ACCUMULATION OF SALT AND PERMEABILITY IN PLANT CELLS

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This paper represents a further report on the progress of investigations originally described by Hoagland and Broyer[1] and by Prevot and Steward.[2] (Compare also investigations of Steward and associates on storage tissues.[3]) These investigations have as their special objective the development of knowledge of the relation of cell metabolism to the accumulation of salt against concentration or activity gradients by root cells. The general technique of experimentation on roots has already been described.[1] As one feature of this technique the internal concentrations of salt were usually determined on sap expressed from the root tissues after killing them by rapid freezing. This procedure is capable of yielding consistent and useful results on the influence of many factors of the internal and external environment on the absorption and accumulation of salt.[4] We are of the opinion that most of the salt recovered in the expressed sap is located in cell vacuoles, large or small. Obviously, however, the sap derived from complex tissues does not represent so definite a component of living cells as does the vacuolar sap that can be removed from large coenocytic algal cells. It is of interest, therefore, to consider another method of studying the movement of salt against gradients in root systems. Investigation of the socalled bleeding sap obtained from the roots of barley or other plants when the shoot is abscised a short distance above the root system provides another method of study. There are various reasons for believing that this sap emerges primarily from xylem vessels.

Comparison of Composition of Exudates and of Expressed Sap from Roots

We have made a number of comparisons of the composition of sap expressed from barley root tissues with that of the exudation fluid, in experiments conducted on plants absorbing salt from dilute solutions of potassium bromide. In one such experiment (Fig. 1), barley plants placed in a dark humid chamber had their roots immersed in aerated distilled water. They were decapitated about 1 inch above the root-stem plate and the bleeding sap collected for

[1] Hoagland, D. R. and Broyer, T. C., *Plant Physiol.*, 1936, **11,** 471.
[2] Prevot, P., and Steward, F. C., *Plant Physiol.*, 1936, **11,** 509.
[3] Steward, F. C., *Tr. Faraday Soc.*, 1937, **33,** 1006.
[4] Broyer, T. C., and Hoagland, D. R., *Am. J. Bot.*, 1940, **27,** 501.

15 minutes to obtain an initial sample of exudate. The experimental salt was then added; *i.e.*, KBr solution of 5 milliequivalents per liter concentration. Successive increments of exudate were collected for analysis. At several intervals root samples were removed, weighed, centrifuged, and frozen for the determination of the K and Br concentrations in the expressed sap. The data presented illustrate that the exudate, as well as the expressed sap of the roots, reflects the power of the actively metabolizing roots to concen-

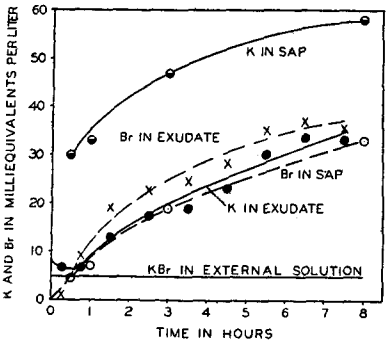

FIG. 1

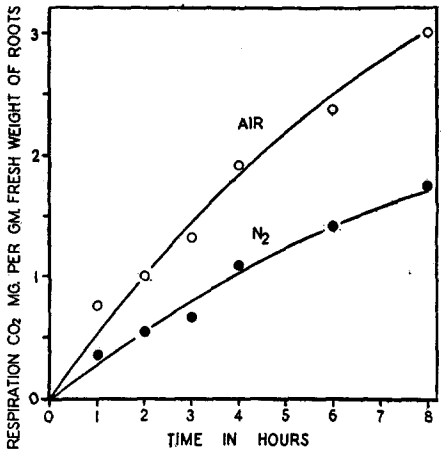

FIG. 2

FIG. 1. Comparison of the concentrations of K and Br in exudates and in expressed saps from roots of decapitated barley plants. Roots of decapitated plants in aerated distilled water (control) and in aerated KBr solution of 5 milliequivalents per liter concentration. The exuding plants were placed in dark, humid chambers.

FIG. 2. Comparison of rates of CO_2 production by excised roots under aerobic and anaerobic conditions. Barley plants grown from Jan. 7th to Jan. 28th (21 days). Experimental culture solutions 50 milliequivalents per liter concentration. Temperature of culture solution $19.0 \pm 0.5°C$. Nitrogen gas purified through pyrogallol. Roots placed in water and pretreated with N_2 gas. Compare Figs. 3*a* and *b*, in which data are from a similar experiment.

trate solutes. Earlier investigators also observed and studied the relations between the composition of exudate fluids and that of the culture medium of the roots. In the recent period, this method of study has assumed greater interest in the light of developments in research on the active transport of solutes by living plant cells.

