THE INFLUENCE OF ONE ION ON THE ACCUMULATION OF ANOTHER BY PLANT CELLS WITH SPECIAL REFERENCE TO EXPERIMENTS WITH *NITELLA**

D. R. HOAGLAND, A. R. DAVIS, AND P. L. HIBBARD

(WITH FOUR FIGURES)

We have previously published the results of experiments on *Nitella* cells, which emphasized the primary importance of illumination and of temperature in determining the accumulation[1] of halogens in the cell sap and at the same time a number of preliminary observations were reported with regard to certain inter-ionic effects in relation to the process of accumulation (**5**). This latter question had also been studied earlier in connection with experiments on the absorption of ions by barley plants (**4**). While it appears that the accumulation of solutes by a plant cell is dependent on the growth or metabolic activities of the cell, it is also clear that the magnitude of the actual accumulation under any given conditions of light and temperature is influenced by the concentration and composition of the culture solution. It is this latter aspect of the question that we now wish to discuss.

The general methods of experimentation heretofore described were used in the present work. *Nitella* cells were immersed in the solutions under investigation and at the end of the experiments the cells were thoroughly rinsed with distilled water and the larger cells (from 1–3 inches in length) were broken individually, the sap being collected in amounts varying between 1 and 25 cc. Since a single cell yielded only one or a few hundredths of a cubic centimeter of sap, each composite sample of sap collected represented a very large number of cells.[2] The method of HIBBARD (**3**), specially developed for this purpose, was employed in analyzing the sap for its halogen content, which procedure made it possible to obtain results of sufficient accuracy even when dealing with very small volumes of sap. (Determinations were made in duplicate or triplicate.)

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[1] This term is used to indicate the process of concentrating solutes in the cell sap, with the attainment of a higher concentration inside than outside, as in the sense of OSTERHOUT.

[2] It is to be noted that different lots of cells may differ in their physiological state, so that quantitative comparisons must usually be made within each experiment.

Since the earlier experiments had shown that the accumulation of halogens in the cell sap is dependent upon adequate illumination and that the process goes on relatively slowly in any case, we have conducted our preesnt experiments with reference to these conditions. In certain instances artificial light of considerable intensity was employed; sometimes the illumination was continuous. The exposure times extended in some instances to two weeks or more, a point especially to be noted, since it indicates that we were dealing with physiological processes involved in mineral nutrition, and not simply with degrees of permeability. When sufficient time is permitted to elapse a perfectly definite accumulation in the cell sap of certain mineral elements, sometimes of relatively large magnitude, can be demonstrated. If a very limited period of time were employed with similar solutions, it might appear that the cells were almost impermeable to electrolytes and thus the processes most vital to nutrition might escape detection.

The relation of concentration to accumulation

The first question to be considered is concerned with the relation between ionic concentrations in the culture medium and in the cell sap. This question was studied by determining how variations in the concentrations of Br ions in the culture medium influenced the accumulation of these ions in the cell sap. Several experiments were performed. In one experiment the accumulation was permitted to continue for one day only, while in another experiment the cells were kept in contact with the bromide containing solutions for over a month. The results of three different experiments are plotted in fig. 1, in which the curves are of a logarithmic type. Thus the factor obtained by dividing internal by external concentration has a much higher value for external solutions of low concentration than for those of high concentration. Special significance is attached to the present data, inasmuch as they reflect directly the conditions existing in the cell sap, but it is interesting and important to recognize that these experiments on *Nitella* cells are entirely consistent with those made on other plants by necessarily less satisfactory methods. Our own results using agricultural plants give evidence of the existence of similar relations between internal and external concentrations of electrolytes; and likewise consistent are the results on storage tissues reported by STILES and KIDD (9). All of the investigations emphasize a principle which is highly important to the student of soils and plants, for the reason that in general the concentrations of essential elements in soil solutions are low, sometimes extremely low, and it is essential that land plants should possess a means of concentrating inorganic elements at a relatively high rate from solutions of low concentration.

