Plant Physiol. (1976) 58, 182–185

# Effects of Inorganic Salts on Tissue Permeability[1]

Received for publication February 19, 1975 and in revised form February 24, 1976

B. W. Poovaiah[2] and A. Carl Leopold[3]
*Department of Horticulture, Purdue University, Lafayette, Indiana 47907*

## ABSTRACT

**Inorganic solutes are shown to alter the permeability of root and leaf tissues. Experiments with beet root tissues reveal that $CaCl_2$ decreases leakage of betacyanin from the tissue, that $(NH_4)_2SO_4$ increases leakage, and that each salt can relieve the effects of the other. A comparison of cations and anions shows a range of effects with the various solutes. Experiments with *Rumex obtusifolius* L. leaf discs reveal that whereas $CaCl_2$ defers the development of senescence, $(NH_4)_2SO_4$ hastens senescence and increases the leakage of materials out of the leaf discs. The solute effect on *Rumex obtusifolius* L. is prevented by gibberellin. $CaCl_2$ can relieve the $(NH_4)_2SO_4$ effect. The results are interpreted as indicating that the inorganic solutes may serve to alter the permeability of membranes through alterations of interactions between water and macromolecules in the tissues; the interpretation is consistent with the evidence for opposite effects of Ca and $NH_4$, the effective concentrations being about $10^{-3}$ M, and the reversibility of the effects of one solute by another of opposite stabilization-destabilization effect.**

It is well known that inorganic salts can have large effects on the configuration of macromolecules; Hofmeister (2) described the range of effects of various salts on protein solubilities, and in a more current context, von Hippel and Wong (16) have described the effects of inorganic salts as an alteration of macromolecular stabilization or destabilization. We have reported that inorganic salts can alter the actions of each of the known plant hormones (3), and have raised the question of whether the alterations of hormonal actions might be related to effects of the solutes on plant membranes. It is the intent of this paper to explore the effects of inorganic salts on some plant membranes, and to consider the possibility that the observed effects might relate to their effects as stabilization or destabilization agents.

## MATERIALS AND METHODS

Beet roots (*Beta vulgaris* L.) were purchased locally and cut into sections 1 cm in diameter and 1 to 2 mm thick. The sections were washed for about 2 hr in several changes of aerated distilled $H_2O$. Five sections were transferred to 10 ml of test solution. Membrane damage was assessed by spectrophotometric determination of the amount of betacyanin pigment that leaked out into the ambient solution over 12- to 18-hr period.

For the *Rumex* assay (*Rumex obtusifolius* L.), following the procedure of Whyte and Luckwill (17), old leaves which were uniformly green were selected from matured plants in the greenhouse. Leaf discs (1 cm) were cut with a cork borer and randomized, and 10 discs were floated in each Petri dish containing 10 ml of solution to be tested in the darkroom at 25 C for about 4 or 5 days. Chl was extracted in ethanol. Ten leaf discs were transferred to 10 ml of ethanol in a closed vial and allowed to stand overnight, and absorbance was read at 665 nm. The absorbance of ambient solution was measured at 280 nm to assess the leakage from leaf discs. All experiments were repeated on at least five different occasions.

## RESULTS

As a simple test of membrane permeability, we used slices of beet root suspended in water, and measured the extent of betacyanin leaking out into the ambient solution as an index of membrane leakiness. As a comparison of an extreme macromolecular destabilizer, $CaCl_2$, and an extreme stabilizer, $(NH_4)_2SO_4$, serial concentrations of these two salts were applied separately to beet root slices (Fig. 1); it can be seen that $CaCl_2$ produced a decrease in the amount of pigment leaking out, whereas $(NH_4)_2SO_4$ caused an increase in the amount.

