Effect of Light on Sodium Influx, Membrane Potential, and Protoplasmic Streaming in Nitella

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The way in which the various activities of a cell relate to the level of available energy can conveniently be studied in photosynthetic cells of size large enough to permit measurements on individual cells. Internodal cells of the Characeae approach ideality for such a purpose in that they are, to a good approximation, right circular cylinders of macroscopic dimensions. Moreover, a certain internal symmetry prevails in that the chloroplasts are uniformly distributed in a single layer in the outer, nonstreaming layer of cytoplasm.

The purpose of this study was to investigate the manner in which light intensity and the composition of the external medium interact to influence a number of cellular activities and properties including sodium influx, membrane potential, the Na, K, and Cl concentrations in the vacuole, and protoplasmic streaming. For this, mature internodal cells of Nitella clavata were placed under a number of environmental conditions in each of which light intensity, solution composition, and temperature were kept constant. The Na influxes and rates of protoplasmic streaming reported here are the constant rates eventually established under a given set of conditions. These constant rates are indicative of a stabilization of certain metabolic processes, but the establishment of steady-state concentrations of the cellular constituents cannot be claimed.

Materials and Methods

Culture Conditions. A sample of Nitella clavata Kutz. kindly provided by Professor L. R. Blinks was propagated vegetatively in the laboratory in a nutrient solution of the following composition, with concentrations expressed in μmoles per liter: macronutrients: 20 KH₂PO₄, 100 K₂SO₄, 1000 NaCl, 2000 NaHCO₃, 1000 CaCl₂, 500 Mg(NO₃)₂, 500 MgSO₄; micronutrients: 2.76 HBO₃, 0.014 (NH₄)₆Mo₇O₂₄, 0.39 KI, 3.6 FeSO₄, 0.91 MnSO₄, 0.76 ZnSO₄, 0.047 CuSO₄, 0.17 CoCl₂. The micronutrient metals were present in chelated form, as the 1,2-diaminocyclohexanetetraacetate complexes (Geigy Chemical Corp.). The stabilities of the metal-chelate complexes were high enough to prevent precipitation of metal hydroxides in the culture solution, pH 8.3. Either incandescent or fluorescent illumination was used at an intensity of about 1500 ergs/cm²/sec, corresponding to about 25 ft-c. The cultures were not aerated.

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In Nitella species the internode consists of a single cell. Internodal cells were harvested by cutting away the neighboring cells, except for several small nodal cells at each end. The latter probably constitute less than 5% of the total volume. For convenience an internodal cell with the attached nodal cells will hereinafter be called an internodal cell. Usually the single cells studied were from the third through seventh internodes. These cells ranged from 25 to 50 mm in length and 500 to 800 μ in diameter.

Protoplasmic Streaming. Streaming rates were measured with a microscope having an eyepiece micrometer. The time required for a small particle to traverse 400 μ was measured with a stopwatch: usually 3 clockings per cell were made. At 25° the streaming rate is about 100 μ/sec with a standard deviation among cells of 3 μ/sec. No change in streaming rate due to illumination from the microscope lamp occurred so long as the observation period was limited to 15 minutes per day. Streaming rates were considered constant when they remained unaltered for at least 1 week.

Sodium Influx. Na²² was used at a specific activity of about 80 mc/mole Na. In all cases except one the influx period was 48 hours: for cells at the lowest light intensity the period was 12 days. In most cases 4 cells were used for each determination. Since the radioassay involved removal of the cells from the constant light and temperature conditions, it was necessary to use a different group of cells for each influx period. Influx was considered to be constant when the mean value showed no change during 3 consecutive 48-hour periods.

Following the influx periods, external Na²² was removed from the cell surface and exchanged from the cell wall by rinsing the cells in solutions identical with the experimental ones except that Na was present only as the stable isotope. Each rinsing consisted of 3 phases of one-half hour each. Each cell was then placed in a 5 ml vial and assayed for gamma radiation in a well scintillation counter (Tracerlab, Inc.). Appropriate corrections for differences in counting geometry due to the variation in cell lengths were made.