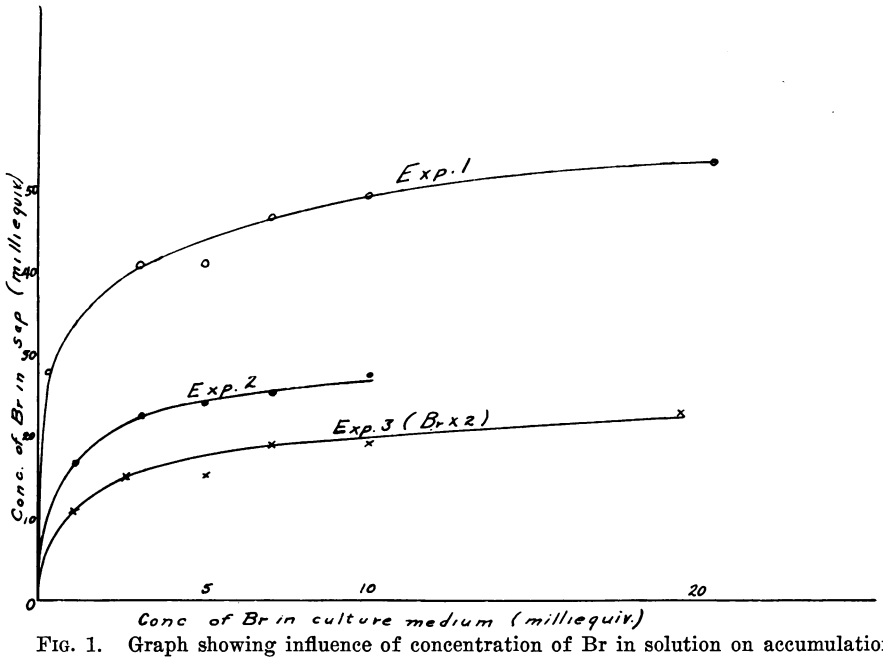

FIG. 1. Graph showing influence of concentration of Br in solution on accumulation of sap. Exp. 1, 9 days' duration. Continuous artificial illumination from two 300-watt lamps; temperature 23°–25° C. Exp. 2, 13 days' duration; diffuse daylight; room temperature. Exp. 3, 27 hours' duration; diffuse daylight plus continuous artificial illumination, two 300-watt lamps. Experiments 1 and 3, without buffer. Exp. 2 with phosphate buffer. Initial pH of all solutions, 5.0–5.4.

If so desired, the data under discussion could be fitted into one of the well known adsorption formulae, but it has not appealed to us that this method of interpretation assists very materially in explaining the mechanism involved, especially when we bear in mind the importance of the metabolic activities of the plant in connection with the ability to concentrate solutes in the interior of cells, as well as the evidence in support of the idea that the chemical elements in question exist in the cell sap primarily in dissociated form, and not for the most part adsorbed on organic compounds. Furthermore, the adsorption formula is of too general a character to serve as a guide to the understanding of such a highly complex series of physiological processes.

Effect of cations and anions on accumulation of Br ions

As another phase of the research we planned to determine to what extent the accumulation of Br ions could be modified by other ions of the same or opposite charge present in the culture solution. First referring to the influence of other anions, experiments were carried out with solutions

containing KBr to which were added KNO_3, KCl, K_2SO_4, or KI in various concentrations (figs. 2–3, tables I–II). The NO_3 and SO_4 ions had no effect whatever in retarding the accumulation of Br ions in the cell sap.[3] In fact the tendency was in the opposite direction, which we attribute to the accelerating influence of the increased K concentration, an effect definitely suggested by experiments to be discussed later in this paper. In marked contrast to NO_3 and SO_4 ions, Cl ions in equivalent concentration signifi-

