Chapter 2

Use of *Nitella* in Biology Laboratory Classrooms

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Introduction

The internodal cells of the characean genera *Nitella* and *Chara* serve as excellent materials for simple demonstrations in biology laboratory classrooms. They can also be used for more detailed exercises in advanced biology courses. Because of their large size (several cm long and approaching one mm in diameter) these algal cells can be readily isolated from the intact plant, which resembles the common horsetail in its general form. Two of the striking features of internodal cells are (a) their ability to secrete acid and base in alternating bands along the cell length, and (b) the very smooth and rapid protoplasmic streaming. The basic procedures for setting up these demonstrations are described below, along with a method which has recently been developed for culturing *Nitella*. *Nitella* and *Chara* can also be collected from local ponds and lakes. In most species of *Chara* the internodal cells are corticated, i.e., have a jacket of small cylindrical cells surrounding them, and therefore are less suitable for these demonstrations.

If a reliable source of *Nitella* is desired, it is best to culture it in the laboratory since the material obtained from biological supply houses does not ship very well. The time invested in culturing will greatly exceed the one or two hours required to set up the above demonstrations. There are, however, some "fringe benefits" which derive from this effort: the open *Nitella* cultures often contain protozoans, small crustaceans, midge fly larvae, small flatworms, and snails. Harmony seems to prevail until the snails grow beyond a certain size and begin devouring the *Nitella*. These can be selectively removed.

Our original stock of *Nitella clavata* was collected in Llano Co., Texas by Dr. Vernon Proctor of Texas Technological College; we thank him for this.

The time required for a student to observe these demonstrations is very short, ordinarily not exceeding a few minutes. However, there is room for much flexibility here, depending on how intensively the phenomena are to be studied. Adequate discussions of acid-base formation and protoplasmic streaming are certainly not going to be easy for the instructor, but the simple beauty of these processes may entice the students into an interest in the underlying complexity. Some guidelines are provided in the annotated bibliography below.

Culturing of *Nitella clavata*

The plants can be propagated vegetatively in open cultures with aeration under cool-white fluorescent illumination of about 500 foot-candles (surface of culture about 50 cm below two 40-watt tubes). A 16-hour photoperiod is suitable. Growth occurs within the temperature range 10–30°C; above 30°C dying off may occur. Ordinary fish tanks can be used as containers. In nature *Nitella* and *Chara* are rooted at the base via rhizoids, but with our culturing

method there is no soil present and no rhizoids develop. Large ornate oospores typically are formed within two weeks if the medium is not renewed, presumably because of the depletion of one or more of the essential elements.

The culture solution, described below, should be renewed each week for dense cultures (average distance between strands less than 2 cm), less often for sparse ones. The plants can be exposed to air for a few minutes when the old culture solution is siphoned out during the renewal process. The solution is a modification of Forsberg's (1965) for *Chara*. Its suitability for other members of the *Characeae* has not been tested.

Culture Solution for *Nitella clavata*

Concentration	*Material*
0.4 mM	KNO_3
1.0 mM	$MgSO_4$
1.0 mM	$NaHCO_3$
1.0 mM	NaCl
1.5 mM	$CaCl_2$
10.0 μM	NaH_2PO_4
10.0 μM	Na_2MoO_4
3.7 μM	H_3BO_3
6.5 ppm	streptomycin sulfate (Sigma Chemical Co.)
1 ml per liter	1000-strength III 59 (see Appendix A)
1 ml per liter	1000-strength IV 76.5 (see Appendix B)

Notes:
1. Stock solutions of each of the materials to be added can be prepared by making them 1000 times the concentration present in the final culture and using 1 ml per liter. For further details see Appendix E.
2. The pH of the culture solution is adjusted to 7.5 ± 0.2 with 1 M HCl if necessary.
3. Faster growth occurs if 0.3 mM "Tris" buffer is present; a 0.3 M stock solution is prepared by neutralizing "Tris" base with 1 M HCl.

Harvesting and Maintenance of Isolated Cells

Nitella plants are laid horizontally in a large tray, immersed in the culture solution. An internodal cell is easily isolated from a strand by cutting away the two adjoining internodal cells and the branch cells at the nodes with a fine dissecting scissors. The isolated cells can then be transferred to a culture

dish containing 100 to 200 ml of a suitable solution. We use a minimal solution designated as Kb (see Appendix C) under the assumption that the cells will have a greater longevity if the rate of metabolism is low. The cells survive for more than a month in this solution when exposed continuously to 50 foot-candles of cool-white illumination. If the solution is renewed every 2 or 3 days, little contamination by microscopic algae occurs.

