PLASMODESMA IN *NITELLA TRANSLUCENS*: STRUCTURE AND ELECTRICAL RESISTANCE

R. M. SPANSWICK AND J. W. F. COSTERTON•
Botany School, University of Cambridge

SUMMARY

Electrical resistance measurements, made with intracellular microelectrodes on a system consisting of two giant internodal cells of *Nitella translucens* joined by a node, show that the specific resistance of the node is 50 times smaller than the specific resistance of the internodal cell plasmalemma. The node is a complex structure of small cells which separate the internodal cells by a single layer at the nearest point of approach. Information on the size and distribution of the plasmodesmata in the node, obtained from electron micrographs, suggests that the specific resistance of the node should be smaller than the observed value by a factor of 330. It must therefore be assumed that there is some restriction on the diffusion of ions in the plasmodesmata.

The electrical coupling between some internodal cells is sufficient for an action potential in one cell to initiate an action potential in the neighbouring cell.

INTRODUCTION

Although much attention has been paid to the transport of ions in phloem and xylem tissues, little consideration has been given to the pathway followed by ions in their passage through other plant tissues. The function of intercellular transport is often attributed to the plasmodesmata but, as Voeller (1964) has pointed out, this is usually done without the support of direct physiological evidence. However, Voeller appears to have overlooked the work of Arisz (Arisz & Schreuder, 1956; Arisz, 1958), who removed the vascular bundles from short lengths of leaves of *Vallisneria spiralis* and obtained transport of chloride through the remaining 'parenchyma bridges'. Since, after a suitable recovery period, no chloride leaks to the external solution, transport must take place via the plasmodesmata.

Further evidence for intercellular transport, uncomplicated by the presence of phloem tissue, has been obtained using the Characeae. Littlefield & Forsberg (1965) have demonstrated a direct intercellular transport of radioactive phosphate in *Chara globularis*, thus confirming the work of Bierberg (1909) who performed similar experiments using dyes. Lou (1955), using external electrodes, showed that the nodal region of *Nitella* possesses a lower resistance than would be expected in the absence of plasmodesmata, and Sibaoka (1966) has also shown that the node has a low resistance.

Problems involving intercellular connexions in animal tissues have been investigated by Loewenstein & Kanno (1964) using intracellular microelectrodes. In this paper we...
describe the application of their technique to adjacent internodal cells of *Nitella translucens* and present electron micrographs showing the structure of the node and the distribution of plasmodesmata in the cell walls of its constituent cells.

**METHODS**

*Electrical measurements*

Two adjacent internodal cells of *Nitella translucens*, each about 3 cm long, were separated from a strand and the leaf cells were removed from the node. The lengths and diameters were measured and the cells were placed in a bath irrigated with artificial pond water containing 0.1 mM KCl, 1.0 mM NaCl, and 0.1 mM CaCl₂. A binocular microscope on an adjustable stand gave magnifications of 24 x to 200 x and provision was made for the insertion of four microelectrodes of the type used previously (Spanswick & Williams, 1964). The arrangement of the electrodes is shown in Fig. 1. Current microelectrodes, I, were inserted at the centre of both internodal cells and a square pulse of current could be passed between either electrode and a

![Fig. 1](image)

Fig. 1. Initial arrangement of the current microelectrodes, I, and the potential microelectrodes, E₁ and E₂, with respect to the internodal cells A and B and the external current electrode, I₄. The external reference electrode is not shown.

Ag/AgCl wire in the external solution. The membrane p.d.'s were measured between microelectrodes E₁ and E₂, and a reference electrode in the external solution. The p.d.'s were fed into two high-input impedance differential amplifiers and displayed on a Tektronix 502A dual-beam oscilloscope. Measurements of the changes in p.d. during the passage of a current were taken from photographs of the oscilloscope display. Corrections for the small changes in p.d. due to the passage of current through the dilute external solution were made by subtracting the changes in p.d. recorded with E₁ and E₂ just outside the cells at the points of insertion. The current (usually 10⁻⁷ A) was measured by using the oscilloscope to record the change in p.d. across a 10⁴ Ω resistor placed in the current microelectrode circuit.

