PASSIVE PERMEATION AND ACTIVE TRANSPORT OF IONS IN PLANT ROOTS\textsuperscript{1,2}

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Active ion transport in plant roots, according to the most widely held view, involves the operation of carriers. The hypothesis is as follows: The ions combine with the carrier molecules and the resulting ion-carrier complexes traverse membranes of limited permeability to the free ions. At the inner surface of the membranes, the ions are released from the carriers. This active process of ion transport depends on metabolism, and is characterized by a high degree of selectivity. Ions taken up by this mechanism are largely nonexchangeable with ambient ions of the same or other species.

The process briefly outlined above is not the only one whereby ions may penetrate plant roots. Cations may be non-metabolically adsorbed on negatively charged surfaces within the root, in stoichiometric exchange for other cations residing on these exchange surfaces. Epstein and Leggett (6) exposed excised barley roots to solutions of radioactive SrCl\textsubscript{2} (Sr\textsuperscript{87}Cl\textsubscript{2}). When, after 60 minutes, the roots were briefly rinsed with water and then exposed to a solution of non-radioactive SrCl\textsubscript{2} of the same concentration, they lost a large part of their Sr\textsuperscript{87} by exchange. Other cations also displaced exchangeable Sr\textsuperscript{87} from the roots. However, the amount lost to water was only a fraction of the amount lost to salts.

When similar experiments were performed on the absorption of sulfate, from solutions of K\textsubscript{2}S\textsuperscript{35}O\textsubscript{4}, a large labile fraction was again observed. However, in this instance, the amounts of S\textsuperscript{35}O\textsubscript{4} lost to water were identical with the amounts lost to non-radioactive sulfate, indicating that no exchanging or displacing ions were needed to effect this removal. It appeared, rather, that a fraction of the S\textsuperscript{35}O\textsubscript{4} ions in the tissue freely diffused out upon transfer of the tissue to water or salt solutions. Another fraction, however, neither diffused out into water, nor exchanged with ambient non-radioactive sulfate. It will be shown that the former process is a manifestation of an "outer region," "water space," or "apparent free space," to which ions have free and reversible access by diffusion. The second irreversible process is active transport of sulfate which will be discussed in greater detail elsewhere (9). The relation between these two processes will be examined. The findings will be shown to apply also to ions other than sulfate.

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Materials and Methods

Radioactive Solutions: Solutions of potassium sulfate labeled with $^35$S ($K_2S{SO}_4$) and of potassium selenate labeled with $^{75}$Se ($K_2Se{SO}_4$) were prepared as described elsewhere (9). Solutions of KH$_2$P*O$_4$ (P$^{32}$O) were prepared in the same way as was K$_2S{SO}_4$.

The procedure followed that described for Sr*Cl$_2$ (6). All radioisotopes were obtained from the Oak Ridge National Laboratory.

Radioactive Assay, Roots: Roots were assayed for radiosulfate as described elsewhere (9).

Solutions: Aliquots of the solutions were pipetted into circular (3.3 cm in diameter and 8 mm deep), $\frac{1}{4}$ oz, seamless tin boxes (made by Buckeye Stamping Company, Columbus, Ohio), containing the following: a circle of lens paper, 3.0 cm in diameter; 1 drop of a 5% solution of "Calgonite" (made by Calgon, Inc., Pittsburgh, Pennsylvania) to act as a spreader; 1 ml of a 0.1 N solution of the non-radioactive salt; and 0.2 ml of a 10% solution of sucrose, which keeps the paper from curling when dry. The samples were dried under infra-red lamps and counted in an internal-sample proportional counter.

Excised Barley Roots: Seeds of barley, var. Atlas 46, were germinated in aerated water for 24 hours, and seedlings grown for 5 days in the dark at 24°C, as described earlier (6), with these two differences: the solution used was Ca (H$_2$PO$_4$)$_2$, $2 \times 10^{-4}$ M, instead of CaSO$_4$, and the plants were left in this solution till the time the roots were rinsed and excised just before the experiment proper, instead of being transferred to water twelve hours earlier.