Influx is expressed as pico-equivalents (10⁻¹² equiv) per square cm of cell surface per second. For calculation of influx small corrections were made for that part of the fast Na fractions which was not removed during the 1.5-hour rinse. Even with this small resistant fraction, Na influx was essentially linear with time, in agreement with the results of Hope and Walker (9). In the present work the amounts of Na remaining in or on the cells after a 30-minute exposure to Na²² followed by a 90-minute exposure...
rinse, were 90 and 170 p-equiv/cm² for the M and C solutions, respectively (steady-state influx subtracted).

Experimental Conditions. For the main work 2 solutions, each containing 3 mM Na, were used: the M solution having the same composition as the macronutrient portion of the nutrient solution described above, with a pH of 8.3; the C solution containing 3.0 mM NaCl, 0.10 mM KCl, 1.0 mM CaCl₂, and 1.0 mM MgSO₄, with a pH of 6.2. In some additional experiments a bicarbonate solution was used, containing 1.0 mM NaHCO₃, 1.0 mM NaCl, 0.05 mM K₂SO₄, 1.0 mM CaCl₂, 1.0 mM MgSO₄, with a pH of 8.0; the total Na concentration is 2.0 mM.

For Na influx measurements quadrant-divided petri dishes were used, with 1 cell in each compartment. With approximately 10 ml of solution in each compartment the solution/cell volume ratio was about 500. During the experiments and pretreatments the cells were kept in solution in the petri dishes, with covers on, in a controlled temperature chamber at 27°. A rather vigorous stirring of the solutions was effected through the vibrations imparted to the chamber by the operation of the compressor. The solutions were not aerated.

The interior walls of the chamber were covered with black paper or black cloth so that a single incandescent lamp at the top could be treated as a point source of light. Radiant flux densities were calculated for the wavelength interval 350 to 750 nm from measurements with a standardized Weston photometer (model 756, with quartz filter) by applying the data of Forsythe and Adams (6). With this arrangement a reading of one ft-c corresponded to a radiant flux density of about 63 ergs/cm² sec. In the text, the terms radiant flux density and light intensity will be used interchangeably. The intensities ranged from 40 to 3400 ergs/cm² sec. Although very low as compared to the levels most frequently used in work on higher plants, they covered the range most favorable for growth of Nitella in the laboratory.

Electrical Potentials. Membrane potentials of cells at 320 and 1800 ergs/cm²/sec were measured with Ag/AgCl microcapillary electrodes filled with 2.5 mM KCl solution, with tip resistances ranging between 0.5 and 1.5 megohms. Potential differences between the 2 microelectrodes immersed in the same solution (C or M) were usually about 10 mv. After insertion of a microelectrode into a cell the potential difference between vacuole and external medium (C or M solution) was measured with a Type 1230-A General Radio Co. electrometer; the input resistance was set at 10⁹ ohms. Since the membrane potentials were about -100 mv, the current passing through the cell was about 10⁻¹⁰ amp or, in terms of net ion transfer, 10⁻¹⁵ equiv/sec. This is negligible in comparison to the approximately 10⁻¹² equiv/ions/sec normally traversing the membrane. The output of the electrometer was connected to a recorder (Esterline-Angus) and potentials were recorded with time. The time required for the potential to stabilize varied between a few minutes and 1 hour, depending presumably on the rate at which sealing around the inserted electrode took place. During a measurement a cell was kept in a small plexiglass trough through which the bathing solution passed. The ground electrode was located downstream from the cell so that KCl diffusing from the tip would not come in contact with the cell. The light intensity was maintained at the appropriate level; the temperature was 24 to 28°.