The data cited in this article indicate that the concentration of mobile ions may be of the same order of magnitude in the exudate and in expressed sap of the roots from which the exudate arises. They are in agreement with re-

sults published earlier from this laboratory.[5] Lundegårdh has recently cited from another point of view several results of similar import.[6] In the present experiments the bromide ion concentrations were nearly the same in the two fluids of different origin (exudate and expressed sap) after a suitable period of time for solute absorption had elapsed. Potassium concentrations remained considerably higher in the expressed sap than in the exudate. Undoubtedly the quantitative relations of solute concentration in root sap and exudate would vary with the time period of the experiment and with factors affecting metabolism. The idea to be stressed here is that the independent method of exudate study leads to essentially the same general point of view concerning the metabolically governed movement of salt against gradients as does the study of expressed sap. This is of interest because the exudate is a fluid obtained without the need of killing and crushing tissues. The exudate from the barley plants contained no protein precipitable by trichloroacetic acid. Phillis and Mason[7] have suggested that the accumulation of solutes by tissues of higher plants may be regarded as a phenomenon of the "solvent capacity" of living protoplasm. The evidence offered in this article, as well as the evidence available from the investigations of *Nitella*, *Valonia*, and other large coenocytic cells does not lead to the view that the activities of living plant cells in concentrating salt can be explained by the concept of "solvent capacity" of protoplasm. Rather a secretory process is indicated.

Respiration in Relation to Salt Accumulation

The intimate association of aerobic metabolism with inorganic solute accumulation by plant cells gives special importance to the processes of aerobic respiration. As a general basis for discussion of this aspect of the problem, it is useful to call attention to the rates of CO_2 production by barley root cells under aerobic and anaerobic conditions. Data from one experiment are presented in Fig. 2.[8] The aerobic CO_2 production is more rapid than the anaerobic, but even in the latter case enough carbonic acid is given off to make possible marked salt accumulation, if this were the determining factor.[9]

[5] Hoagland, D. R., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 181.

Hoagland, D. R., and Broyer, T. C., *Am. J. Bot.*, 1940, **27**, 173.

[6] Lundegårdh, H., *Planta*, 1940, **31**, 184.

[7] Phillis, E., and Mason, T. G., *Ann. Bot.*, 1940, **4**, N.S., 645.

[8] Also see footnote 1 and data in Tables I and III.

[9] Unpublished data with excised barley roots show that a small aerobic accumulation of salt can occur at low temperature (0–5°C.) under which conditions the concomitant CO_2 production may be only 20 per cent of that at normal temperatures (20–25°C.) The rate of CO_2 production under anaerobic conditions is frequently 50 to 60 per cent of that under aerobic conditions, at similar, normal temperatures,

Yet anaerobic respiration is ineffective for this purpose. The respiratory quotient for well aerated cells is close to 1, provided an excess absorption of cation or anion does not occur. If an excess of an ion of one sign of charge is absorbed then through adjustments in organic acid metabolism the respiratory quotient may be altered (Ulrich).[10]

Because of the importance of aerobic respiration in relation to salt accumulation, it is an obvious step to investigate the influence on salt accumulation of respiratory inhibitors. A systematic study of roots from this point of view is going forward. In several experiments already completed potassium cyanide in relatively low concentration (0.5 to 10 milliequivalents per liter) was added to aerated solutions of potassium bromide in which barley roots were immersed. The cyanide suspended completely, or almost completely, the accumulation of K and Br ions, without apparent injury to the roots, except for a development of brown color in higher concentrations of cyanide (Table I, Experiment 1). The effect of cyanide on salt accumulation was to a certain extent reversible (Table I, Experiment 2). The lower effective concentrations of cyanide (0.5 to 1.0 milliequivalents per liter) did not depress CO_2 production very greatly, but the more significant oxygen consumption value may show greater depression, according to certain unpublished data by Machlis, of this laboratory. The results obtained with cyanide suggest an induced anaerobiosis. The general suggestion is that a metal catalyzed respiratory system is linked in some way with the process of salt accumulation, although preliminary experiments have not revealed the exact nature of the system involved.

It is conceivable that a hydrogen acceptor which would modify the course of respiration would likewise influence the accumulation of electrolytes. The effects of methylene blue were studied from this viewpoint. The presence of this hydrogen acceptor in the nutrient medium in very low concentrations (1×10^{-5} to 1×10^{-4} molar) had little influence on either CO_2 production or electrolyte absorption. Methylene blue concentrations of 1×10^{-3} molar which were effective in inhibiting salt accumulation (Table II), did not depress the average rate of CO_2 production over an experimental period of 4 hours. Although the methylene blue-treated tissues (1×10^{-3} M) frequently appeared to be turgid, a tissue injury was apparent in the general loss in fresh weight and solute by the roots during this interval of time. These effects were irreversible. Methylene blue increased the permeability of the cells, as indi-

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but appreciable salt accumulation does not occur. Since under low oxygen tensions the respiratory quotient increases, oxygen absorption would be a more satisfactory index of respiratory metabolism in relation to absorption of salt (compare data on respiration of plant tissues by Merry and Goddard, see Merry, J., and Goddard, D. R., *Proc. Rochester Acad. Sc.*, 1941, **8**, 28) than CO_2 produced.