The transferring of internodal cells from one container to another should be done gently with a fine dissecting forceps, the tips of which have been covered with fine polyethylene tubing ("Intramedic," obtainable at Fisher Scientific and other companies); the tubing should extend slightly beyond the metal tips so that a soft grasp is possible.

Demonstration of External Acid and Base Formation

The formation of acid and base on the external surface of *Nitella* cells in alternating bands is promoted by light. The acid and base are readily detected through use of the pH indicator dye phenol red (Fig. 2.1). Two internodal cells are placed in a 60-mm petri dish top containing 25 ml of Kb solution plus 0.1 mM K salt of phenol red, pH 6.9 (see Appendix D). At pH 6.9 the solution color is orange; at lower pH it is yellow and at higher pH it is a deep red.

The recommended lighting is 150 foot-candles of cool-white; this is based on Lucas's (1975) finding that an intensity of 5 Wm^{-2} was saturating for OH^- efflux in *Chara corallina*. After 5 or 10 minutes of bright light the acid and alkaline banding pattern should be quite evident. The solution can then be gently stirred to once again render it uniformly orange, and the process may be repeated. A surge of acid formation generally occurs shortly after the cells are placed in darkness. White paper beneath the dish provides a suitable background.

The OH^- and H^+ effluxes observed in this way are large fluxes related to the general regulation of ionic traffic through the membrane (see the annotated bibliography). In nature calcium carbonate often precipitates on the cell surface in the alkaline zones; the $CaCO_3$ bands are called incrustations.

Demonstration of Protoplasmic Streaming

One or two internodal cells are placed in a 60-cm petri dish bottom with enough Kb solution to cover them. The dish is placed on the stage of a monocular microscope and the cells observed with the 10X objective. An alternative is to use a greater depth of solution and immerse the objective (a 20X objective is also suitable). The microscope should be focussed on the