Vacuolar Ion Concentrations. From the same groups of cells as used for potential measurements, vacuolar sap samples were obtained from the largest cells. This was done by puncturing the cells with a sharp needle and gently pressing the cell walls with a forceps so that the clear cell sap ran onto a small polyethylene square. Five microliters were then immediately taken up in capillary tubing (Microcaps, Drummond Scientific Co.) and emptied into 3 ml of distilled water. Na and K were analyzed by flame photometry. Chloride was determined amperometrically with a Cotlove Chloridometer (Buchler Instruments, Inc.) On smaller cells the osmotic value of the cell sap was measured plasmolytically with mannanol solutions; this was taken to be 96% of the mannanol concentration at which definite pockets of separation of protoplasm from the cell wall occurred, since in most cases a very slight wrinkling of the protoplasm surface was observed at such a concentration. Pretreatments and treatment of the batches of cells on which potential measurements and vacuolar cell sap analyses were carried out were essentially the same as those for the corresponding cells in the Na influx study, except that the temperature was 23° instead of 27°.

Results

Na Influx. Figure 1 shows that Na influx is dependent on light intensity and the extent of this dependence is influenced by the composition of the

![Fig. 1. Light dependence of Na influx in Nitella clavata at 27° in the M and C solutions, each containing 3 mM Na. Each point: mean influx for 6 or 12 cells; variation expressed as standard error of mean.](https://www.plantphysiol.org/upload_files/paper/1964/1116/19641116fig01.jpg)
medium. The M solution (containing nitrate, phosphate, and bicarbonate) has a characteristically different effect on Na movement. For both solutions there are threshold light requirements, followed by a linear dependency on illumination. The inhibition of Na influx at the highest intensity is associated with visible changes in the arrangement of the chloroplasts, to be described below. The leveling off of Na influx in C cells is not associated with any such changes.

Some additional experiments under somewhat different conditions indicate that if bicarbonate is added to a solution of the C type Na influx is stimulated to about the same extent as in the M solution. In this work the Na concentration was 2.0 mM (as NaCl in the C type solution; as 1.0 mM NaCl plus 1.0 mM NaHCO₃ in the bicarbonate solution). Light was 2500 ergs/cm² sec, temperature 23°. For cells in the C type solution the mean Na influx was 0.038 ± .003 while that for the bicarbonate solution was 0.083 ± .009. As a check, an influx determination of cells in the regular C solution (3.0 mM NaCl) was carried out under these conditions, giving a value of 0.070 ± 0.005, about the same as expected at 27° (fig 1).

**Potentials and Vacular Ion Concentrations.** The membrane potentials of cells maintained at 320 and 1800 ergs/cm² sec and the vacuolar ion concentrations are reported in table I, along with results on the osmotic values of cell sap. There is no effect of light on the potential. Transient effects were observed at much higher intensities [cf. Brown (3)], but they are not relevant here. The small difference between M and C cells in membrane potential is less noteworthy than the deviation of both from their K equilibrium potentials of -152 and -170 mv, respectively; each is 45 to 50 mv more positive. Although in many instances with various cells the membrane potential does appear to be essentially a K diffusion potential (4), other factors must be involved here.

No large differences in the vacuolar concentrations of Na, K, and Cl are present among the 4 groups of cells; the K concentrations for the M cells at both light intensities are slightly higher, reflecting the higher external K concentration. Table I also indicates that the osmolarity of the cell sap is largely accounted for by Na, K, and Cl. Nitrate was apparently not accumulated to an appreciable extent in the vacuole in M cells. This is in agreement with the results of Hoagland and Davis (7), which also indicate that Ca, Mg, and SO₄ may be present in the vacuole in *Nitella clavata* in measurable quantities.

**Protoplasmic Streaming.** Figure 2 indicates that the dependence of protoplasmic streaming on light is limited to intensities below 700 ergs/cm² sec. In contrast to Na influx this dependence is much less marked. So long as a cell has sufficient light for survival, the streaming rate is at least 67 μ/sec at 27°. No difference between the M and C cells was evident except for the slight depression of the streaming rate of M cells at 3400 ergs/cm² sec.