[10] Ulrich, A., *Am. J. Bot.*, 1941, **28**, 526.

TABLE I

Effects of Aeration and of Cyanide on the Absorption of K and Br by Excised Barley Roots

Experimental conditions	Composition of expressed sap per liter		Net absorption per liter		Respiration rate in amount of CO_2 per hr. per gm. fresh weight tissue
	K	Br	K	Br	
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*mg.*
Experiment 1					
Control roots (initial status)	26.8	0.0			
Distilled water; aerated	25.2	0.0	−1.6	0.0	0.29
Distilled water; N_2 gas	21.7	0.0	−5.1	0.0	0.16
KBr 5 m. eq. per l.; aerated	55.9	22.3	29.1	22.3	0.32
KBr 5 m. eq. per l.; N_2 gas	26.7	3.2	−0.1	3.2	0.12
KCN 0.5 m. eq. per l.; aerated	25.6	0.0	−1.2	0.0	0.29
KCN 1.0 m. eq. per l.; aerated	22.5	0.0	−4.3	0.0	0.20
KCN 3.0 m. eq. per l.; aerated	27.3	0.0	0.5	0.0	0.08
KBr 5 m. eq. per l., plus KCN 0.5 m. eq. per l.; aerated	26.4	4.7	−0.4	4.7	0.27
KBr 5 m. eq. per l., plus KCN 1.0 m. eq. per l.; aerated	24.3	0.0	−2.5	0.0	0.19
KBr m. eq. per l., plus KCN 3.0 m. eq. per l.; aerated	31.8	0.8	5.0	0.8	0.10
Experiment 2					
Control roots (initial status)	18.6	0.0			
KBr 5 m. eq. per l.	44.5	33.8	25.9	33.8	
KBr 5 m. eq. per l., plus KCN 10.0 m. eq. per l.	23.9	2.1	5.3	2.1	
KBr 5 m. eq. per l., plus KCN 10.0 m. eq. per l., followed by washing, then KBr 5 m. eq. per l.	31.9	18.0	13.3	18.0	
Experiment 3					
Control roots (initial status)	25.0	0.0			
KBr 50 m. eq. per l., plus KCN 1.0 m. eq. per l.	29.2	6.8	4.2	6.8	

Experiment 1: Barley plants grown from Oct. 15 to Nov. 8 (24 days). Experimental periods 8 hours. pH of cyanid cultures about 7.0. Nitrogen gas purified through pyrogallol. Temperature of culture solutions 22°C.

Experiment 2: Barley plants grown from June 3 to June 24 (21 days). Experimental periods each 5 hours. All culture solutions aerated. Temperatures of culture solutions 21.5± 0.5°C.

Experiment 3: Barley plants grown from Dec. 30 to Jan. 30 (31 days). Experimental periods 8 hours. Culture solution aerated. Temperature of culture solution, 17.0 ± 0.5°C.

cated by outward movement of solutes, and CO_2 production continued, but disturbance of the protoplasmic mechanism prevented salt accumulation.

The influence of oxygen deficiency in preventing salt accumulation might be associated with the formation of by-products of fermentative processes, such as alcohol or lactic acid. An experiment was carried out in which these substances were added to the culture solution, under aerobic and anaerobic conditions (Table III). Under aerobic conditions neither the alcohol (0.01 M) nor the lactic acid (0.001 M) had the inhibiting effect on the entrance of salt into the tissue that was produced by oxygen deprivation. It may be that oxidation of the lactic acid or alcohol was sufficiently rapid to prevent any

TABLE II

Effects of Methylene Blue on the Absorption of K and Br by Excised Barley Roots

Experimental conditions	Composition of expressed sap per liter		Net absorption per liter		Respiration rate in amount of CO_2 per hr. per gm. fresh weight tissue
	K	Br	K	Br	
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*mg.*
Experiment 1					
Control roots (initial status)	22.3	0.0			
Distilled water	21.0	0.0	−1.3	0.0	0.29
Methylene blue 0.001 M	8.7	0.0	−13.6	0.0	0.32
KBr 5 m. eq. per l.	37.9	7.85	15.6	7.85	0.39
KBr 5 m. eq. per l. plus methylene blue 0.001 M	12.5	2.04	−9.8	2.04	0.43
KBr 5 m. eq. per l. plus methylene blue 0.001 M, followed by washing, then KBr 5 m. eq. per l.	11.8	4.05	−10.5	4.05	0.26*

Experiment 1: Barley plants grown from Apr. 8 to May 2 (24 days). Experimental periods each 4 hours. Culture solutions aerated. Temperatures of culture solutions 21.5 ± 0.5°C. At the termination of each experimental period all roots appeared to be turgid; losses in fresh weight of tissue during the experiment were not significant. Sugar losses from roots treated with methylene blue were significantly greater than those accountable to normal respiratory processes.