A simplified equivalent circuit for the experimental system is given in Fig. 2. It is assumed that, because of their small surface area, a negligible proportion of the current passes directly between the nodal cells and the external solution. This assumption was checked by measuring the small p.d. change in the external solution produced by the passage of a current pulse. The p.d. change at a given point is proportional to the amount of current leaving the cell at that point. If a large amount is leaving through the node the p.d. change should pass through a maximum as the external electrode moves across the node. There was, in fact, a smooth transition at
the node with no maximum and therefore the assumption seems justified. It also suggests that when the leaf cells are removed the plasmodesmata connecting them to the nodal cells seal over. In theory, all three resistances in the network may be determined by measuring the changes in p.d. produced by passing a measured pulse of current between each internodal cell and the external solution (see Appendix). In practice, the measurements are complicated by (a) the resistance of the vacuolar sap, and (b) the tonoplast resistances.

The resistance of the vacuolar sap causes attenuation of the p.d. change with distance from the current microelectrode. Dr J. Hogg (personal communication) has shown that if the p.d. measurement is made at a distance $0.42l$ from the current electrode, where $l$ is the half length of the cell, it may be used in calculating the membrane resistance without significant error. The p.d. electrodes were therefore inserted at a distance $0.42l$ from the current electrodes and hence at a distance $0.29 \times 2l$ from the node. The amount of attenuation in these experiments is small and therefore the p.d. change measured at this distance from the node in the cell adjacent to the one

![Fig. 2. Equivalent circuit of the experimental system. The points A and B represent the cytoplasm of cells A and B, and O the external solution; $r_A$ is the resistance of the plasmalemma of cell A, $r_B$ is the resistance of the plasmalemma of cell B, and $r_N$ is the resistance of the node.](image-url)

into which current is being injected, is very close to the value at a distance $0.42 \times 2l$ where it should theoretically be measured. It is therefore justifiable to use the values of the p.d. changes measured at points a distance $0.42l$ from the current electrodes for calculating the resistances of the two cells from equations (3) and (4) (see Appendix). In calculating the resistance of the node, however, the change in p.d. across the node must be used. This will be equal to the difference in the measured p.d. changes only if there is no attenuation between the p.d. electrodes and the node. Additional measurements were made to determine the magnitude of the attenuation and showed that in most cells it was negligible and in no case was it greater than 10%.

Corrections must be made for the tonoplast resistances because the p.d. measurements were made with the microelectrode tips in the vacuoles. Ideally, the measurements should be made with the p.d. microelectrode tips in the cytoplasm but it is very difficult to do this before the initiation of the sealing process described by Walker (1955). Measurements were therefore made about 3 h after the insertion of the p.d. microelectrodes into the vacuoles and later corrected for the tonoplast resistances by separate measurements of the p.d. changes across the tonoplasts.
Since the equations in the Appendix assume a linear voltage/current relationship, error could arise from non-linearity, especially as the p.d. changes in the two cells were commonly as different as 20 mV and 4 mV. In the present work hyperpolarizing currents were used and, as may be seen from the example in Fig. 3, the relationship is accurately linear.

**Electron microscopy**

The nodes and adjoining internodal cells were fixed and embedded for electron microscopy by several different methods. Some cells were fixed in unbuffered 2% potassium permanganate at 0°C for 2 h or in unbuffered 2% osmium tetroxide at room temperature for 2 h, washed, dehydrated in graded ethanol solutions, infiltrated with propylene oxide, and embedded in Araldite (Glauert, 1962). Others were fixed for 60–90 min at room temperature in phosphate-buffered 5% glutaraldehyde, at pH 6.6–7.0 and tonicity 0.3–0.5 M. Following fixation in the glutaraldehyde solution, the cells were washed 5 times in phosphate buffer made up with sucrose to the same molarity as the fixative. The cells were then post-fixed in 2% osmium tetroxide in phosphate buffer, at the same molarity and pH as the glutaraldehyde fixative solution, at room temperature for 1 h. They were then washed a further 5 times, dehydrated, infiltrated, and embedded as described above.

Thin sections were cut using an L.K.B. Ultratome. In studying the structure of the node, serial sections were cut and drawings were made of the pattern of cell walls, which were clearly visible when the sections were viewed in reflected light. Once the pattern of cell walls at a given level was established, thick sections were taken until the pattern changed significantly, and then thin (approximately 600 Å) sections were again cut and sketched. Longitudinal sections through the node were also cut and examined by both light and electron microscopy. In this way we were able to establish the spatial relationships of the cells in the node, and to correlate this information with their fine structure.
Sections for electron microscopy were collected on uncoated grids, and stained with both uranyl acetate (Huxley & Zubay, 1961) and lead citrate (Reynolds, 1963). The stained sections were then supported by a thin film of evaporated carbon, and examined and photographed using an AEI EM6 electron microscope.