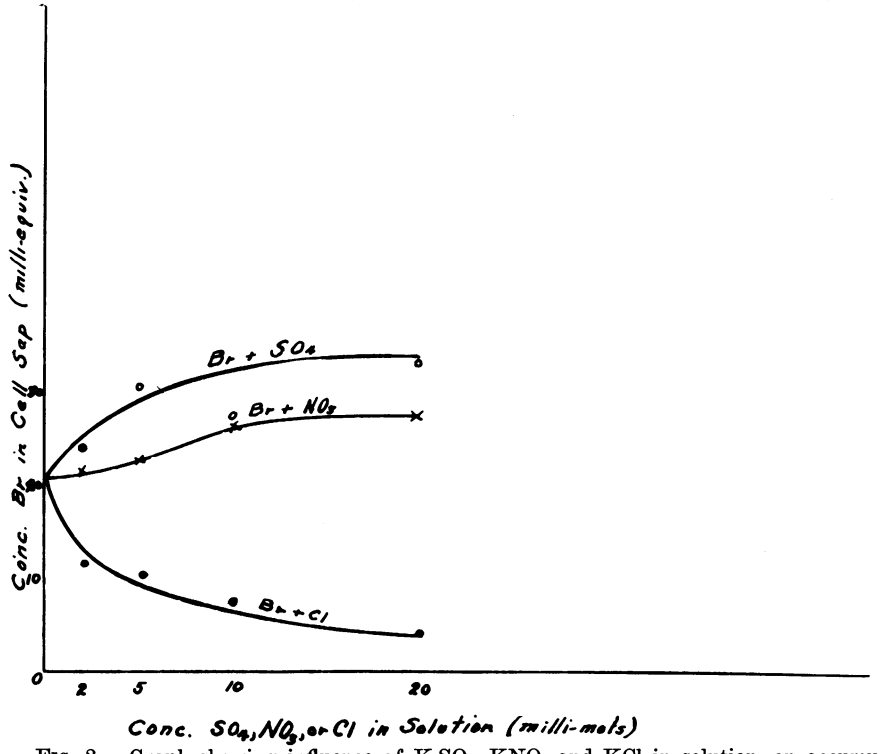

FIG. 2. Graph showing influence of K_2SO_4, KNO_3 and KCl in solution, on accumulation of Br in cell sap; 4 days' duration; continuous artificial illumination with two 300-watt lamps; temperature 23°–24° C. Initial pH of all solutions, approximately 5.0. Phosphate buffer solutions used, containing 0.005 M. KBr to which were added K_2SO_4, or KCl as indicated.

cantly retarded the accumulation of Br in the cell sap and I ions were also definitely effective in the same way, although not to the same degree as Cl ions. A pronounced retardation in the accumulation of Br ions occurred as a result of the presence in the culture solution of Cl ions in a concentration as low as 0.002 molar.

[3] Experiments in which the accumulation of Br from unbuffered solutions and from solutions buffered with phosphate suggest that phosphate ions fall in this respect in the same category with SO_4 or NO_3 ions.

Evidently in these experiments, we have a very clear illustration of one anion influencing the intake and accumulation of another anion. These effects occur in very dilute solutions and with the exception of the higher concentrations of iodide, apparently do not involve injury; therefore it is doubtful whether we should refer to them as phenomena of antagonism. Naturally this depends entirely on the definition one may desire to apply to

