absorption by high-salt roots, in most of the experiments now under consideration, cannot be ascribed merely to deficiency of carbohydrate. The root cells must have a certain upper limit for salt concentration even when they remain in an active state of metabolism. Conceivably maximum distention of the cell wall or a salt saturation of protoplasmic constituents limits salt absorption, but the mechanism is still obscure.

TIME EFFECTS

Preliminary experiments using an earlier technique, with older root systems of moderate potentiality for salt accumulation, had shown that absorption of salt (KBr) by excised roots reached an approximate maximum within 24 hours, after which a net loss of potassium from the tissues as a whole took place, without concurrent loss of Br, within total period of 48 hours (fig. 7). More recently a time experiment was carried out with young root systems, according to the principal technique outlined earlier in this article (figs. 8, 9).

The plants, consisting of 168 in each set, were first grown in a complete culture solution from June 30 to July 19. Each set of roots was then placed in a 4-liter bottle containing 3 liters of an external solution having an initial

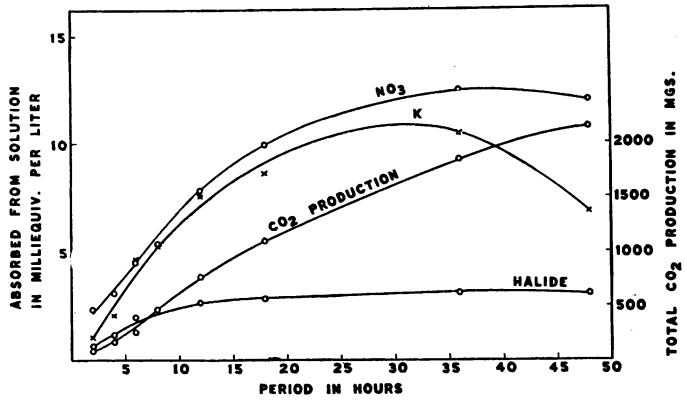

FIG. 8. Time effects on absorption of salt by young excised barley root systems, based on analysis of external solutions.

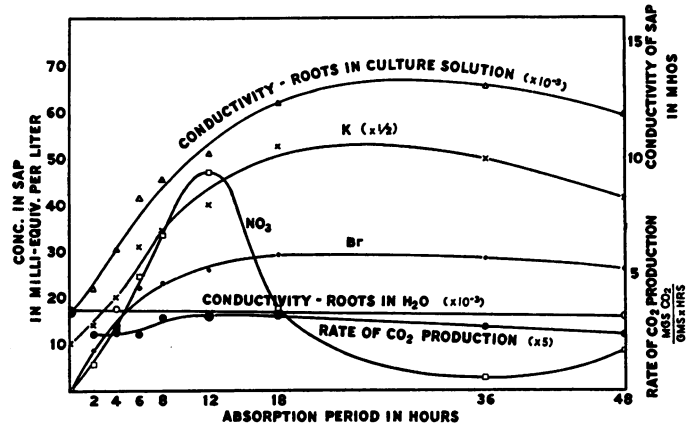

FIG. 9. Time effects on accumulation of salt in sap by excised barley root systems.

composition of 0.005 M KBr, 0.005 M KNO_3 and 0.002 M $Ca(NO_3)_2$. Change of solution was made following the 18-hour period, to maintain as far as possible the original concentration. The temperature of the solution was held at 26° ± 1° C.

The CO_2 production was expressed in milligrams of CO_2 produced by each set of root tissues and includes CO_2 evolved and CO_2 from HCO_3 formed in the culture solution.

The points for the 24-hour period are omitted since we have reason to believe that the activity of this set of roots was depressed by some accidental circumstance effective during the initial growth period. The insertion of these points would not modify the general trend of the curves nor the conclusions reached.

Residual solutions and sap expressed from the tissues were analyzed for K, Br, and NO_3. Total sugar content of expressed sap was also determined. Figure 9 presents evidence of significant accumulation of salt within a few hours, with concentrations in the sap rising rapidly during the first 12 to 18 hours. Approximately maximum values for concentrations of K and Br in the expressed sap were attained within this period.

The concentration of NO_3 in the sap increased very rapidly during the period of 16 hours, then fell off abruptly. The absorption of NO_3 continued (as evidenced by the observations on the culture solution, fig. 8), but at the point indicated by the break in the curve, the rate of NO_3 reduction in the tissues greatly exceeded the rate of NO_3 accumulation. The factors controlling reduction of NO_3 need further elucidation, but the suggestion is made that the increasing concentrations of K in the sap influenced the reducing properties of the tissues (ECKERSON, 7). Apparently, unknown factors associated with the seasonal conditions under which plants are grown also modify the ability of the tissues to reduce nitrate. The reduction of nitrate was especially rapid in the experiment now cited. The results on NO_3 absorption emphasize the necessity for examining not only the residual culture solution, but also the tissues, in order to understand the relation of NO_3 absorption to accumulation of NO_3, as such, in the sap. This point is of interest in connection with the study of the relation of oxygen tensions to NO_3 absorption versus NO_3 accumulation.

The significance of data on CO_2 production in relation to sugar loss during a period of salt accumulation is being further investigated, but attention is now directed to the fact that the rate of CO_2 production continued almost undiminished, at least for some time, after the tissues had attained their maximum salt content. The amount of sugar remaining at this point was still relatively large, and adequate for rapid accumulation of salt by low-salt tissues, if we may judge from results of the earlier experiments on the effects of sugar depletion on salt accumulation. The presumption is that any

actively growing cells in the high-salt root system continued to absorb salt after an apparent maximum salt concentration had been attained by the root system as a whole, but this limited absorption would not be determinable from the examination of a mass of root tissues in which most of the cells had attained a high and perhaps maximum salt concentration, particularly if some cells were losing salt, and compensating for gains by actively growing cells.