Standard Experimental Procedure: The roots were prepared and absorption experiments performed essentially as described earlier (6). One gm portions of excised roots were weighed out (after blotting on washed, dry cheesecloth to remove water adhering to the surface) and transferred to 50 ml water in 250 x 22 mm Pyrex test tubes. To initiate the absorption experiment proper, the water was decanted and replaced by 50 ml solution of a salt containing a radioactively labeled ion. The solutions were previously adjusted to pH 4.0 with 0.1 N HCl. The shift in pH during the absorption period seldom exceeded 0.1 unit. At the end of the absorption period, the solution containing the radioactively labeled ion was decanted, the roots were removed from their tube by means of a hooked glass rod, blotted on cheesecloth to remove solution adhering to the surface, and transferred to a new tube containing 50.0 ml water or solution of non-radioactive salt. Aeration was continuous, both during the absorption and desorption periods. Following the desorption period, an aliquot of the solution in the desorption tube was taken for assay of the amount of radioactively labeled ion lost by the roots. In some cases, the roots themselves were assayed. All treatments were set up in duplicate, except those of the experiment shown in figure 1, which were not replicated. The following is a summary of the relevant experimental conditions: roots, 1.00 gm fresh weight; volume, 50 ml; temperature, 30°C; pH, 4.0; aeration, continuous.

Results

Figure 1 presents the results of an experiment on the loss of radiosulfate to water and non-radioactive sulfate. The roots were exposed to a 20 meq/l solution of K$_2S{SO}_4$ for 90 minutes, then blotted and transferred to water or a 20 meq/l solution of CaSO$_4$. There occurred an exit of S*O$_4$ from the roots which was essentially completed in 60 minutes. A fraction of the S*O$_4$ taken up during the initial 90 minutes was retained by the roots, no further loss occurring after the first 60 minutes in water or non-radioactive sulfate. A comparison of the present experiment with that presented in figure 6, Epstein and Leggett (6), shows that the principal difference between the two was that in the latter experiment, only a fraction of the labile Sr* was lost to water, whereas in the present case, the loss of S*O$_4$ to water equalled the loss to non-radioactive SO$_4$. Diffusion, rather than ion exchange seemed to be the factor responsible for this loss. (In preliminary experiments, the roots were briefly rinsed with water, after the absorption period in radiosulfate, in order to remove the solution adhering to the surface of the root. But when it became apparent that diffusion was involved, this procedure was abandoned in favor of blotting.) The findings pointed to the existence, in barley roots, of a region or space to which ions have free and reversible access by diffusion. Terminology and findings for other types of cells and tissues will be considered in the discussion. In this paper, the region accessible by diffusion will be called the "outer" space of the root, and the region to which the ions are transported by the active mechanism, and where they are no longer subject to loss by diffusion or exchange with ambient ions of the same or other ionic species, will be called the "inner" space. On the assumption that at equilibrium, the concentration of the ions in the "outer" space is the same as their concentration in the ambient solution, the "outer" space is defined as...
ambient concentration (in meq/l)
ambient concentration (in ml/gm fresh wt)
ambient concentration (in meq/gm fresh wt),

\[
\mu \text{eq diffusible ion/gm fresh wt}
\]

"ambient concentration" referring to the concentration of the ion during the initial, or absorption, period.

In the experiment shown in figure 1, the amount of \( S^\circ O_4 \) lost to water or non-radioactive \( S^\circ O_4 \) was 4.45 \( \mu \text{eq/gm fresh wt} \). The concentration of \( K_2S^\circ O_4 \) during the initial, or absorption, period, was 20 meq/l, or 20 \( \mu \text{eq/ml} \). Hence the volume of the "outer" space was \( 4.45/20 = 0.23 \text{ ml/gm fresh wt} \). The water content of this tissue is 94%, so that over 24% of the total tissue water was accessible to the ions by diffusion.

In this experiment, the amount of labile or "outer" space \( S^\circ O_4 \) was determined by the difference in \( S^\circ O_4 \) content between blotted roots and roots which had been held in water or non-radioactive sulfate for 60 minutes or longer. This has the disadvantage that two root samples are required for each determination: a sample of blotted roots for determination of total \( S^\circ O_4 \), and a sample of roots which had been exposed to water or non-radioactive solution for 60 minutes for determination of "inner" space \( S^\circ O_4 \). A different method was therefore used for measuring the "outer" space in the other experiments reported here. The roots were held in the radioactive solution for 60 minutes, blotted, transferred to 50.0 ml water, and after 60 minutes, an aliquot (usually 5 ml) was taken for assay of \( S^\circ O_4 \) lost from the roots. Use of non-radioactive sulfate for the desorbing period was discontinued when the finding shown in figure 1, viz, identical loss to water and non-radioactive sulfate, was verified in many experiments, both for high (20 meq/l) and low (0.5 meq/l) concentrations of \( S^\circ O_4 \) during the absorption period, and of \( S^\circ O_4 \) during the desorption period.