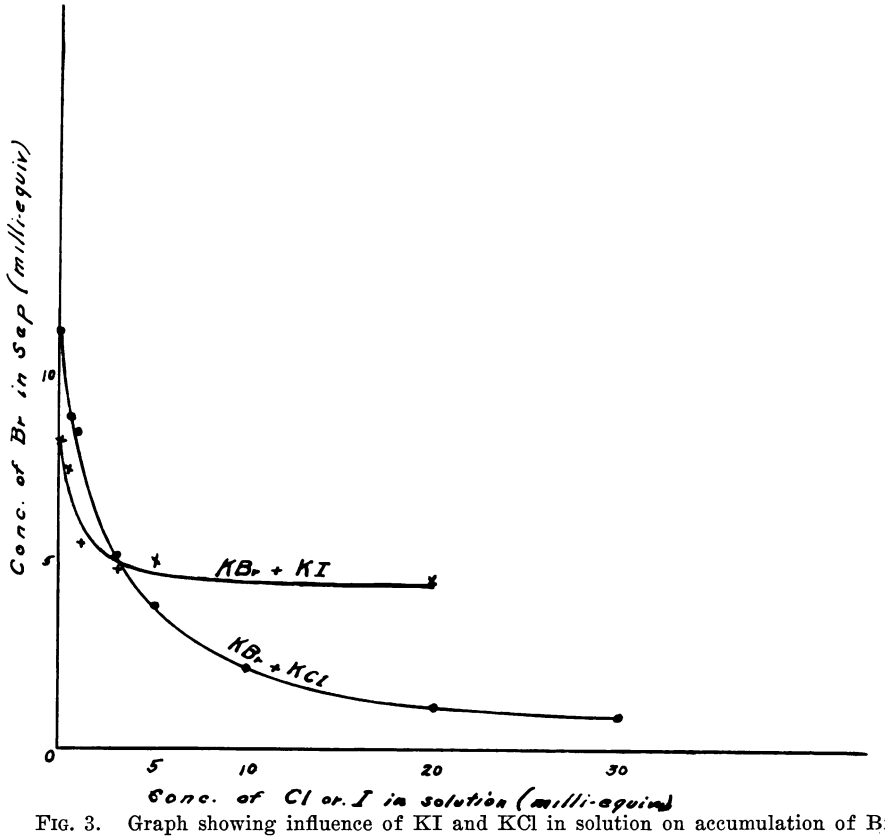

FIG. 3. Graph showing influence of KI and KCl in solution on accumulation of Br in cell sap. Exp. 1, 0.001 M. KBr solutions to which were added KI as indicated. Five days' duration; diffuse daylight plus continuous artificial illumination from two 300-watt lamps. Temperature, 20°–25° C. Initial pH, 5.6–5.8. Final pH, 5.8–6.2. Exp. 2, 0.001 M. KBr solutions to which were added KCl as indicated. Nine days' duration; illumination as in exp. 1; temperature 20°–25° C.; pH values similar to those of exp. 1.

the term "antagonism." When the cells were exposed to certain solutions of iodides during a period of ten days or more, a definite toxicity was observed in several of the experiments, whether caused directly by the accumulation of I ions, or by the formation of molecular iodine, subsequently. The presence of Br ions delayed or inhibited such toxicity. Pos-

sibly this might be considered as an example of antagonism occurring in dilute solution and becoming manifest only after a considerable interval of time.

TABLE I

EFFECT OF OTHER ANIONS ON ACCUMULATION OF Br IONS IN CELL SAP (Br PRESENT IN ALL SOLUTIONS AS 0.005 M. KBr)

CONDITIONS OF EXPOSURE	CONCENTRATION OF ADDED SALT (MOLAL)	CONCENTRATION OF Br IN SAP (MILLIEQUIVALENT)
	Experiment I	
Diffuse daylight, 10 day period, phosphate buffer solution* used. Room temperature.	none	15.2
	K_2SO_4 0.005	19.3
	KCl 0.005	7.3
	KI 0.005	8.2
	Experiment II	
Diffuse daylight, 12 day period, phosphate buffer solutions* used. Initial pH 5.0, final 5.8–5.9. Room temperature.	none	32.0
	K_2SO_4 0.005	35.0
	KNO_3 0.005	31.8
	KCl 0.005	13.8
	KI 0.005	15.5

* General buffer solution
0.002 M. $Ca(H_2PO_4)_2$
0.005 M. KH_2PO_4
Initial PH of such solutions usually between 5.0 and 5.4 and final pH between 5.6 and 6.0.