If these opposite effects are, in fact, related to destabilization and stabilization effects, then it should be possible to relieve the effects of each salt with increasing amounts of the other. In order to test that possibility, a series of beet root sections was treated with $3 \times 10^{-3}$ M $(NH_4)_2SO_4$, and the effects of increasing amounts of $CaCl_2$ were examined (Fig. 2). It can be seen that whereas the $(NH_4)_2SO_4$ produced a marked increase in the amount of pigment leaking out of the root sections, the further addition of $CaCl_2$ at slightly above $10^{-3}$ M $CaCl_2$ brought the leakage back down to the level of the sections in water alone. The converse experiment was carried out as shown in Figure 3; here, a group of sections was treated with $CaCl_2$ at $10^{-3}$ M concentration, and the effects of further additions of $(NH_4)_2SO_4$ were examined. It can be seen that the $CaCl_2$ alone reduced the leakage of pigment by about half; the further addition of $(NH_4)_2SO_4$ brought the amount of leakage back to the value of water controls. These two experiments indicate that the effects of each of these two salts may be erased by the simultaneous application of the other salt.

As further evidence about the possibility that destabilization-stabilization effects were causing the leakage responses observed, we made a comparison of various cations and various anions. An array of 10 cations was added as their chlorides, each at $3 \times 10^{-3}$ M concentration, and the amount of pigment leaking out of the beet sections is shown in Table I; here again, ammonium caused the greatest leakage, and Ca depressed leakage by more than half. The monovalent ions K, Li, and Na gave a graded series of effects toward a lessening of leakage, and the divalent cations Mg, Ca, Ba, Sr, and Mn produced greater depression of leakage in that order; the trivalent lanthanum was markedly more effective in depressing leakage, as expected for a "supercalcium" (7). Various anions were tested as the K salts at a concentration of $3 \times 10^{-3}$ M, and the comparative effectiveness of the five anions is shown in Table II. Relatively little effects were obtained for phosphate, chloride, and sulfate, and a slight

[1] Journal Paper No. 5813, Purdue University Agricultural Experiment Station, Lafayette, Ind. 47907.

[2] Present address: Department of Horticulture, Washington State University, Pullman, Wash. 99163.

[3] Present address: Graduate College, University of Nebraska, Lincoln, Neb. 68583.

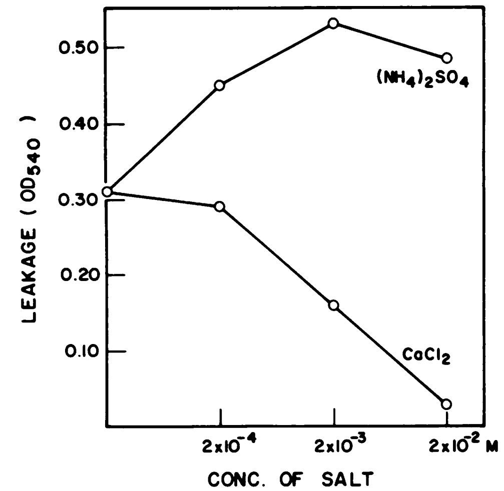


FIG. 1. Effects of $(NH_4)_2SO_4$ and $CaCl_2$ on leakage of betacyanin from beet slices.

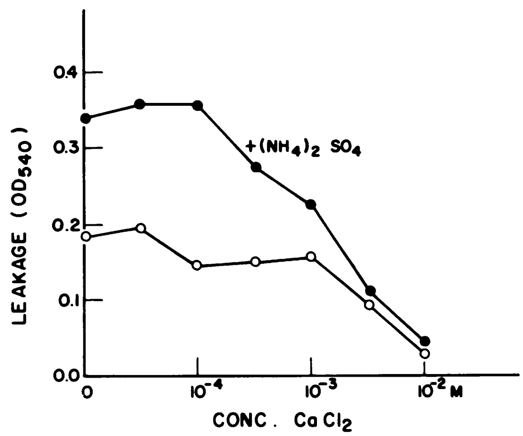


FIG. 2. Promotion of betacyanin leakage from beet slices with 3 mM $(NH_4)_2SO_4$ and the reversal of its effect with increasing concentrations of $CaCl_2$.

reduction of leakage was obtained with nitrate and carbonate. Collectively, these cation and anion experiments indicate that the leakiness effects are not specific to special cations or anions, but that graded series of effects can be obtained with a wide array of anions and cations.