RESULTS

Node, plasmalemma, and tonoplast resistances

When the microelectrodes had been in the positions shown in Fig. 1 for about 3 h a current pulse was passed from the current electrode in cell A to the current electrode in the external solution (A → O) and the changes in p.d. recorded. The p.d. measurements were repeated for a pulse passed between cell B and the external solution (B → O). The p.d. electrode in cell A was then removed, inserted in the cytoplasm of cell B, and current pulses were again injected into the cells. The p.d. changes thus recorded across the tonoplast were subtracted from the corresponding p.d. changes previously recorded by the electrode in the vacuole of cell B. This gave the p.d. changes across the plasmalemma (V_B and V_a) for insertion in equations (3) and (4).

The microelectrode was then removed from the cytoplasm and inserted in the vacuole of cell B near the node. About 1 h later the p.d. measurements were repeated and any attenuation of the p.d. changes between the electrodes was used to correct the change in p.d. across the node (V_N), which is equal to (V_A - V_b) or (V_B - V_a) if the attenuation is zero.

Finally the p.d. electrode at the node was reinserted in its original position in cell A and a corresponding set of measurements of tonoplast resistance and attenuation in cell A was made with the p.d. electrode from cell B.

The specific resistances for 9 pairs of cells, calculated using the equations in the Appendix, are given in Table 1. The two values of the nodal resistance correspond to

<table>
<thead>
<tr>
<th>Experiment</th>
<th>R_A</th>
<th>R_B</th>
<th>R_N, current A → O</th>
<th>R_N, current B → O</th>
<th>R_T^A</th>
<th>R_T^B</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>68</td>
<td>60</td>
<td>2.3</td>
<td>3.9</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>23</td>
<td>131</td>
<td>86</td>
<td>0.8</td>
<td>0.8</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>64</td>
<td>57</td>
<td>1.9</td>
<td>2.6</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>25</td>
<td>77</td>
<td>134</td>
<td>1.7</td>
<td>1.2</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>26</td>
<td>193</td>
<td>103</td>
<td>1.5</td>
<td>1.3</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>43</td>
<td>38</td>
<td>1.7</td>
<td>1.6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>28</td>
<td>117</td>
<td>67</td>
<td>2.6</td>
<td>2.3</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>29</td>
<td>62</td>
<td>58</td>
<td>1.4</td>
<td>1.3</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>30</td>
<td>134</td>
<td>104</td>
<td>0.9</td>
<td>0.9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>99</td>
<td>79</td>
<td>1.6 (4)</td>
<td>1.7 (6)</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

a Tonoplast resistance in cell B assumed to be equal to that in cell A.
equations (5) and (6). Cell B was usually smaller, and probably younger, than cell A, but the difference between the mean nodal resistances for currents $A \to O$ and $B \to O$ is not significant. The differences in the individual experiments probably give an indication of the cumulative experimental error rather than evidence for rectification.

**Transmission of the action potential**

In some pairs of cells an action potential in one internodal cell would occasionally trigger off an action potential in the neighbouring internodal cell. Examples of both possibilities in the same pair of cells are given in Fig. 5. This phenomenon has previously been observed by Drs J. Hogg & R. J. Johnston (personal communication), and Sibaoka (1966) has recently published similar observations.

**Table 2. Thicknesses of the different cell walls within the node of**

*N. translucens* **at various stages of maturity**

<table>
<thead>
<tr>
<th>Type of node</th>
<th>Type of wall</th>
<th>Exposed wall of upper internodal cell</th>
<th>Internodal/nodal cell wall</th>
<th>Nodal/nodal cell wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature node (upper internodal cell &lt; 1 cm long)</td>
<td>Range of widths (μm)</td>
<td>0.60—0.83</td>
<td>0.22—0.29</td>
<td>0.07—0.11</td>
</tr>
<tr>
<td></td>
<td>Mean width (μm)</td>
<td>0.72</td>
<td>0.24</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>No. of measurements</td>
<td>5</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Developing node (upper internodal cell 1—3 cm long)</td>
<td>Range of widths (μm)</td>
<td>1.0—3.0</td>
<td>None</td>
<td>0.13—0.42</td>
</tr>
<tr>
<td></td>
<td>Mean width (μm)</td>
<td>1.71</td>
<td>measured</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>No. of measurements</td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mature node (upper internodal cell &gt; 3 cm long)</td>
<td>Range of widths (μm)</td>
<td>6.94—8.32</td>
<td>1.18—2.50</td>
<td>0.37—0.92</td>
</tr>
<tr>
<td></td>
<td>Mean width (μm)</td>
<td>7.27</td>
<td>1.66</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>No. of measurements</td>
<td>6</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