* This figure represents the respiration rate obtained during the secondary treatment. The value during the primary treatment was 0.40.

interference with protoplasmic reactions. Or, as Blinks[11] suggests, on the basis of the work of Osterhout and Hill,[12] it is conceivable that under anaerobiosis there may exist as an influential factor, not the formation of a deleterious product of metabolism, but rather the failure of synthesis of some protoplasmically active compound.

[11] Blinks, L. R., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 204.

[12] Osterhout, W. J. V., and Hill, S. E., *Proc. Soc. Exp. Biol. and Med.*, 1934–35, **32**, 715.

Another approach to the possibility that absorption of salt might be retarded or prevented through by-products of fermentative processes was made. It was considered that these by-products might not be formed in significant quantities anaerobically under conditions of low temperature. If permeability *per se* were not impaired or not essentially low at all times, then ions should move readily into the cell *with* a positive inward concentration gradient at a low temperature. In one experiment, the results of which are presented in Table IV, the effects of passing air and N_2 gas through the culture solution on the absorption of K and Br with a concentration gradient were compared.

TABLE III

Effects of Lactate and of Ethanol on the Absorption of K and Br by Excised Barley Roots

Experimental conditions	Composition of expressed sap per liter		Net absorption per liter		Respiration rate in amount of CO_2 per hr. per gm. fresh weight tissue
	K	Br	K	Br	
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*mg.*
Control roots (initial status)	23.2	0.0			
KBr 50 m. eq. per l., plus K lactate 0.001 M (pH 5); aerated	77.1	38.7	53.9	38.7	0.33
KBr 50 m. eq. per l., plus K lactate 0.001 M (pH 5); N_2 gas	27.9	8.1	4.7	8.1	0.19
KBr 50 m. eq. per l., plus ethyl alcohol 0.01 M, aerated	73.0	33.5	49.8	33.5	0.34
KBr 50 m. eq. per l., plus ethyl alcohol 0.01 M; N_2 gas	16.9	7.9	−6.3	7.9	0.19

Barley plants grown from Aug. 28 to Sept. 18 (21 days). Experimental periods 12 hours. Temperature of culture solutions 23.5 ± 0.5°C. Nitrogen gas purified through pyrogallol. All tissues were turgid at the termination of the experiment. Fresh weights and sugar contents of tissues were maintained. Roots placed in water and pretreated with N_2 gas.

The extremely slow entry of those ions, either at the lower or the higher temperature, when N_2 gas was passed, does not supply evidence for a temperature controlled effect on the formation of substances influencing permeability. However, the modifying influence of low temperatures on the viscosity of protoplasm and membrane structure must not be disregarded.

Thus far, except for the temperature experiment just discussed, we have had in mind studies of salt accumulation from dilute solutions (5 milliequivalents per liter or less) in which the initial concentration of potassium in the culture solution was much lower than the initial concentration of K in the roots. Bromide although not initially present in the roots, rapidly rose to a concentration higher than that of the external solution, and the potassium was concomitantly accumulated against the existent concentration gradient. Other

experiments have now been made with solutions of much higher concentration of K and Br in the culture medium, but not so high as to induce harmful osmotic disturbances. These concentrations were 50 or 60 milliequivalents per liter of KBr. The inward gradient of K was then positive from the beginning.

Effects on inward salt movement were compared for aerated solutions and solutions through which purified nitrogen gas was passed (Figs. 3*a* and 3*b*). Anaerobically very little potassium entered the roots, not withstanding that a marked inward gradient existed for this ion, while aerobically the potassium was rapidly absorbed and even accumulated to a higher concentration than that of the relatively strong external solution. Bromide ions moved inward

TABLE IV

Effects of Temperature and Aeration on the Absorption of K and Br by Excised Barley Roots

Experimental conditions	Composition of expressed sap per liter		Net absorption per liter	
	K	Br	K	Br
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*
Control roots (initial status)	22.0	0.0		
KBr 60 m. eq. per l., 0.75°C; aerated	40.5	16.1	18.5	16.1
KBr 60 m. eq. per l., 0.75°C; N_2 gas	27.8	9.7	5.8	9.7
KBr 60 m. eq. per l., 18.5°C; aerated	80.4	50.0	58.4	50.0
KBr 60 m. eq. per l., 18.5°C; N_2 gas	29.2	10.7	7.2	10.7

Barley plants grown from Nov. 4 to Nov. 27 (23 days). Experimental periods 12 hours. Nitrogen gas purified through pyrogallol. Temperature of culture solutions 0.75 ± 0.05°C. and 18.5 ± 0.5°C. The specific conductivities of the expressed sap followed directly the changes in K and Br. Roots placed in water and pretreated with N_2 gas.

only to a slight degree when the cells were deprived of oxygen, but entry was rapid when the cells were adequately aerated. When CO_2 was passed through the culture solution, the absorption curves for K and Br were similar to those for N_2 gas (Figs. 3*a* and 3*b*). Further, it is apparent from the data of Table I, Experiment 3, that the suppression of entry of KBr into the cells even with an inward concentration gradient, under aerobic, cyanide treatment, was likewise similar to the anaerobic effect obtained when oxygen was withheld (N_2 gas treatment). Incidentally, attention may be called to the relative rates of aerobic accumulation of potassium and bromide ions (Fig. 3*a*). As frequently happens in the early stages of salt absorption by roots of high absorbing capacity, potassium ions were absorbed more rapidly than the associated anions. This involves ion exchanges which have been discussed elsewhere.[13]

[13] Broyer, T. C., and Overstreet, R., *Am. J. Bot.*, 1940, **27**, 425.