The results obtained with Br and I give an opportunity for relating potential rapidity of accumulation and reciprocal ion effects. It appears that anions which themselves are incapable of rapid accumulation are also incapable of having a marked influence on the accumulation of other anions. For example, sulphate ions, which are capable of only slow accumulation, do not retard the accumulation of Br appreciably. While it is possible for I ions to accumulate in the cell sap in considerable concentration, several experiments indicate that the accumulation of Br ions may be decreased through the presence of I ions in the solution, without the latter ions being accumulated in the sap in an amount equivalent to the decrease of Br ions. Obviously there exists a reciprocal relation between Br and I ions, so that the sum of the equivalents of halogens accumulated from the single salt solutions may be much larger than the corresponding value for the mixed solution. This means, of course, a mutual hindrance to penetration into

TABLE II

RELATIONS BETWEEN BR AND I IN ACCUMULATION

CONDITIONS OF EXPOSURE	BR IN SOLUTION (INITIAL) MILLI-EQUIV.	I IN SOLUTION (INITIAL) MILLI-EQUIV.	BR IN SAP MILLI-EQUIV.	I IN SAP MILLI-EQUIV.
Experiment I†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination for 6 days. Total period 10 days.	5.0	.....	58.6	.....
	.....	5.0	.....	19.9
	5.0	5.0	32.6	1.5
Experiment II†				
In light chamber.* 1,800 watts continuous illumination. Period 10 days.	5.0	.....	55.5	.....
	.....	5.0	.....	30.1
	5.0	5.0	41.5	6.8
Experiment III†				
Diffuse daylight and artificial illumination (2–300-watt lamps) 10 days, and 5 days in light chamber,* 3,000 watts. Continuous illumination throughout.	5.0	.....	61.7	.....
	5.0	5.0	46.6	4.0
Experiment IV†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination, 11-day period.	5.0	.....	22.0	.....
	.....	5.0	.....	6.0
	5.0	5.0	24.7	3.1

* A glass chamber 56 in. × 26 in. with lights evenly distributed outside chamber about 1 ft. from glass.

† Experiments I and II used phosphate buffer solution (table I) pH values 5.4–6.0. Experiments III and IV no buffer used. Similar pH values. Temperature in experiments not controlled, but varied between 20–25° C. Comparisons to be made within each individual experiment. In experiment II one lot of cells immersed in KI solution were too severely injured to be used. In experiment III nearly all cells in KI solution were killed. Cells in KI plus KBr solution in good condition.

the sap occurring somewhere in the protoplasmic layer. Apparently anions which do not have the potentiality of rapid accumulation in the sap are also incapable of penetrating the protoplasm to such an extent as to interfere with the accumulation of other ions. In comparing Br and I it will be observed that Br had a much greater relative effect on the accumulation

of I than I had on the accumulation of Br, and that Br could be accumulated by the cell more rapidly than I. If we can compare these results with the well known antagonistic effects between cations it is clear that anions may "antagonize" each other in an equally significant way, even in very dilute solutions.

We shall discuss next the possible influence of cations on the accumulation of anions. To test this possibility, experiments were made with solutions of various bromides and the accumulation of Br ions in the cell sap determined. It is clear from fig. 4 that the type of cation employed did