Some further experiments were done with another tissue: the 1-cm discs of *Rumex* leaves. We had previously reported that the senescence of corn leaf discs was associated with a marked increase in leakiness, as measured by apparent free space and by permeability to tritiated water (8). It is known that the senescence of *Rumex* leaves is deferred by gibberellin (17), and that $CaCl_2$ also markedly slowed the development of senescence (8) and abscission (9). In the present experiments, *Rumex* leaf discs were treated with various concentrations of $(NH_4)_2SO_4$ to determine whether this stabilizing salt would have effects opposite to those of $CaCl_2$. Effects were measured in the presence and in the absence of GA. Chl content was used as a measure of senescence, and the relative absorbancy of the ambient solution at 280 nm was taken as an indication of the amount of leakage of organic materials from the leaf discs.

The data in Figure 4 indicate that $(NH_4)_2SO_4$ at concentrations of $10^{-3}$ M and above enhanced the rate of leaf senescence, as indicated by the greater loss of Chl, than in control pieces. While GA deferred senescence effectively, the addition of $(NH_4)_2SO_4$ in the presence of the GA did not alter the rate of leaf senescence. The measurements of leakage out of the leaf discs indicate that the $(NH_4)_2SO_4$ concentrations of $3 \times 10^{-3}$ M or above also caused an increase in leakage out of the leaf sections. Even in the presence of GA, an enhancement of leakage by $(NH_4)_2SO_4$ was evident at the higher concentrations.

If the enhancement of the rate of leaf senescence and of leakage by $(NH_4)_2SO_4$ is related to the solute effecting the destabilization-stabilization state of the membranes, then one would expect that $CaCl_2$ additions might relieve the effects of the $(NH_4)_2SO_4$. Experiments in which various concentrations of $CaCl_2$ were added in the incubation medium are presented in Figure 5, from which it can be seen that even in the presence of the $(NH_4)_2SO_4$, $CaCl_2$ was able to defer the rate of senescence, as indicated by the maintenance of higher Chl contents in the leaf discs. The lower figure indicates that the leakage of materials absorbing at 280 nm was stimulated by $(NH_4)_2SO_4$ as was seen previously in Figure 4, but the $CaCl_2$ additions relieved the effects of the $3 \times 10^{-3}$ M $(NH_4)_2SO_4$ both with respect to Chl retention (Figure 5, above) and limiting the development of leakiness (Fig. 5, below).

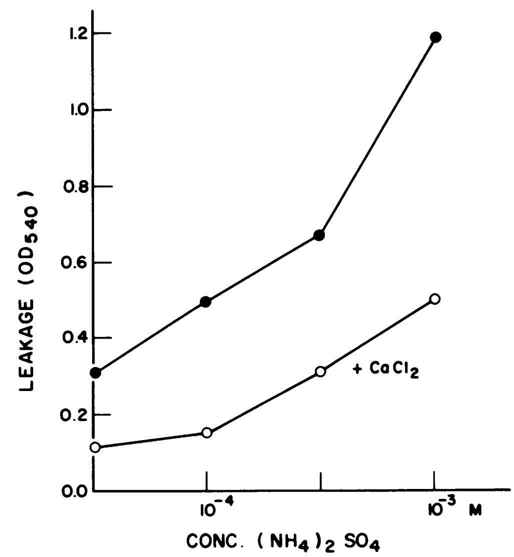


FIG. 3. Inhibition of betacyanin leakage from beet slices with 1 mM $CaCl_2$ and the reversal of its effect with increasing concentrations of $(NH_4)_2SO_4$.