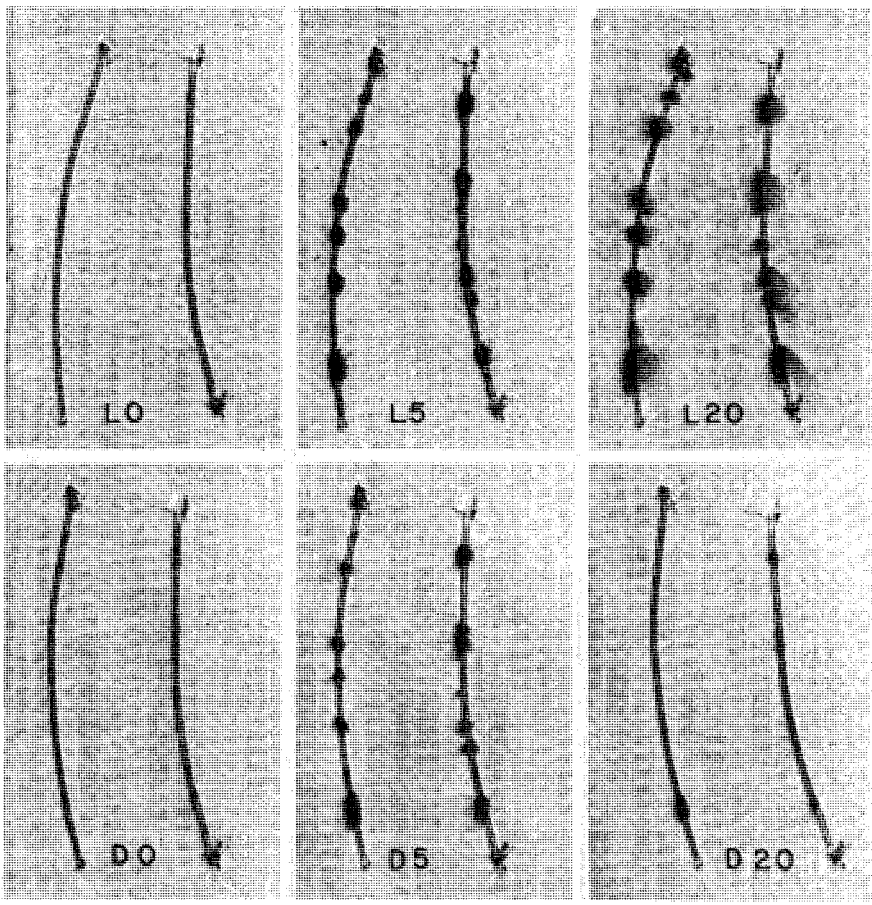

Figure 2.1. Accumulation of base (dark areas) and acid (light areas) on the external surface of two internodal cells of *Nitella clavata*. Upper sequence: cumulative amounts of base and acid produced in light after zero, 5, and 20 minutes following 20 minutes of dark pretreatment. Lower sequence: decreasing rates of base and acid formation in darkness immediately following the 20-minute exposure to light above; each picture shows the amounts of base and acid formed during the 5-minute interval preceding the picture. The pH indicator dye phenol red was used for detection of base and acid (see text for solution composition and lighting). Reproduced by permission of the *Journal of General Physiology*.

stationary chloroplast layer; the streaming endoplasm is just beneath this and is still within the focal depth. The smooth movement of particles and loose chloroplasts in the endoplasm can easily be seen. The streaming endoplasm is in the form of a continuous band which proceeds along the length of the cell on one side and doubles back on the other.

To demonstrate that either metabolism or a viscosity change, or both, are involved in the slowing down of the streaming rate upon cooling, a small piece of ice may be added to the dish. The temperature dependence is quite pronounced. It is possible to measure the velocity of streaming if a calibrated eyepiece micrometer and a stopwatch are available.

A sudden cessation of streaming usually means that the cell has received a mechanical shock strong enough to trigger an action potential. At room temperature, about 10 minutes may be required before the normal rate of streaming resumes following an action potential.

Use of *Nitella* in Membrane Physiology Exercises

Only brief mention will be made of two types of exercises for which internodal cells are especially suited, because of their large size. These are (a) measurement of resting membrane potentials and action potentials, with intracellular microelectrodes; (b) measurement of ion fluxes by means of radioactive tracers. Such exercises are quite complex both theoretically and in their practical details and require specialized equipment. It is essential for the instructor to have acquired a good working knowledge of the methodology and to have tested out all the practical aspects of the measurements. A summer spent with an expert in this discipline is highly recommended. These exercises should be limited to students with adequate backgrounds in physics and chemistry.

References

A. General

Hope, A.B.; Walker, N.A. The physiology of giant algal cells. London: Cambridge University Press; 1975.

This book concentrates on the biophysical aspects of giant cells, especially *Chara* and *Nitella*. Most of the coverage is devoted to membrane phenomena including acid and base formation. There is a chapter on protoplasmic streaming.

B. Acid-Base Formation

Lucas, W.J. The influence of light intensity on the activation and operation of the hydroxyl efflux system of *Chara corallina*. J. Exp. Botany 26:347–360; 1975.

The light-saturation of the OH^- efflux occurs at about 5 Wm^{-2}, equivalent to about 150 foot-candles of cool-white fluorescent illumination.

Lucas, W.J. Plasmalemma transport of HCO_3^- and OH^- in *Chara corallina*: non-antiporter systems. J. Exp. Botany 27: 19–31; 1976.

This work indicates that although there is a 1:1 stoichiometry of OH^- efflux and HCO_3^- influx, the two transport processes can occur in different regions of the cell surface. Thus, a common exchange mechanism is not responsible for the observed ratio. This suggests that other anions may also be absorbed via their own mechanisms.

Ryan, T.E.; Barr, C.E.; Zorn, J.P. Potassium transference in *Nitella*. J. Gen. Physiol. 72:203–218; 1978.

The possible relationship of K^+ uptake to H^+ extrusion is discussed. This appears to be more complex than the simple 1:1 stoichiometry observed between HCO_3^- uptake and OH^- efflux. Taken together with Lucas's work, the versatility of characean cells in taking up essential cations and anions as made possible by the equivalent release of H^+ and OH^-, respectively, is indicated. This is a concept that is currently the subject of active investigation.

Spear, D.J.; Barr, J.K.; Barr, C.E. Localization of hydrogen ion and chloride ion fluxes in *Nitella*. J. Gen. Physiol. 54:397–414; 1969.

This is the initial work on acid-base formation by characean cells and contains black and white photographs demonstrating the process in light.

C. Protoplasmic Streaming

Kamiya, N. Physics and chemistry of protoplasmic streaming. Ann. Rev. Plant Physiol. 11:323–340; 1960.

This is a comprehensive review containing information on the absolute value of the motive force of streaming, and discussion of the possible mechanisms involved in its generation.