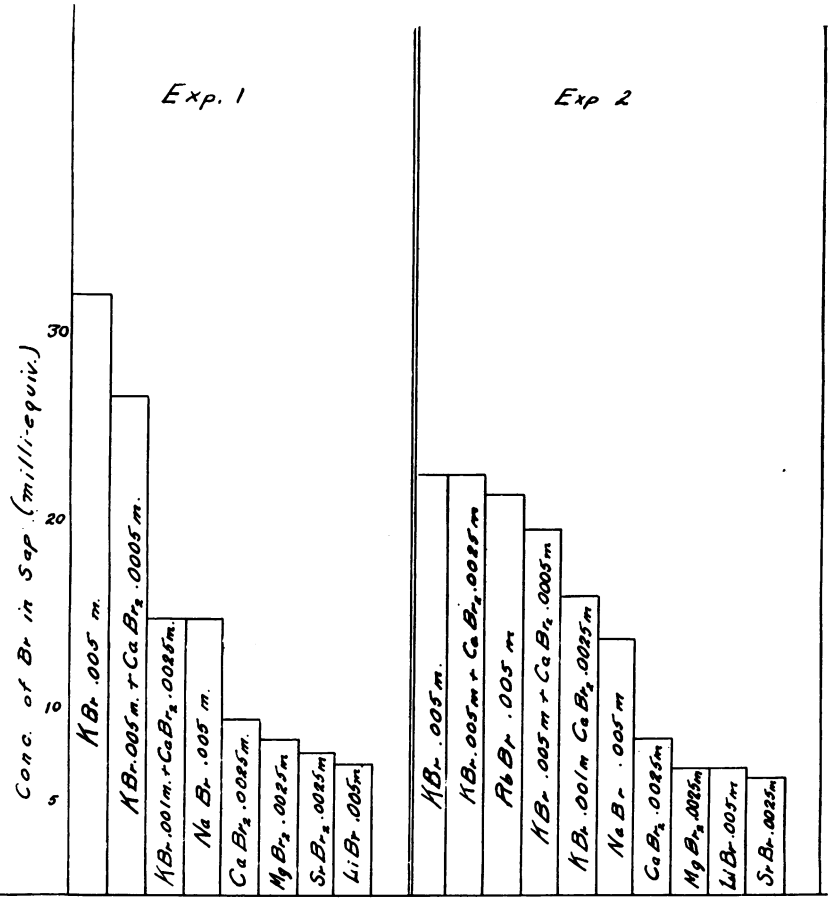

FIG. 4. Graphic representation of accumulation of Br in cell sap from solutions of various bromides. Exp. 1, three days' duration; daylight supplemented by artificial illumination at night from two 300-watt lamps; room temperature (20°–25° C.). Exp. 2, two days' duration; daylight plus continuous artificial illumination from two 300-watt lamps; room temperature (20°–25° C.). Initial pH of all solutions similar (5.6–5.8).

have a marked influence on the concentration of Br attained in the sap after a given exposure to the bromide solutions. The cations fall in three general groups, K and Rb; Na; Ca, Mg, Sr, Li, in the order of decreasing Br accumulation. These general relations were entirely consistent in two experiments, using different periods of time and conditions of exposure. Duplicate determinations of Br were made on each sample of sap with excellent agreement.

In several instances mixtures of KBr and $CaBr_2$ were used. In these cases (fig. 4) K had the dominant influence so that considerable excess of Ca had to be present in order to lower markedly the accumulation of Br from KBr solutions. These relations are not entirely similar to those ordinarily met with in experiments on antagonism, in which very small proportions of Ca may cause great changes in the physiological properties of a solution. In the present study the rate of accumulation of the anion was conditioned primarily by the presence in suitable concentration of a cation capable of ready penetration and accumulation rather than upon alterations in the protoplasm resulting from different proportions of mono- and di-valent ions. Probably a distinction should be made between cation-anion relations and those just discussed with reference to anions. The cation-anion relation may be electrostatic in nature and occur at outer surfaces.

In discussing the cation-anion relations it is suggestive to recall an experiment in which *Nitella* cells were allowed to accumulate Cl from a KCl solution to a concentration significantly greater than that normally existing in the cell sap. When these cells containing the increased amount of Cl were placed in a KBr solution they did not accumulate Br nearly so rapidly as similar cells taken from tap water. The Br which was accumulated by the former cells was accounted for by a nearly equivalent displacement of Cl from the sap, whereas the cells with the normal Cl content accumulated Br as a result both of exchange of Br for Cl and of simultaneous intake of K and Br. With the information now at hand these results can be explained on the assumption that the accumulation of Cl in the preliminary period was accompanied by an accumulation of K to a point approaching equilibrium, so that in the subsequent period of exposure to a KBr solution the cells could not readily accumulate an additional amount of K, thus restricting the Br accumulation to that represented by an exchange of Cl and Br. If this reasoning be correct we have another interesting example of the influence of the accumulation of the cation on the accumulation of the anion.

Since cation-anion and anion-anion interrelations exist it might be anticipated that one cation could influence the accumulation of another cation in dilute solutions, and we believe this to be true. However, it is not prac-

ticable at present to obtain satisfactory quantitative evidence with *Nitella* cells. The most rapidly accumulated cations, K and Rb, are exceedingly difficult to separate by any analytical method, while Cs is probably toxic. In experiments with plants of the agricultural type, in which accumulation of most ions proceeds sufficiently rapidly, it is possible to obtain very definite evidence of inter-ionic relations between cations in dilute solutions of the ordinary type.