Table I. *Comparative Effects of Cations on Leakage of Betacyanin from Beet Slices*

Each cation supplied as the chloride at $3 \times 10^{-3}$ M concentration.

| Solute supplied | Betacyanin leakage ($OD_{540}$) |
|---|---|
| $H_2O$ | 0.266 ± 0.040 |
| Ammonium | 1.300 ± 0.200 |
| Potassium | 0.325 ± 0.040 |
| Lithium | 0.200 ± 0.008 |
| Sodium | 0.180 ± 0.006 |
| Magnesium | 0.160 ± 0.030 |
| Calcium | 0.115 ± 0.008 |
| Barium | 0.111 ± 0.003 |
| Strontium | 0.098 ± 0.009 |
| Manganese | 0.030 ± 0.003 |
| Lanthanum | 0.017 ± 0.004 |

Table II. *Comparative Effects of Five Anions on Leakage of Betacyanin from Beet Slices*

Each anion supplied as the potassium salt at $3 \times 10^{-3}$ M concentration.

| Solute supplied | Betacyanin leakage ($OD_{540}$) |
|---|---|
| $H_2O$ | 0.266 ± 0.040 |
| Phosphate | 0.360 ± 0.014 |
| Chloride | 0.280 ± 0.058 |
| Sulfate | 0.227 ± 0.013 |
| Nitrate | 0.133 ± 0.005 |
| Carbonate | 0.103 ± 0.002 |

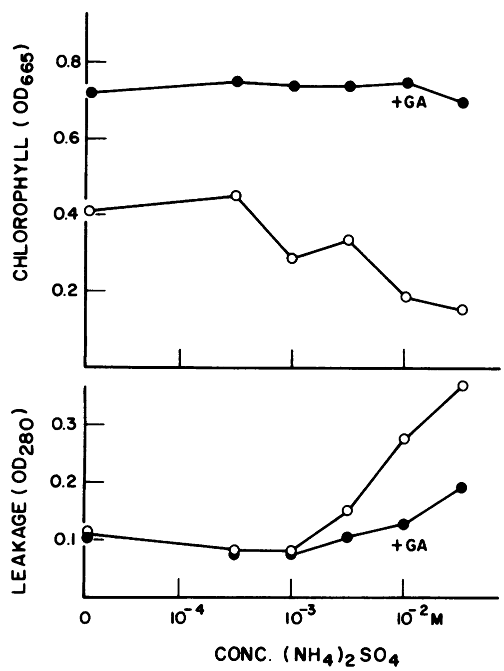


FIG. 4. Effects of $(NH_4)_2SO_4$ solutions on the Chl content of *Rumex* leaf discs, alone and in the presence of $10^{-5}$ M GA (upper). Effects of $(NH_4)_2SO_4$ solutions on the leakage (280 nm) of materials into the ambient solution, alone and in the presence of $10^{-5}$ M GA (lower).

Collectively, the experiments in Figures 4 and 5 indicate that $(NH_4)_2SO_4$ can enhance the rate of leaf senescence except in the presence of GA, that this enhanced senescence is associated with an increase in leakage of materials out of the leaf discs, and that $CaCl_2$ can relieve the effects of $(NH_4)_2SO_4$, both with respect to the effects on senescence and on leakiness.

## DISCUSSION

The experiments reported here have shown that Ca could reduce the leakiness of membranes to betacyanin and to solutes absorbing at a wavelength of 280 nm, that $(NH_4)_2SO_4$ could enhance such leakiness, and that interactions of each salt in the presence of the other were obtainable. We suggest that these results, for beet root pieces and for *Rumex* leaf discs, are consistent with the interpretation that the solute effects are associated with destabilization-stabilization effects of the solutes; and the relative effects of arrays of cations and anions in bringing about changes in leakiness also support the interpretation of destabilization-stabilization effects.