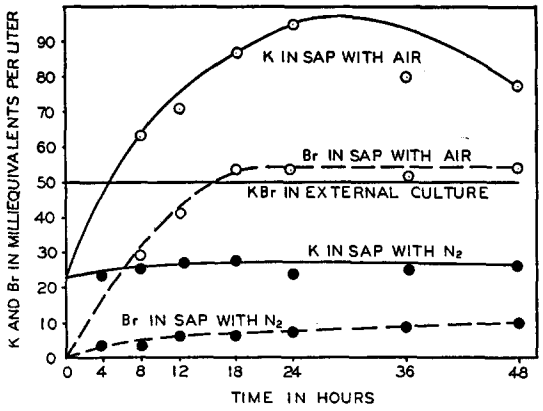

FIG. 3*a*

FIG. 3*a*. Effects of air and N_2 on the absorption of K and Br by excised barley roots, under conditions of an inward concentration gradient.

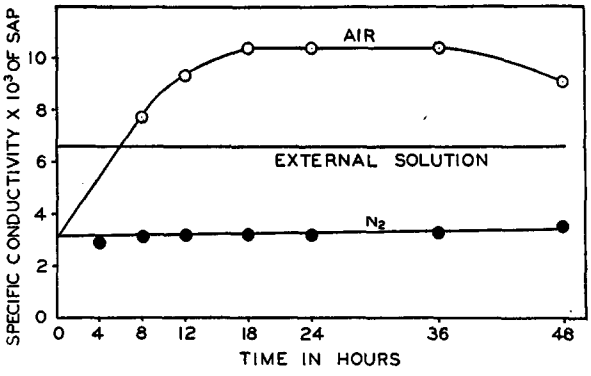

FIG. 3*b*

FIG. 3*b*. Effects of air and N_2 on the absorption of K and Br by excised barley roots, under conditions of an inward concentration gradient, as measured by electrical conductivity.

FIGS. 3*a* and 3*b*. Barley grown from June 23 to July 15 (22 days). Temperature of culture solutions 22.0 ± 0.5°C. Nitrogen gas purified through pyrogallol. Roots placed in water and pretreated with N_2 gas. Roots in 48 hour aerated condition were slightly flaccid at time of removal; roots of all other sets were turgid. Compare Fig. 2, which data are from a similar experiment.

Under an anaerobic environment the roots may lose to distilled water a small amount of potassium. This does not occur to a significant degree under an aerobic environment, as long as cells remain in healthy condition. The outward movement of ions under anaerobiosis, over limited experimental

periods, is nevertheless slight in comparison with the loss from markedly injured cells, and sometimes it is not certain that any net efflux occurs. When it does, it is still possible that some, if not all, of the loss may have originated in senescent cells, breaking down more rapidly because of oxygen deficiency. Conceivably many of the cells retain their solutes completely for a considerable period even though they are unable to accumulate more solutes. Undoubtedly over a long period of time oxygen is essential to maintain barley root cells in a state enabling them to retain their solutes, but they can maintain without oxygen supply a salt concentration higher inside than outside for 48 hours or more.

The data already discussed raise various questions of permeability, in the restricted sense of the term, *versus* active transport of salt or ions. The extensive work of Blinks[14] on *Halicystis* cells requires attention in this connection. He studied the effects of oxygen on bioelectric potentials and in relation to ionic discrimination by the protoplasm. The effects of oxygen deprivation of sufficient degree in decreasing or abolishing the normally maintained bioelectrical potential, were very striking. Over a considerable period of time, the effects were reversible. This investigator also measured electrical resistance across the protoplasm (by a direct current method) of cells supplied with oxygen in comparison with cells deprived of oxygen. The resistance was found to be far greater for the cells deprived of oxygen. These observations were interpreted in terms of decrease in permeability of some protoplasmic surface or decrease of mobility of ions in the protoplasm in general, when aerobic metabolism is abolished.