It does not follow from the foregoing statements that large accumulations of Br ions may not take place from solutions of salts, the cations of which accumulate very slowly, for the contrary is true. Under such circumstances, Br ions penetrate into the cell chiefly in exchange for Cl ions, and the latter enter the outside solution, as has been pointed out in a previous paper. (When Br is accumulated from KBr solutions, simultaneous accumulation of K and Br is involved as well as exchange between Cl and Br.) Furthermore there is the possibility that ions may enter the cell to some slight extent in conjunction with H or OH ions, although the available data do not indicate that this method of accumulation is of primary importance in the experiments under discussion.

The influence of hydrogen-ion concentration on the accumulation of Br ions

The rôle of hydrogen-ion concentration in the accumulation of other ions is undoubtedly a very complicated one. When we used masses of very small young cells and examined the culture solution, instead of the cells themselves, we found that the removal of Br or Cl ions was much more pronounced from an acid solution (pH 5–6) than from an alkaline one (pH 8–9). A similar effect was shown for the entrance of NO_3 into large cells by direct examination of the cell sap of individual cells. We have not been able to show, however, equally marked effects of hydrogen-ion concentration on the accumulation of Br by large cells, although the indications are that a pH of 6–7 is most favorable to the accumulation. It may be noted that M. M. Brooks (1) in studying the penetration of methylene blue into *Vallonia* cells finds that the rate and not the equilibrium value is influenced by reaction. Our results do not necessarily give an accurate idea of rates.

It is certain that large accumulations of Br ions may take place at any of the pH values experimented with, namely, pH 5–8 (table III). In passing, attention is called to the observation that Br ions may become much more concentrated in the cell sap than in the culture solution even when the pH value of the latter is the same as that of the cell sap, so that no gradient of H or OH ions is necessarily required for the process of accumulation. This fact may be of interest in connection with hypotheses concern-

TABLE III

EFFECT OF HYDROGEN-ION CONCENTRATION ON ACCUMULATION OF Br

CONDITIONS OF EXPERIMENT	INITIAL pH	FINAL pH	CONCENTRATION OF Br IN SAP MILLIEQUIV.
Experiment I*			
Diffuse daylight plus continuous artificial illumination (2–300-watt lamps), 5-day period. Room temperature.	5.4	5.6	20.7
	6.4	6.8	31.7
	8.2	7.6	20.6
Experiment II*			
Light chamber, 3,000 watts continuous illumination, 3-day period. Temperature 20–25° C.	5.4	.....	30.6
	6.4	.....	35.0
	7.6	.....	27.9
Experiment III*			
Diffuse daylight, 3-day period. Room temperature.	5.5	5.9	7.4
	6.4	6.6	17.7
	7.4	7.4	16.9
	8.6	7.4	12.7
Experiment IV*			
Diffuse daylight, 5-day period. Room temperature.	5.2	6.0	15.6
	6.0	6.6	31.0
	7.0	7.2	34.4
	8.0	7.9	27.8
	8.3	7.9	19.5
Experiment V*			
Continuous artificial illumination (2–300-watt lamps), 3-day period. Average temperature, 24° C.	4.9	5.5	19.3
	6.0	6.0	24.4
	6.9	6.9	26.4
	7.8	7.8	30.4

* KH_2PO_4 plus NaOH used to make buffer solution. Experiments I, II, III, KH_2PO_4 0.001 M. Experiments IV, V, K_2PO_4 0.0025 M. KBr 0.005 M. present in all solutions.

ing the mechanism of accumulation. It is also worthy of comment that the accumulation of both cations and anions takes place on the alkaline side of the average isoelectric point of the proteins present in the cell, as determined by PEARSALL (**8**). There does not seem to be available any explana-

tion of ion accumulation on a simple basis of ion protein combinations. It would be difficult to assume that individual proteins are available of such widely different isoelectric points as to permit the simultaneous formation of both cation and anion compounds at the pH values involved.[4]