**The histology of the node**

The node, essentially, forms a set of sockets which accommodate the internodal and leaf cells. It allows the two opposed internodal cells to come within one nodal-cell width of each other, and allows the leaf cells to come within one nodal-cell width of the upper internodal cell (Fig. 6). The walls of a *Nitella* cell vary in thickness with the age of the cell and with its position in the node. The outer walls of the outer cells of the node are thick, as are the exposed walls of the internodal and leaf cells (Figs. 6, 14). The unexposed walls of the internodal and leaf cells (the internodal/nodal cell wall, and the leaf cell/nodal cell wall) are thinner (Figs. 6, 11), and the walls of the cells within the node (the nodal/nodal cell walls) are very much thinner (Figs. 1, 6—8, 14). The data in Table 2 illustrate how the cell-wall thicknesses increase as the node grows, but their relative thicknesses remain roughly constant. The nodes used in measurements of electrical resistance were of the mature type.
Location of the plasmodesmata

Plasmodesmata do not, of course, occur in the outside walls of the nodal cells (Fig. 14), or in the exposed walls of the internodal or leaf cells, but they do penetrate almost all of the unexposed walls of the node. Specifically, they are found in very large numbers in the walls between nodal cells (Figs. 7-10), and in the walls between the internodal and nodal cells (Figs. 10, 11) from a few microns inside the point at which the internodal cells become enclosed in the socket provided by the node. In these respects, and in all others covered in this work, the walls between the leaf cells and the nodal cells were identical with those between the internodal and nodal cells.

Structure of the plasmodesmata

The plasmodesma is a membrane-enclosed cytoplasmic connexion between two adjacent cells which traverses an aperture in the intervening cell wall. In some preparations it is readily seen that the plasmodesmata do not completely fill the aperture in the cell wall but appear to be constricted due to plasmolysis and the attendant separation of their cytoplasmic origins (Figs. 7, 9-11). In embeddings fixed in unbuffered osmium tetroxide and in buffered glutaraldehyde at 0.3 M, there was less plasmolysis and the cytoplasmic connexions were seen more nearly to fill the apertures in the walls (Fig. 12). Therefore we believe that, in the living cell, the cytoplasmic connexion fills the available space in the aperture, and, for this reason, the effective diameter of the actual connexion between the cells was calculated to be the diameter of the aperture minus twice the thickness of the membrane. The diameters of the plasmodesmata were measured in a large number of different micrographs, in sections cut at various planes, and the averages of these values are given in Table 3. These values show a remarkable uniformity in the dimensions of the openings of the plasmodesmata in different types of walls, and in nodes of varying maturity. As the node develops, and its cell walls grow, the dimensions of the openings of the plasmodesmata remain constant.

The membrane of the plasmodesma can be seen to be derived from the plasmalemma of one cell, and to merge with the plasmalemma of the connected cell (Figs. 7, 9). Elements of the endoplasmic reticulum are found in the cytoplasm near the origins of the plasmodesmata (Figs. 12, 16) but they are only rarely seen to be associated with them. In cross-section we occasionally see membrane systems within the plasmodesmata (Fig. 13) similar to those described by López-Sáez, Giménez-Martin & Risueño (1966), but this is not a regular structural arrangement in our material. Microtubules (Figs. 7, 12) are also seen in cross-section in the areas from which the plasmodesmata originate but there is no demonstrable association between them. A complex structure was found at the origin of some plasmodesmata (Fig. 7) in only one preparation whose overall preservation was unsatisfactory and we consider it to be an artifact.