The data we have cited on root tissues could likewise be considered as indicating a greatly decreased cell permeability as a consequence of anaerobiosis, or of the action of respiratory poisons like cyanide. On the other hand, at least as far as the root experiments are concerned, one may conceive that a pumping mechanism is made inoperative when some component of the aerobic respiratory system fails under oxygen deficit, or when an inhibitor of aerobic respiration is in action. In other words, the cells may be relatively impermeable, at least at the vacuolar membrane, under these circumstances (until protoplasmic breakdown occurs) as well as when active aerobic metabolism is taking place, but the latter process permits the solute to be actively secreted, as a net effect, into the vacuole. There would still remain for elucidation the ionic exchanges that can take place, especially when anions are involved. (Compare early observations on Br-Cl exchanges in *Nitella*.[15]) On this point it may be noted, however, that certain experiments on roots with

[14] Blinks, L. R., Darsie, M. L., Jr., and Skow, R. K., *J. Gen. Physiol.*, 1938, **22**, 255.

[15] Hoagland, D. R., Hibbard, P. L., and Davis, A. R., *J. Gen. Physiol.*, 1926, **10**, 121.

radioactive bromide indicate that while considerable exchange of radioactive for non-radioactive bromide ions can occur, aerobically, (data on anaerobic conditions are not completed) at the same time the net inward movement of bromide greatly surpasses the exchange of ions.

Collander[16] has shown in experiments on *Chara* and *Tolypellopsis* cells that the exchange of cations between sap and solution is extremely slow and he believes that this means a high degree of impermeability in the protoplasm or its membranes. Overstreet and Broyer[17] have published data on root cells indicating that only about 10 per cent of the potassium present in the cells is readily exchangeable in experiments with isotopes. It is not certain that even this limited exchange occurs between vacuole and solution. About 10 per cent of the potassium is not recoverable in composite expressed sap.[18]

One rather crude concept would be that the salt or its ions are forced into the vacuole, although membrane properties prevent them from readily diffusing out into distilled water. Another concept is that of a dynamic system in which the salt or ions of the salt are actively transported to the vacuole and the solutes moving outward are carried back, except as certain ionic exchanges meanwhile take place. We are, of course, aware that these speculative views imply merely that the mechanism of salt accumulation remains an unsolved problem. In any event, the system of the higher plant as a whole involves other complications. Salt previously accumulated in root cells may be removed inward and then be carried upward to be accumulated by cells of the shoot. This polarized movement out of root cells also requires much further investigation. (Compare Crafts and Broyer.[19])

Experiments on Nitella Cells

Additional light on some of the problems of salt accumulation comes from recent experiments on *Nitella* cells in which radioactive isotopes were employed. Brooks[20] was the first to apply this technique to *Nitella* cells. A rough separation of cell sap, protoplasm, and cell wall was made. Brooks found that radioactive rubidium entered the protoplasm rapidly, although subsequent movement into the vacuole was very slow under the conditions of his experiments. The metabolic activity and capacity for accumulating additional total salt are at a far lower rate level in *Nitella* than in roots of the type referred to in this article. We have recently conducted other experiments on *Nitella* with radioactive isotopes, to supplement as directly as possible the experiments on roots and to carry further early studies on *Nitella* in this laboratory.[21]

[16] Collander, R., *Protoplasma*, 1939, **33**, 215.

[17] Overstreet, R., and Broyer, T. C., *Proc. Nat. Acad. Sc.*, 1940, **26**, 16.

[18] Broyer, T. C., and Hoagland, D. R., *Am. J. Bot.*, 1940, **27**, 501.

[19] Crafts, A. S., and Broyer, T. C., *Am. J. Bot.*, 1938, **25**, 529.

[20] Brooks, S. C., *J. Cell. and Comp. Physiol.*, 1938, **11**, 247.

[21] Hoagland, D. R., and Davis, A. R., *Protoplasma*, 1929, **6**, 610.

Nitella cells were grown in pond water. Nodal cells and the terminal bud were removed (in Experiments 1 and 2 to be cited) from the main axis of the thallus. The large internodal cells were replaced into vessels containing pond water to allow them to reestablish equilibrium with their normal environment. These filaments (or in Experiment 3, untreated group of cells) were placed into solutions containing radioactive salt and subjected to either continuous light or darkness, with air or N_2 gas continuously passed through the bathing medium. The light intensity, when applied, was 200 watts (daylight, fluorescent lamps) at approximately 2 feet. Large internodal cells were individually broken and the vacuolar sap collected. This was centrifuged and an aliquot of the clear supernatant sap counted for its radioactivity. After removing the sap, the residues from the debranched filaments, including protoplasm and cell walls (Brooks has shown the latter to possess little or no capacity for accumulation) were dried and leached to volume, with hot acidulated water, to remove the radioactive ions sought. Counts on aliquots of the filtered leach were converted for tabular comparison to activity per milliliter of residual sap, based on the loss in weight on drying the residue.