**Cytological Observations.** In cells kept in the M solution at 3400 ergs/cm² sec an unusual condition of the chloroplasts occurred. Normally the chloroplasts are embedded very regularly in a single layer in the outer, nonstreaming cytoplasm (just inside

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**Table 1**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Light ergs/cm² sec</th>
<th>Membrane potentials*, mv</th>
<th>Vacular Ion Concentrations**, mM</th>
<th>Osmolarity of cell sap**, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>C</td>
<td>320</td>
<td>-122 ± 2</td>
<td>33 ± 4</td>
<td>77 ± 3</td>
</tr>
<tr>
<td></td>
<td>1800</td>
<td>-120 ± 2</td>
<td>39 ± 6</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>M</td>
<td>320</td>
<td>-108 ± 4</td>
<td>38 ± 3</td>
<td>86 ± 3</td>
</tr>
<tr>
<td></td>
<td>1800</td>
<td>-104 ± 4</td>
<td>31 ± 5</td>
<td>81 ± 4</td>
</tr>
</tbody>
</table>

* Mean for 8 to 12 cells ± standard error.

** Mean for 6 cells ± standard error.
the plasmalemma), blanketing perhaps 80% of the protoplast surface. The only exception to this is the presence of 2 white lines running the length of the cell, each white line being a zone about 20 μ across having no chloroplasts. In the abnormal cells chloroplasts were observed in the white lines, making these zones difficult to distinguish from the regular field of chloroplasts. In addition, some chloroplasts were missing from their regular fixed positions, apparently having moved into the inner protoplasm.

Besides this unusual disposition of the chloroplasts, the inner protoplasm had a somewhat jerky movement and was slightly retarded. These abnormalities, along with the low Na influx, indicate a rather general derangement of metabolism in the cell. The roughly 10 to 15% mortality rate of cells in the M solution under these conditions is also consistent with this interpretation.

**Attainment of Constant Na Influxes and Streaming Rates.** Na influxes usually became constant in about 1 week; in a few cases 2 to 3 weeks were needed. The constancy of the influxes of cells damaged by light at 3400 ergs/cm² sec is somewhat in doubt because of the uncertain stability of these cells. The attainment of constant streaming rates required 5 weeks in cells at 100 to 200 ergs/cm² sec, with shorter times needed at higher intensities. Initially these cells had maximum streaming rates.

**Variation among Cells.** The relative variation of Na influx among cells was large but rather constant over the entire range of light intensities, the coefficient of variation being about 0.3 in each case. Figure 2 indicates that considerable variability among cells also existed in the ability to survive at low light intensities, but this was not influenced by the composition of the solution. Some cells survived at 80 ergs/cm² sec whereas others required greater than 170 ergs/cm² sec. This degree of variation is roughly the same as that for the Na influx.

**Discussion**

**Na Influx, Light, and Membrane Potentials.** Although the strong correlation of Na influx with light intensity suggests an active transport mechanism, a net passive movement of Na into the cell should occur because of the existing electrochemical potential gradient. The electrical potential for which Na would be at equilibrium across the membrane is given by the following expression (4):

\[ E_{Na} = \frac{RT}{F} \ln \left( \frac{[Na]_o}{[Na]_i} \right) \]

For both M and C cells \( E_{Na} \) is about —62 mv. Since the actual potentials were more negative than this, the net passive Na flux will be inward. The difference in electrochemical potential (Δμ) between the interior and exterior of the cell for an ion is the driving force for net passive movement of the ion (2). Since both the electrical potential and the vacuolar Na concentration were unaffected by light (table I), it is clear that the light dependence of Na influx is not due to changes in ΔμNa. It is possible that changes in the sodium permeability of the plasmalemma occur in response to light, allowing a faster passive movement in brighter light. Blinks (1) reported that the electrical resistance of the plasmalemma of *Nitella clavata* could be reduced four or fivefold by exposing the cells to full sunlight for a day or more. The effect of light on the electrical resistance of the plasmalemma under the conditions of our experiments remains to be examined.