As will be seen in Figs. 7–9 the plasmodesmata always traverse the walls between nodal cells in straight apertures of uniform diameter. In contrast, the apertures through which the plasmodesmata penetrate the walls between nodal and internodal
Table 3. The diameters of the lumens of the plasmodesmata of different walls of N. trans- 
lucent, at various stages of maturation, as derived from the diameters of the apertures 
in the cell walls

<table>
<thead>
<tr>
<th>Type of node</th>
<th>Type of wall</th>
<th>Type of section</th>
<th>No. of measurements</th>
<th>Average diameter (Å)</th>
<th>Corrected† diameter (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature node (upper</td>
<td>Wall between inter-</td>
<td>Cross-section</td>
<td>10</td>
<td>960</td>
<td>800</td>
</tr>
<tr>
<td>internodal cell</td>
<td>nodal and nodal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 cm long</td>
<td></td>
<td>Cross-section</td>
<td>9</td>
<td>1020</td>
<td>860</td>
</tr>
<tr>
<td>Overall average</td>
<td>Wall between nodal</td>
<td>Cross-section</td>
<td>26</td>
<td>1210</td>
<td>1050</td>
</tr>
<tr>
<td>(s.d. = 170 Å)</td>
<td>cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall average</td>
<td>Wall between nodal</td>
<td>Cross-section</td>
<td>37</td>
<td>670</td>
<td>510</td>
</tr>
<tr>
<td>(s.d. = 58 Å)</td>
<td>cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developing node (upper</td>
<td>Wall between nodal</td>
<td>Cross-section</td>
<td>16</td>
<td>750</td>
<td>590</td>
</tr>
<tr>
<td>internodal cell</td>
<td>nodal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 cm long</td>
<td>(s.d. = 121 Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature node (upper</td>
<td>Wall between inter-</td>
<td>Longitudinal</td>
<td>13</td>
<td>690</td>
<td>530</td>
</tr>
<tr>
<td>internodal cell</td>
<td>nodal and nodal cells</td>
<td>section</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3 cm long</td>
<td></td>
<td>Oblique section</td>
<td>12</td>
<td>730</td>
<td>570</td>
</tr>
<tr>
<td>Overall average</td>
<td>Wall between nodal</td>
<td>Cross-section</td>
<td>4</td>
<td>710</td>
<td>550</td>
</tr>
<tr>
<td>(s.d. = 57 Å)</td>
<td>cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall average</td>
<td>Oblique section</td>
<td></td>
<td>3</td>
<td>780</td>
<td>620</td>
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<tr>
<td>(s.d. = 47 Å)</td>
<td>Longitudinal section</td>
<td></td>
<td>21</td>
<td>620</td>
<td>460</td>
</tr>
<tr>
<td></td>
<td>Longitudinal section</td>
<td></td>
<td>19</td>
<td>730</td>
<td>570</td>
</tr>
</tbody>
</table>

† This value is arrived at by subtracting twice the thickness of the membrane of the plasmo-
desma (80 Å) from the diameter of the aperture to give the actual diameter of the lumen of 
the structure.

Fig. 11 illustrates the structure of the plasmodesmata in the wall between the internodal 
and nodal cells of the immature node, and it will be seen that the aperture widens apprecia-
tively just under its narrow surface opening, making the measurement of the outside opening difficult, 
especially in cross-section. For this reason the measurements obtained may be artificially high.

Cells, and those between nodal and leaf cells, are complex and anastomosing, and 
have characteristic median sinuses. In immature nodes these sinuses are simple and 
individual, but in mature nodes, they coalesce to produce very large cavities in the 
median area of the cell wall (Fig. 11). It is interesting to note that the diameters of the 
openings of these plasmodesmata are uniform, and remain constant as the node 
matures. The morphology of the plasmodesmata is illustrated in Fig. 4. When cal-
culating the extent of physical communication between cells we used the diameter of 
the opening of the aperture of the plasmodesma and ignored the central anastomoses 
and sinuses.
The extent of physical communication between cells in the node

Some of the sections obtained from this material gave a very large number of cross-sectional profiles of plasmodesmata (Figs. 10, 12, 13). In sections whose plane was not exactly at right angles to the axis of the aperture in the cell wall the smallest dimension of the plasmodesma was used, since this more nearly represents its true diameter. The percentage of the total surface area of the cell wall comprised by the openings of the plasmodesmata is tabulated in Table 4.

Fig. 4. The morphology of the plasmodesmata.