If ions move into and out of a certain space within the tissue, reaching equality of concentration with the ambient solution, measurement of the volume of this space would not be expected to depend on the particular ambient concentration used (see equation 1). Figure 2 (left) shows that this expectation did not seem to be borne out by the experimental findings. While at the high concentrations of \( K_2S^\circ O_4 \), the volume of the "outer" space was constant at 0.22 ml/gm fresh wt, it increased at progressively lower concentrations, reaching a value of 0.32 ml at 1 meq/l \( K_2S^\circ O_4 \). This finding suggested a labile binding of sulfate which, at low concentrations, would give an apparent or false increase in the measured "space" by contributing to the sulfate released to water. At high concentrations, the amount contributed would be a negligible percentage of the sulfate released, and correct measurements of the "outer" space would result under these conditions.

Experiments were performed at low concentrations of \( K_2S^\circ O_4 \), in the presence of high concentrations (20 meq/l) of KCl and KH\(_2\)PO\(_4\), in the expectation that excess chloride or phosphate would displace sulfate from the postulated binding sites. It was found, however, that even in the presence of these added anions, the measured "outer" space was still high (of the order of 0.3 ml), when measured by means of \( K_2S^\circ O_4 \) at low concentrations (1 or 2 meq/l). However, \( K_2SeO_4 \) at increasing concentrations progressively lowered the measured magnitude of the space which eventually reached the same value (0.24 ml in this experiment) obtained when the space was measured with \( K_2S^\circ O_4 \) at 20 meq/l, in the absence of \( K_2SeO_4 \) (fig 2, right). These results are evidence for a labile binding of sulfate and for competition by selenate in this process. So far as measurements of the "outer" space by means of sulfate are concerned, this binding introduces an error by apparently increasing the measured space at low sulfate concentrations.

The above findings suggested a means whereby the "outer" space could be measured without interference by the labile sulfate binding even at low sulfate con-

![Figure 2](left). Magnitude of the "outer" space of barley roots as affected by the concentration of \( K_2S^\circ O_4 \), used in measuring it (left), and by the concentration of \( K_2SeO_4 \), at a low concentration of \( K_2S^\circ O_4 \) (2 meq/l) (right).

![Figure 3](right). Amounts of labeled sulfate in the "outer" space of barley roots as a function of the concentration of \( K_2S^\circ O_4 \) in the ambient solution. \( K_2SeO_4 \), 20 meq/l throughout.
TABLE I
Determination of the "Outer" Space of Barley Roots, Using Various Conc of K2SeO4

<table>
<thead>
<tr>
<th>Conc K2SeO4</th>
<th>&quot;Outer&quot; space S*O4</th>
<th>&quot;Outer&quot; space</th>
<th>meq/gm fresh wt</th>
<th>ml/gm fresh wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.02</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.31</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>5.88</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>11.33</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

20 meq/l K2SeO4 was used throughout.

TABLE II
"Outer" Space of Barley Roots as a Function of the pH of the Solution (20 meq/l K2SeO4) Measuring It

<table>
<thead>
<tr>
<th>pH*</th>
<th>&quot;Outer&quot; space ml/gm fresh wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.22</td>
</tr>
<tr>
<td>4.5</td>
<td>0.23</td>
</tr>
<tr>
<td>5.9</td>
<td>0.20</td>
</tr>
<tr>
<td>7.0</td>
<td>0.23</td>
</tr>
<tr>
<td>7.7</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* Adjusted as follows: pH 4.0, by means of 0.1 N HCl, as in all other experiments. All other samples, 0.05 M NaH2PO4-Na2HPO4 buffer (final conc). Shift in pH during the experiment was less than 0.1 unit in every case.

TABLE III
Measurement of the "Outer" Space of Barley Roots by Means of Several Ions*

<table>
<thead>
<tr>
<th>Ion</th>
<th>Conc of Ion</th>
<th>&quot;Outer&quot; space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>meq/l</td>
<td>ml/gm fresh wt</td>
</tr>
<tr>
<td>S*O4</td>
<td>20</td>
<td>0.25</td>
</tr>
<tr>
<td>Sr*O4</td>
<td>20</td>
<td>0.22</td>
</tr>
<tr>
<td>H2P*O4</td>
<td>20</td>
<td>0.24</td>
</tr>
<tr>
<td>Ca*</td>
<td>1</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* The anions were used as the potassium salts. Ca* was present as Ca*Cl2. SrCl2 at a conc of 20 meq/l was present in addition to the Ca*Cl2. See text for explanation.

excess Sr would effectively prevent significant quantities of Ca* from occupying the cation exchange surfaces. It was found for all ions that equilibration was complete in 60 minutes, and absorption and desorption periods of 60 minutes were used. Table III shows that the magnitude of the space was essentially independent of the particular ion used in measuring it.