General discussion

It will be remarked that we have explained all of our data in terms of ions. We are, of course, aware that the hypothesis has been advanced that only undissociated molecules are able to penetrate into living cells. Certain experiments with weak acids and with dyes have been made which lend support to this hypothesis (**6, 7**). (Some work by M. M. BROOKS (**1**) on dyes tends rather to an interpretation in terms of ions.) Concerning these data we do not know to what extent they bear on the results we have presented. As we have suggested previously, in the systems containing the weak acids employed, gaseous components were present and it is not certain that they can be disregarded. In the solutions with which we have experimented, the salts were possibly completely dissociated according to present theories, and in the sap the salts also must have existed primarily in dissociated form for reasons discussed elsewhere. It would require very complicated assumptions to explain our results on any other than an ionic basis. For example, we have to consider the unequal accumulation of the ions of a salt such as $CaBr_2$, the Br accumulation in this case being accompanied by a marked loss of Cl from the cell. An explanation is required also for the very different effects of different anions on the accumulation of Br. In fact, all of the observations seem to be most easily understood by assuming that ionic processes are involved. It is true, however, that the actual mechanism of accumulation is as yet unsolved, so that it would be unwarranted to make positive statements, especially as the physical chemistry of even simple ionic systems appears to be in a stage of further development.

Although the data we have discussed in this paper are confined to one algal organism, we feel that the general relations which have been shown to exist between the cell sap and the culture medium apply to a wide range of plants. Indeed our experiments on agricultural plants are consistent with this statement. With regard to the comparative rates of accumulation of specific ions it would seem that this question is not wholly one of ionic properties, but involves the type of plant metabolism as well. For example, many agricultural plants are capable of very rapid accumulation of nitrate,

[4] Note, however, recent article by G. E. BRIGGS and A. H. K. PETRIE, Biochemical Jour., Vol. XXII, No. 4, pp. 1071–1082 (1928) also J. Davidson, Jour. Agr. Res. Vol. 35, No. 4, pp. 335–346 (1927).

but this does not seem to be true of *Nitella* cells. If we consider the cation relations, as they are reflected by the present experiments, the physiological order would be consistent in a general way with the mobilities of the ions, but with the anions the matter is in doubt. A complex ion like NO_3 or SO_4, capable also of reduction, is very difficult to place on a purely electrochemical basis.[5] Comparing the results on other plant cells, obtained by STILES and KIDD (**10**) and by several other investigators, there is a general agreement as to the order of the ions, with the exception of the NO_3 ion, as already mentioned. Recently, COOPER and WILSON (**2**) have suggested that the electromotive force is the most important characteristic of the ion to be considered, but the details of their researches are as yet unavailable.

Summary

1. Further experiments on *Nitella* cells are reported pertaining to the accumulation of halogens in the cell sap. Analyses were made of composite samples of sap obtained from individual cells. Consideration was given to the maintenance of conditions of illumination essential to the process of accumulation.

2. The relation between Br ion concentrations in the culture medium and in the cell sap was of a logarithmic type. This is in agreement with results obtained on other plant cells using other electrolytes.

3. The accumulation of Br ions was significantly retarded by Cl or I ions also present in the culture medium, but not by SO_4 NO_3 or PO_4 ions. These and other ionic effects discussed occurred in very dilute solutions. Consideration is given to the relation between the retarding effects of one ion on another and potentiality of accumulation in the sap.

4. The accumulation of Br ions was definitely influenced by the nature of the cation, being most rapid when solutions of KBr or RbBr were used, and least rapid with solutions of LiBr, $CaBr_2$, $SrBr_2$ and $MgBr_2$.

5. With regard to the effects of hydrogen-ion concentration, marked accumulation of Br ions took place at reactions varying between pH 5 and 8.

6. Taking all the data into consideration it is found very difficult to explain them on any other than an ionic basis.

LABORATORY OF PLANT NUTRITION,
UNIVERSITY OF CALIFORNIA.

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