Nagai, R.; Hayama, T. Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. J. Cell Sci. 36:121–136; 1979.

This report brings up to date the current ideas on the mechanism of protoplasmic streaming, especially in terms of an actin-myosin type of interaction. The roles of ATP and Ca^{++} are discussed.

D. Culturing of *Nitella* and *Chara*

Forsberg, C. Nutritional studies of *Chara* in axenic cultures. Physiol. Plant. 18:275–290; 1965.

Nitella clavata will not grow in the Forsberg medium but will grow in the modified version of it described in this article.

APPENDIX A
1000-Strength III 59

To make one liter of the above solution:

1. To 600 ml distilled water in a liter beaker add 1.89 g $FeCl_3 \cdot 6H_2O$ and 19.1 g nitrilotriacetic acid. While stirring magnetically, add enough 1.0 M NaOH to bring the pH to 7.0 after all materials have been dissolved. About 300 ml of NaOH solution are required.
2. Add to the above 50 ml of a solution made by dissolving 8.80 g $ZnSO_4 \cdot 7H_2O$, 0.142 g $MnCl_2 \cdot 4H_2O$, 0.162 g $CoCl_2 \cdot 6H_2O$ and 0.198 g $CuSO_4$ (anhydrous) in one liter of distilled water. Adjust the pH of this solution to 4.5 with HCl.

3. Adjust the final pH to 7.0 with the NaOH solution.
4. Bring the volume to 1.0 liter. Keep refrigerated.

APPENDIX B
1000-Strength IV 76.5

To make one liter of the above solution:

1. Add to 400 ml distilled water in a liter beaker 4.78 g nitrilotriacetic acid and 5.0 g humic acid (Aldrich Ghemical Co., Cat. No. 1675–2).
2. While the above is stirring magnetically, add enough 1.0 M NaOH to bring the pH to 9.3. About 75 ml of the NaOH solution are required.
3. Boil for one hour with magnetic stirring. A watch glass is used to cover the beaker to prevent excessive evaporation.
4. Allow to cool overnight.
5. Centrifuge to remove the particulate matter. Because of the large volume, 50-ml plastic centrifuge tubes are recommended with exactly 40 ml placed in each tube.
6. Collect all of the supernatant and bring the volume to 1.0 liter. The final pH is about 8.9. Refrigerate.

APPENDIX C
Kb Solution

Concentration	*Material*
1.0 mM	KCl
0.1 mM	NaCl
0.1 mM	$CaCl_2$
0.1 mM	$MgCl_2$
0.1 mM	$KHCO_3$

This solution is most conveniently made from two stock solutions, each 100-strength:

(a) a solution containing 100 mM KCl plus 10 mM each of NaCl, $CaCl_2$, and $MgCl_2$.
(b) a 10 mM $KHCO_3$ solution.

Add 10 ml of (a) and 10 ml of (b) to 980 ml distilled H_2O. Adjust to pH 6.0 with 1 mM HCl.

APPENDIX D
Stock Solution of Phenol Red

A 100-strength stock solution of the potassium salt of phenol red, pH 6.9, is made by neutralizing 1 millimole of phenol red (0.354 g) in 80 ml H_2O with KOH. If 0.1 M KOH is used, the volume required to bring the pH to 6.9 is less than 10 ml. The final volume is adjusted to 100 ml.

For the acid-base exercise, 1.0 ml of this stock solution is added to 100 ml of Kb solution.

APPENDIX E

1000-Strength Stock Solutions Used in Preparing Culture Solution
(Each solution made separately and kept in its own container)

Concentration of Stock Solution	*Material*	*Grams per Liter*
0.4 M	KNO_3	40.4
1.0 M	$MgSO_4$(anhydr.)	120.4
1.0 M	$NaHCO_3$(anhydr.)	84.0
1.0 M	NaCl	58.4
1.5 M	$CaCl_2 \cdot 2H_2O$	220.5
0.01 M	$NaH_2PO_4 \cdot H_2O$	1.38
0.01 M	$Na_2MoO_4 \cdot 2H_2O$	2.42
0.0037 M	H_3BO_3	0.23
6500 ppm	streptomycin sulfate (Sigma Chemical Co.)	6.5

Note: Add 1 ml of each of the above solutions to 990 ml of distilled water to make one liter of culture solution. Besides these 1 ml each of the 1000-strength III 59 and IV 76.5 stock solutions are to be added (See Appendices A and B for the preparation of these).