Further comparisons of salt accumulation by root and storage tissues

The low-salt root tissues had a remarkably high capacity for rapid salt accumulation, greatly surpassing discs of potato tuber in this respect. This difference in behavior can be explained in part by the fact that the potato tuber tissues have a relatively high initial salt content, while root tissues of the kind under consideration are very low in salt for reasons given earlier. The potato tissues, following an initial period of salt loss, continue to absorb salt (KBr) from dilute solution without any approach to a steady state for a period of more than 100 hours (**35**), in contrast to the young root systems which very rapidly attain an approximately maximum average concentration of salt, and in a later period may suffer a net loss of salt.

The writers are of the opinion that the nature of salt accumulation is fundamentally the same for storage tissues and root tissues, but at present it is useful to recognize two aspects of the general problem: (a) the very rapid initial accumulation of salt by low-salt root tissues, and (b) the gradual and sustained salt accumulation by discs of aerated potato tuber tissues. The relatively high-salt concentration characteristic of the latter limits the initial rate of salt accumulation, while, on the other hand, root systems of the type used in our experiments lack the capacity for long sustained salt accumulation because, apart from relatively few cells in the apex, the root cells do not retain the potentiality for constructive metabolism associated with growth. They differ, therefore, in this respect from the surface cells of the discs of potato tuber (**36**). Prevot and Steward (**28**), however, have shown that segments of very young primary roots of barley, relatively high in salt, give time curves for bromide accumulation which have a general similarity to the corresponding curves for bromide accumulation by potato tuber tissues.

One difficulty in comparing salt accumulation by discs of storage tissues and by root systems is that it is not now feasible to assign salt absorption by roots to cells having a particular position with reference to external surfaces. By the use of discs of controlled thickness, Steward *et al.* (**40**) demonstrated that the accumulation of bromide by discs of potato tuber is limited almost entirely to cells lying near the surface, which receive an adequate supply of oxygen and have a very high rate of respiration. Probably the accumulating

cells of these young roots (the cortex is primarily involved) are not limited by the ability of oxygen to diffuse rapidly enough into this relatively shallow zone of cells, which is permeated by intercellular air spaces.

Carbohydrate relations also demand consideration. The potato tuber has a large reserve of carbohydrate available for respiration and other metabolic processes, while the sugar supply of the root tissues is rapidly depleted under conditions of aeration favoring the most rapid accumulation of salt.

The low-salt root tissues were restricted in their initial supply, not only of potassium, but also of nitrogen and phosphorus. Deficiency of either of the latter two elements might limit some phase of metabolism essential to growth and sustained salt absorption over a long period. One might also speculate on the factors associated with shoot growth, apart from carbohydrate synthesis and removal of salt from the root system, which influence the growth activities and salt-accumulating power of the root over extended periods.

Discussion

The relation of metabolic activities of cells to salt accumulation is strikingly illustrated by the experiments on excised root tissues, which afford strong support to the main conclusions of STEWARD, and BERRY and STEWARD, based on experiments with storage tissues, and at the same time present for study certain additional phases of the general question of salt accumulation. A reexamination of many problems of salt absorption and accumulation by roots is demanded from the point of view of cell metabolism and growth. Relations between ions in absorption, concentration relations, nutrient deficiencies, utilization of various forms of nitrogen, etc., together with the control of those variables which determine rates and kinds of metabolism, require further investigation. Oxygen-supplying power of the culture medium, temperature, rate of translocation of carbohydrate from shoot to root, as well as internal factors of cell growth and metabolism, are indispensably concerned with the processes of salt absorption.

Justification is found for emphasizing once more that accumulation of salt by the living cell is not merely a question of permeability. Indeed, many of the methods used in the study of permeability are of such a nature as to preclude the possibility of obtaining data on the absorption and accumulation of solutes by normal metabolizing cells. Emphasis is often placed on the slight permeability of living cells to strong electrolytes. It is now apparent that this statement requires modification with reference to some, if not all, types of plant cells, when initial salt concentration and metabolic activities of the cells are given consideration. Potassium salts can enter the root cell with relatively great rapidity under favorable conditions.

The problem of salt absorption and accumulation by root cells, and also by the living cells of the shoot, is fundamental to the study of the transloca-

tion of mineral elements. These processes also have a direct bearing on certain aspects of root pressure. New points of view with respect to general root metabolism and to certain questions of developmental anatomy arise.

Summary

1. A technique is described for the study of salt accumulation by excised barley roots, which are especially adapted to the investigation of certain general problems of salt accumulation. It is shown that under proper conditions, which are defined, potassium salts accumulate very rapidly in the sap of excised roots, against concentration gradients.

2. A number of variables must be carefully controlled or evaluated in experiments on salt accumulation by excised roots, particularly: (a) age of roots and proportion of actively metabolizing cells, (b) initial salt content of roots, dependent on supply of nutrient salts provided during preliminary growth period, (c) seasonal effects on development of root system, (d) available carbohydrate, (e) variability of material.

3. Accumulation of salt is associated with active aerobic respiration of the roots. An adequate supply of oxygen is indispensable for both cation and anion accumulation. Data pertaining to effects of varying oxygen tensions on salt accumulation are discussed.

4. Temperature coefficients of salt accumulation were found to be of a high order, but evidence of this can be obtained only under experimental conditions carefully chosen to eliminate certain complicating factors.

5. The experiments on excised roots lead to fundamental conclusions similar to those derived from experiments on salt accumulation by storage tissues, and additional aspects of the problem of salt accumulation are presented for study.

6. Attention is directed to the importance of the results on salt accumulation by root cells relative to other problems such as root pressure, translocation of salts, and the general metabolism of root cells.

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