DISCUSSION

The findings presented in this paper indicate, for barley roots grown under the conditions of these experiments, that there occurs a movement of ions between the ambient solution and a compartment or space (the "outer" space) within the root occupying approximately 23% of the total tissue volume. This movement of ions has the following characteristics: 1) After transfer to a new solution, equilibrium is established within 60 minutes; 2) the evidence is consistent with the conclusion that at equilibrium, the concentration of the ions in the "outer" space equals their concentration in the ambient solution; 3) there is no competition among ions for the space; 4) there

Regions to which various solutes have free and reversible access by diffusion have been recognized in a variety of cells and tissues. Conway and Downey (2) have described an "outer region" or "space" of the yeast cell which is fully accessible to a great variety of solutes, including inorganic ions. The space has a water content of approximately one-tenth the whole cell volume. Cowie et al (3), in experiments with Escherichia coli, found that K and Na ions rapidly entered the entire "water space" of the cell (approxi-
mately 75% of the cell volume), reaching equality of concentration with the ambient solution in less than 5 minutes. Entry of the ions into the water space was reversible, and not a function of metabolism. Roberts et al (10), using the same organism, reported similar findings for sulfate, phosphate, manganese, glucose-1-phosphate, fructose-1,6-diphosphate, and amino acids. Whittam and Davies (11) found that a large fraction of the sodium in guinea pig kidney cortex tissue exchanged at very rapid rates and was indistinguishable from the sodium in the spaces outside the cells. On the assumption that the concentration of the ion in the space was the same as in the medium, the "outer space" occupied approximately 50% of the tissue at 37°C, suggesting that the barrier to the exchange of the rest of the sodium is not the outer cell membrane but may lie within the cell.

Hope and Stevens (8) were the first to recognize this phenomenon in the roots of higher plants (beans) and termed the space accessible by diffusion the "apparent free space" (AFS). Hope (7) examined the relation between the measured space and the concentration of the KCl solution used for measuring it. The "space" progressively increased with increasing concentration of KCl, indicating a Donnan equilibrium between the root cell cytoplasm and the medium. Butler (1), using wheat roots, and chloride, phosphate, and mannitol for measuring the space, found values ranging from 24 to 34% of the tissue, but there was no trend in the magnitude of the measured space with changes in the concentration of the solute used in measuring it.

It should be recognized that the variety of terminology notwithstanding, the meaning of the terms used is similar, viz., a space accessible to certain solutes by diffusion. When the solute is specified, it is convenient to speak of "sulfate space," "phosphate space," etc. For a general term, we have used "outer" space, following Conway and Downey (2) who apparently were the first to describe such a space 4 in a plant cell (yeast). The term facilitates the distinction between this space and the "inner" space—the region or regions to which the ions are transported by the active carrier mechanism, and where they are no longer subject to loss by diffusion or exchange with ambient ions of the same or other ion species. "Outer" space is defined here strictly in operational terms, no attempt being made to identify it with particular regions within the tissue or cells. The term is meant to convey the conclusion that this space stands in communication with, and correspondence to, the external solution, so far as the test solutes are concerned.

3 The "water space" in _E. coli_ described by Cowie et al (3) has the additional characteristic that its volume equals the total cell water, whereas the other spaces accessible by diffusion discussed here represent only a fraction of the total cell or tissue water.

4 "Outer" space as viewed here differs from "apparent free space" in that ions restrained by labile binding and exchange effects are separately accounted for, so that the "apparent" contribution to the space is eliminated. The external solution and the solution in the "outer" space of the tissue form a continuum.