We shall refer here only to several experiments with radioactive rubidium or bromide ions. The data now at hand give evidence in accordance with the conclusion of Brooks, that at first there is an accumulation of the radioactive ions in the protoplasm, but the present data suggest continued movement of the ions into the vacuole (Table V, Experiment 1) until in course of time the concentration in the vacuole (at least for Br) exceeds that in the protoplasm (Table V, Experiment 2). If this latter relation can be substantiated,[22] it would imply a secretory process, operating from protoplasm to vacuole. Under an aerobic condition, the radioactive ions accumulated in the vacuole to a definitely higher concentration than that in the external solution, but this concentrating effect did not appear under an anaerobic condition, which was attained by placing cells in the dark with N_2 gas passed through the solution (Table V, Experiment 3). Cells in the light, of course, produce oxygen and can accumulate salt, even if no air is passed through the solution, although there is a suggestion from one experiment that aerated cells in the light have the greatest activity for the inward movement of salt. Preliminary data by Blinks[23] further suggest that entry of ions into the vacuoles of *Halicystis* cells is influenced by oxygen supply. In an aerobic environment radioactive rubidium entered the cells until vacuolar sap concentration finally exceeded protoplasmic concentration.

Some of the evidence on *Nitella* cells from the work of Brooks and others

[22] At present it is not possible to evaluate the errors resulting from contamination of cell residue by external solution or vacuolar sap. Contamination would also be present if attempts were made to remove protoplasm according to the technique of Brooks.

[23] Private communication.

may mean that the vacuolar membrane has but very slight permeability. Nevertheless, by the pump-like action, additional increments of salt may move into the vacuole under sufficiently favorable metabolic conditions. We believe that movement of this kind is particularly fast in actively metabolizing root cells. Certainly if the root system as a whole is considered, very rapid accumu-

TABLE V

Absorption of Rb and Br* Ions by Nitella*

Experimental conditions	Br* in counts per min. per ml.			Br* ratios in counts per min. per ml.			Spec. cond. in mhos × 10^{-3}	
	Sap		External solution	Vacuole	Vacuole	Residue	Vacuolar sap	External solution
	Vacuole	Residue		Residue	External solution	External solution		
Experiment 1								
Rb*Cl aerated, light 1 day.....	82	417	33	0.2	2.5	12.6		
Rb*Cl aerated, light 10 days...	400	545	33	0.7	12.1	16.5		
Experiment 2								
Control cells (initial status)....							6.9	
KBr* aerated, light 23 hrs.....	5180	3840	280	1.4	19	14	8.9	0.2
KBr* aerated, light 71 hrs.....	12000	6750	280	1.8	43	24	10.4	0.2
Experiment 3								
Rb*Cl, aerated, light.....	74	—	30	—	2.5	—		
Rb*Cl, N_2 gas, dark.....	23	—	24	—	0.7	—		

Experiment 1: *Nitella* cells from pond water. Nodal cells and terminal buds removed from cells of main axis of thallus. Only these latter cells were used in the absorption studies. The experimental external solution was Rb*Cl 1.0 milliequivalent per liter.

Experiment 2: *Nitella* cells from pond water. Nodal cells and terminal buds removed from cells of main axis of thallus. Only these latter cells were used in the absorption studies. The experimental external solution was KBr* 1.18 milliequivalents per liter.

Experiment 3: *Nitella* cells from pond water. A composite group of cells was used during the absorption period. Vacuolar sap was obtained from large cells of the composite sample. The experimental external solution was Rb*Cl 5 milliequivalents per liter.

lation of salt in the root exudate can take place. Within less than half an hour mobile ions can be transferred to the exudate in concentrations higher than those of the solution from which absorption takes place.

Studies on Fluids Obtained by Suction from Tomato Roots

Another way of attacking the general problem will now be considered. Sturdy young tomato plants were decapitated several inches above the junction of stem and root and by attaching a tube to the cut stem, suction was applied

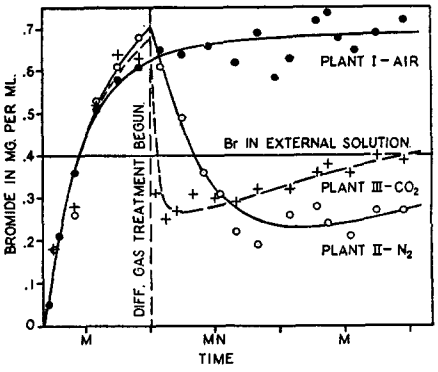

FIG. 4*a*

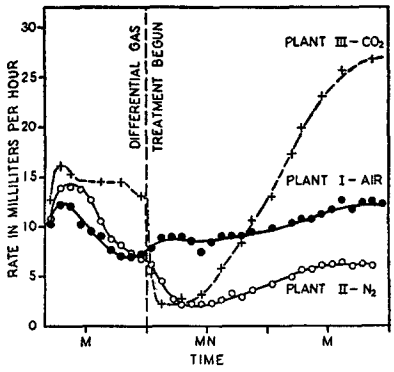

FIG. 4*b*

FIG. 4*a*. Effects of differential gas treatments on the concentration of bromide ions in exudates from decapitated tomato plants.

FIG. 4*b*. Effects of differential gas treatments on the rate of exudation by decapitated tomato plants.