**Na Influx, Light, and Bicarbonate.** The differences between M and C cells in the extent to which light influences Na influx suggest that at least a part of Na influx is connected with the assimilation of metabolizable ions. Our results with the bicarbonate solution indicate that an enhancing effect on Na influx comparable to that of the M solution is obtainable with this ion. The mechanism by which bicarbonate interacts with light to influence Na influx is not clear, but some connection with photosynthesis would seem most likely. Scott (13) found that caesium uptake by *Rhodophyceae palmata* occurred only in light, and then only when CO₂ was present in the aerating gas. Scott's results do not establish the participation of bicarbonate per se in caesium uptake, but they do clearly indicate that either carbon influx or carbon assimilation is necessary for Cs influx. Our results suggest that a similar situation may exist for Na influx in *Nitella*.

**Protoplasmic Streaming.** Since protoplasmic streaming attains a maximum rate at the low light intensity of 700 ergs/cm² sec, it may be reasoned that this process has a small energy requirement. Kamiya and Kuroda (10) experimentally found the motive force of streaming in a *Nitella* species to be 1.6 dyne/cm², from which the energy requirement for a streaming rate of 107 μ/sec may be calculated:

\[ 1.6 \text{ dyne/cm}^2 \times 0.0107 \text{ cm/sec} = 0.017 \text{ erg/cm}^2 \text{ sec} \]

Assuming an absorption spectrum like that of Chlorella (5), we found that Nitella cells absorbed about 66% of the incident light. For a photosynthetic efficiency of 20%, the work done in streaming would account for only 0.04% of the energy supply at 1000 ergs/cm² sec.

There appears to be no close connection between the rate of streaming and Na influx. The indifference of streaming to the composition of the external solution is to be expected because of the controlled internal environment of the cell. The slightly retarded, jerky streaming of cells in M solution and bright light is probably attributable to a general derangement of metabolism, rather than to specific effects of the metabolizable ions themselves, as discussed above.

**Concluding Remarks**

The results from this and other studies (8, 9, 11, 12) indicate that in photosynthetic organisms the magnitudes of ion fluxes in both directions across the plasmalemma are correlated with light intensity. The nature of the coordination of these fluxes with metab-
The electrochemical potential transport up in response to passive fluxes. However, the magnitude of Na influx in Nitella cannot be explained by changes in the electrochemical potential difference across the plasmalemma, since this was found to remain constant despite changes in light intensity. If Na influx is to be explained on a passive basis, changes in permeability in response to light must occur. Measurements of the electrical resistance of the plasmalemma at different light intensities in the presence and absence of bicarbonate may help clarify this.

Summary

Na\(^{2+}\) influx, membrane electrical potentials, vacuolar ion concentrations, and protoplasmic streaming were measured in nongrowing cells of *Nitella clavata* at several light intensities. Na influx and protoplasmic streaming rates were followed until constant; this required from several days to several weeks depending on the process and the light intensity. Na influx was strongly correlated with light intensity although light had no effect on the membrane potential or the ionic composition of the vacuolar sap. The results do not distinguish between a passive movement of Na into the cell accelerated by a light-induced increase in permeability and an active transport mechanism operative in the same direction as the electrochemical gradient. A faster entry of Na\(^{2+}\) into cells occurred at the higher light intensities when bicarbonate was present in the medium.

Protoplasmic streaming showed a dependence on light only at intensities below 700 ergs/cm\(^2\) sec. At the lowest light intensities streaming rates were always at least 60% of the maximum rate. There was no influence of the ionic composition of the external solution on streaming.

Acknowledgments

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Literature Cited