The only alternative to the interpretation of the demonstrated reversible ion movements as due to diffusion would be one based on a labile binding of the ions, which, on transfer of the tissue to water, would dissociate from the binding entities. This possibility deserves careful consideration in view of the fact that with sulfate, such binding was actually demonstrated at low K2S2O4 concentrations. The conclusive test that distinguishes between binding and the lack of it is applied by means of experiments on ion competition. Similar ions tend to compete for identical binding entities, but ions do not compete with one another in diffusion through water. The sulfate uptake giving an apparent or false increase in the measured space was diminished by progressively higher concentrations of selenate, and effectively abolished at a selenate concentration of 20 meq/l. The sulfate concentration in the "outer" space, on the other hand, reached equality of concentration with the ambient solution regardless of the selenate concentration. The space was the same when measured with K2S2O4 at 20 meq/l, without selenate (fig 2), as when measured at 1 meq/l or 50 meq/l in the presence of selenate at 20 meq/l (fig 3). This test is the justification for considering the increase in the "space" at low sulfate concentrations, in the absence of selenate, as due to a phenomenon different in kind from diffusion into the outer space proper. Before concluding that a certain uptake involves an "outer" or "free" space, the possibility of labile binding should be ruled out.

The labile binding of sulfate giving rise to the spurious high values for the measured space at low concentrations of sulfate should not be confused with active absorption of sulfate. The processes have in common the fact that selenate competes with sulfate, but following active transport into the "inner" space, sulfate is far from labile, neither being lost to water nor subject to exchange with ambient sulfate (fig 1).

We shall finally examine the relation between diffusion of ions in the "outer" space to active transport of the ions (i.e., their transfer into the "inner" space). It is obvious from the results that the effective interface between the ambient solution and the "inner" space cannot be identified with the epidermis. Rather, the conclusion emerges that the cells of the root, by their active transport mechanism, absorb ions directly from a solution already within the tissue, viz., the solution in the "outer" space, which, in turn, is in equilibrium with the ambient solution. Evidence for this conclusion is presented in figure 4.

Following a 60-minute absorption period in K2S2O4 at 20 meq/l, the roots were blotted and transferred to tubes containing 50.0 ml water. They were not, however, placed in the water, but in the space above the water, whereupon the tubes were stoppered. The roots were thus confined in a small volume (50 ml) of humid air, and could neither gain nor lose sulfate. Immediately after the transfer, and at hourly intervals thereafter, samples were transferred into the
water, and after 60 minutes, aliquots were taken for determination of "outer" space sulfate, and the roots themselves were assayed for "inner" space sulfate. The roots steadily gained "inner" space, or "absorbed" sulfate, at the expense of "outer" space sulfate, with no change in total sulfate. The rate of the process corresponds to the rate of active absorption from a solution of K₂S²O₄ at 20 meq/l. By contrast, cations exchangeably adsorbed on the exchange surfaces of the root, in the absence of an external reservoir of the ions in the solution, are absorbed very slowly by the active mechanism, the exchange spots effectively competing with the carriers for the limited supply of ions (Epstein and Leggett (6), fig. 6).

The picture of the "outer" space of plant roots, as it emerges from the studies on diffusion (1, 7, 8, and the present paper), together with the studies on cation exchange (6), is that of a sponge made of cation exchange material. Both anions and cations freely diffuse in the water, and cations exchange on the negatively charged spots of the exchange surfaces. Donnan effects may or may not be pronounced, depending on the concentration of the cation exchange spots, the salt concentration in the ambient solution, and its pH. Within this matrix lie the "inner" spaces, separated from the "outer" space by membranes or barriers across which operate the carriers effecting active ion transport.

The view presented here resolves a paradox apparently inhering in the findings on active ion transport. The evidence and kinetic interpretation presented earlier (4, 5, 6) and in the present paper is that the over-all process of absorption by the active carrier mechanism is essentially irreversible, and that ions so absorbed are nonexchangeable with other ions of the same or other ionic species. How, then, can cells in the interior of the root tissue absorb ions which have previously been absorbed by cells nearer the ambient solution, if this previous absorption was essentially irreversible? Any satisfactory scheme of active ion transport must operate at the cellular and sub-cellular level. Certainly, the process cannot be ascribed to the epidermis cells exclusively—the only cells apparently in direct contact with the ambient solution. The paradox of which we spoke is that of a one-membrane scheme and a multi-membrane tissue.

The evidence for the "outer" space resolves this difficulty by providing for access of the ions to the membranes surrounding the "inner" spaces of the individual cells without requiring them to pass through the "inner" spaces of other cells. Rather, the "inner" spaces of the individual cells, through the active carrier mechanism, absorb ions from a common pool—the solution in the "outer" space, which is in equilibrium with the ambient solution.