The possibility that the solutes may be having effects through alterations of pH was set aside for two reasons: *(a)* the pH of the salt solutions was monitored routinely before and after the tissue incubations, and no correlation of leakiness with pH was found; *(b)* a pH range from 3.4 to 8.6 was tested using MES and tris buffer combinations, and over the range of pH 5 to 7, there was no pH effect on beet leakage; at pH 4.5 or below, there was a slight increase in leakage of betacyanin.

There are numerous precedents for thinking that inorganic solutes can alter the characteristics of membranes. In the first place, the various structural members of membranes are each macromolecules, and Hofmeister's early work (2) and the more recent experiments of von Hippel and Wong (16) have defined the relative effects of a wide range of cations and anions in altering the characteristics of proteins and other macromolecules. The solute effects on macromolecular structures, according to von Hippel and Schleich (15), may involve changes in surface charges on the macromolecular surface, and changes in the water lattice interacting with the macromolecule. Gary-Bobo (1) made synthetic membranes of cephalin and showed that Ca could markedly depress the permeability. Levin *et al.* (4) have made synthetic vesicles with lecithin, and through the use of NMR resonance measurements, they found that Ca and stronger destabilizing solutes could greatly depress the permeability of vesicles, apparently through alterations of the lecithin and its interactions with the surrounding water lattice. At a more structural level, Marinos (5) has shown that Ca deficiencies in plants resulted in striking losses of membrane integrity as shown in electron microscopic photographs. Murakami and Packer (6) have likewise shown that chloroplast membranes produced much lighter membrane stacking in the presence of Ca or Mg than in the presence of various monovalent cations. Van Steveninck (14) has shown that beet root tissue pieces became markedly more permeable to K after EDTA treatment, and that Ca and other divalent cations could restore the low natural level of permeability to K. There are several references to ammonium effects on leakage, with Rehfeld and Jensen (12) having shown

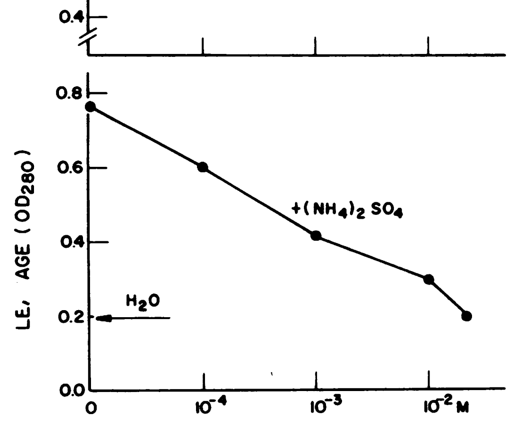


FIG. 5. Promotion of leakage (280 nm) into ambient solution and the loss of Chl with 10 mM $(NH_4)_2SO_4$ in leaf discs of *Rumex* and the effects of $CaCl_2$ on these changes.

that ammonium salts could increase the leakage of photosynthates out of cotton leaf cells, and Ca having the opposite effect. In some experiments with dynamic pumping systems, Satter *et al.* (13) have shown that the rhythmic movements of *Albizzia* pulvini are caused to exercise an opening action in the presence of calcium salts, a closing action in the presence of ammonium salts, and lesser or intermediate effects of other cations in the Hofmeister series. These experiments can be interpreted as indicating that inorganic solutes of the Hofmeister series can increase permeability of membranes or decrease the permeability, depending upon the stabilization or destabilization effects of the solutes. Through the experiments on interactions between solutes, our experiments have added strength to the concept of solute effects being through the destabilization-stabilization effects.

Our special interest in the solute alterations of membrane functions lies in the area of hormonal action; if hormones act through an attachment to a site of action on a membrane, then solute alterations of membrane characteristics might be expected to alter hormonal effectiveness. We have shown that each of the plant hormones is altered in effectiveness by calcium and ammonium salts (3, 11), and that the attachment of auxin to membrane pieces from corn coleoptiles can also be altered by these salts (10).

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