FIGS. 4*a* and 4*b*. Tomato plants grown with aeration in a culture solution of 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

Fe as iron tartrate; 1 ml. of 0.5 per cent solution per liter of culture solution.

Micronutrients as required.

Growth period from Oct. 10 to Nov. 26 (47 days). Experimental culture solution 5 milliequivalents per liter. Gases were continuously passed through the solutions. All roots were aerated until 6 P.M. Differential gas treatments of air, N_2 and CO_2 were then applied. Suction equal to 740 mm. Hg was continuously applied (except during observations on volume of exudate and during removal of increments of exuded fluid) throughout the experiment. At the termination of the experiment the abilities of the plants to develop an exudation pressure (as a measure of the state of the root systems) were noted. The following results were obtained: aerated cultures, appreciable and maintained exudation pressure; N_2 treated cultures, a weak yet maintained pressure; CO_2 treated cultures, no exudation pressure over an extended interval of time. The roots in the CO_2 treated culture were flaccid at the termination of suction exudation.

and the fluid obtained examined under various conditions in the root environment. This is essentially the method described by Kramer.[24]

The results of one experiment are presented in two graphs (Figs. 4 *a*

[24] Kramer, P. J., *Am. J. Bot.*, 1940, **27**, 216.

and 4 *b*). The first sections of the curves represent data obtained on fluid recovered in the period during which air was passed through the solution in contact with the roots. Then one plant continued to receive aeration, one was subjected to nitrogen gas, and one to carbon dioxide gas. In the two latter cases, an abrupt downward trend in the concentration curves for bromide is evident (Fig. 4 *a*). The concentration of this ion in the recovered fluid became much lower than that of the external solution, whereas during the aerobic period it was much higher. There followed an upward trend in the CO_2 curve until the concentration of bromide attained approximate equality with that of the solution. The upward trend of the N_2 curve, within the time period of the experiment, was less marked and the concentration of bromide in the fluid recovered by suction remained lower than that of the external solution. The other figure (Fig. 4 *b*) represents volumes of solution recovered over various time intervals (compare Kramer). Similar trends in the curves for the several treatments are observable. The injurious effect of the CO_2 treatment was manifest in the latter stages of the experiment, but in the nitrogen-treated plants similar effects were much less apparent.

The most obvious interpretation of these experimental results is that the effects of N_2 and CO_2 cause initially a decrease in root permeability to inorganic solutes and to water, and also in power to accumulate solutes; followed by a protoplasmic breakdown with increase of permeability, *without* power of solute accumulation on the part of the cells.

DISCUSSION

If all the various lines of evidence are brought together, the general picture would seem to be that permeability, metabolism, and accumulation of salt are so intimately interrelated that generally it becomes impossible to disentangle the several aspects of the phenomena. Blinks has emphasized, on the basis of his bioelectric measurements on *Halicystis* cells that permeability and metabolism may be connected through the relation of metabolism to the formation and maintenance of protoplasmic membranes. As we have already pointed out, in the root systems of plants there are still other reactions to explain in the polarized movement of ions out of the root cells. As the subject develops and the results of new types of experiments become available, the general conclusion is reinforced that the absorption and movement of ions in the plant, in nearly all their aspects, require an increasing understanding of the metabolism of plant cells. The limitations of many earlier experiments on permeability of cells in their application to problems of plant nutrition become clear. It has seemed especially appropriate to submit this brief discussion on accumulation of inorganic solutes and permeability in plant cells for publication in a volume dedicated to Dr. Osterhout, who has for so many years contributed to this field of inquiry. The article has been prepared with the

hope that the data presented may serve a purpose as an aid to stimulating further discussion of the problems of plants absorbing their inorganic nutrients from dilute media.

SUMMARY

1. Comparisons are made of concentrations of K and Br in exudates of barley roots and in expressed sap from roots, under conditions favorable for aerobic metabolism. Both methods lead to the same general viewpoint concerning metabolically governed transport of solutes by living plant cells.

2. Cyanide in low concentration prevented salt accumulation by barley roots. Methylene blue, without decrease of CO_2 production by roots, destroyed power of salt accumulation.

3. K and Br ions entered roots to only a slight extent under an anaerobic condition, even with an inward gradient of ionic concentration.

4. Lactate or alcohol, under aerobic conditions, did not prevent rapid accumulation of salt by root cells.

5. Experiments on fluids obtained by suction from tomato roots gave evidence of loss of salt-accumulating power under the influence of N_2 gas or CO_2 gas, together with probable effects on cell permeability.

6. Several experiments on *Nitella* cells in which radioactive isotopes were used are reported. Bromide gradually moved into vacuolar sap until the concentration appeared to exceed that of the protoplasm, on the basis of the results of the several types of experiments. Accumulation of salt in the vacuole did not occur anaerobically.

7. Some views of interrelations of permeability, salt accumulation, and metabolism are suggested for further discussion.