**Summary**

Experiments are described demonstrating a passive and reversible permeation of barley root tissue by sulfate and other inorganic ions through diffusion. The volume so accessible (the "outer" space of the tissue), as measured with sulfate, has the following properties.

1. Its magnitude is approximately 23 % of the volume of the tissue.
2. Migration of the ions into this space is freely reversible; upon transfer of the tissue to water, the ions diffuse out of the space again.
3. Equilibration with the ambient medium is reached in 60 minutes.
4. At equilibrium, the concentration of the ions in the "outer" space of the tissue equals their concentration in the ambient medium.
5. There is no competition among different ions for the space.
6. There is no pH effect. The space was freely accessible to selenate, phosphate, and calcium ions, in addition to sulfate.

The relation is examined between diffusion of ions in this "outer" space of the tissue and their absorption by the active ion transport mechanism, i.e., their transfer into the "inner" space. It is shown that the immediate substrate for absorption by the active mechanism is the solution in the "outer" space of the tissue, this solution in turn being in equilibrium with the ambient solution.

**Literature Cited**

6. Epstein, E. and Leggett, J. E. The absorption of...
Developmental abnormalities induced by chemicals provide a useful approach to the study of plant development if the precise manner in which the formative substance affects metabolism can be established. The experimental object for such a study should preferably be capable of growth under aseptic conditions, morphologically simple so that formative effects may be easily defined, and so constructed that translocation effects of the substance in question are minimized. The common duckweed, *Lemna minor* L., satisfies all these requirements. In addition, its rapid vegetative reproduction insures a large and genetically uniform source of material. A good account of the development of Lemna has been given by Ashby et al. (3), and of its growth in aseptic conditions by Gorham (15).

Compounds believed to act as antimetabolites might be expected to exert comparatively easily identifiable biochemical activities. Preliminary tests of a number of such compounds on Lemna showed that benzimidazole (BZ), reported to be an adenine antagonist (32), could induce a profound morphological modification without being toxic (fig 1). This was of particular interest since adenine has been implicated in leaf development and in the action of auxin (4, 28). An investigation of the action of BZ on Lemna was thus undertaken, and developed primarily as a comparison of the physiological characteristics of normal and BZ-modified plants.

**Materials and Methods**

The sterile clone of Lemna used was kindly provided by Dr. Hempstead Castle. Experimental and stock cultures were grown in 125 ml Erlemeyer flasks containing 50 ml of Gorham’s (15) mineral medium.


A constant temperature of 20°C was employed, and continuous daylight fluorescent light of 150 to 500 fc intensity, depending on the experiment. BZ was obtained from Krishel Laboratories, Portland, Oregon, and several other sources. It was dissolved by heating with the medium. The pH was adjusted with 0.1 N HCl or NaOH before autoclaving, and the change caused by autoclaving, with or without BZ, was 0.1 pH unit or less in the range pH 4.0 to 6.5.

Experimental cultures were started with one “colony” (3 to 4 adhering plants) in each flask and allowed to grow for 10 to 28 days. Multiplication rate, defined by Gorham (15) as the increase in the logarithm (base 10) of plant number per day, of normal plants in the simple mineral medium ranged from 0.060 to 0.150, depending upon light intensity. In order to avoid arbitrary judgments all visible outgrowths from older plants, no matter how small, were counted when plant numbers were determined. Several replicate flasks, usually five, were used for each experimental treatment.

Respiration experiments and enzyme assays were carried out using standard manometric techniques (31) at a water-bath temperature of 27°C. For the respiration experiments, healthy plants were selected, randomized, and transferred with care to Warburg vessels, 50 or 100 being placed in the main chamber (volume about 15 ml) of each vessel with 2 or 3 ml of Gorham’s medium. Two-tenths ml of 20% KOH or of 60% diethanolamine with folded filter paper in the center-well was used to absorb CO₂. The sensitivities of normal and BZ-modified plants to the respiratory inhibitors cyanide, azide, carbon monoxide, phenylthiourea (PTU) (10, 14) 2,4-dinitrophenol and iodoacetate were compared; comparisons were based on the relative change of the oxygen uptake of each lot of plants from its rate before treatment. Cyanide was used by removing the vessels from the manometers and adding the solution after the control period. This was done to prevent volatile HCN from acting prematurely. It was found that when 60% diethanolamine (DEA) was used in the center-well instead of KOH the absorption of HCN, and thus the diminution of the cyanide inhibition with time, could be almost abol-