DISCUSSION

The resistance measurements (Table 2) make it clear that the specific resistance of the node is about fifty times smaller than that of the internodal cell plasmalemma. The obvious interpretation of this fact is that the plasmodesmata observed in the electron micrographs provide a direct pathway for the diffusion of ions between the internodal cells. This takes place via the nodal cells, since the systematic sectioning of the node has shown that the internodal cells are separated from each other, at the nearest point of approach, by a single layer of nodal cells. The leaf cells are, similarly, separated
from the internodal cells by a single layer of nodal cells. We note that all of the cell walls not actually exposed to the environment are penetrated by plasmodesmata whose openings comprise as much as 14.7% of the surface of the walls which they traverse, and apparently provide such extensive physical communication between the cells as to render the organism a virtual syncytium.

Table 4. The percentage of the area of different cell walls comprised by the openings of the plasmodesmata in immature and mature nodes of N. translucens

<table>
<thead>
<tr>
<th>Type of node</th>
<th>Type of wall</th>
<th>Area surveyed (μ²)</th>
<th>No. of plasmodesmata</th>
<th>Average diameter (Å) minus 160 Å*</th>
<th>Wall area comprised by openings of plasmodesmata (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>Nodal/nodal</td>
<td>1.12</td>
<td>81</td>
<td>510</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>Internodal/nodal</td>
<td>18.17</td>
<td>124</td>
<td>800</td>
<td>3.63</td>
</tr>
<tr>
<td>Mature</td>
<td>Nodal/nodal</td>
<td>3.75†</td>
<td>17</td>
<td>400</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Internodal/nodal</td>
<td>7.25†</td>
<td>18</td>
<td>550</td>
<td>0.59</td>
</tr>
</tbody>
</table>

* In the living cell the cytoplasmic connexion wholly occupies the aperture in the wall, and the diameter of its lumen would therefore be the diameter of the aperture minus twice the thickness of the enclosing membrane (80 Å).

† These values are obtained from longitudinal and oblique sections. The area of cell-wall surface surveyed was taken as the length of the part of the wall, as measured from the photograph, times the thickness of the section (500–700 Å), with a correction for oblique sections. Since the photographs would record plasmodesmata if more than 25% of their cross-sectional diameter fell within the section, we are, in fact, sampling an area wider than the section thickness. We therefore calculate the width of the strip of cell-wall surface surveyed as being the section thickness plus two times 75% of the average diameter of the plasmodesmata.

As Porter & Machado (1960) and Frey-Wyssling, López-Sáez & Mühlethaler (1964) point out, the pattern of plasmodesma formation is established at the time of the formation of the cell plate, and it is interesting to see that, in the nodes of N. translucens, the increase in cell-wall surface area involved in the maturation of the node is accompanied by a decrease in the number of plasmodesmata per unit area of cell-wall surface. The diameters of the openings of the plasmodesmata do not increase as the cell wall grows.

In those tortuous plasmodesmata which traverse the walls between nodal and internodal cells, the plasmodesma expands to fill even the very large spaces of the median sinuses. However, for the purposes of making a quantitative comparison between the structural and electrical measurements we will assume that the plasmodesmata are cylinders 1.7 μ in length whose openings occupy 0.59% of the area of the walls between the nodal cells and the two adjoining internodal cells. We will make a further simplification and assume that the internodal cells are separated by a single layer of nodal cells over the whole area of the node. The concentrations of the major ions in the cytoplasm of N. translucens are 119 mM K+, 14 mM Na+, and 65 mM Cl− (Spanswick & Williams, 1964). The conductivity of the cytoplasm may therefore be taken to be approximately equal to that of 100 mM KCl; that is, 0.011 Ω⁻¹ cm⁻¹. On this
basis it is possible to predict that the specific resistance of the node should be $5.2 \, \Omega \, \text{cm}^2$. When this is compared with the measured value of $1700 \, \Omega \, \text{cm}^2$ it is evident that there is a discrepancy of a factor of about 330 between the measured resistance and the value predicted on the basis of structural evidence. It is unlikely that the cytoplasm of the nodal cells contributes more than $1 \, \Omega \, \text{cm}^2$ to the specific resistance of the node, and the size of the discrepancy therefore suggests that the plasmodesmata are partly blocked. Frey-Wyssling et al. (1964) and López-Sáez, Giménez-Martin & Risueño (1966) have reported that elements of the endoplasmic reticulum traverse the plasmodesmata as a central core. We have seen membrane systems within the lumen of the plasmodesma in a very small proportion of our observations, of which Fig. 13 is an example, but this is by no means a common finding in our material. However, our conclusions concerning the structure of the plasmodesma are the most 'open' possible, since its lumen is essentially empty and it is possible that part of this lumen is occupied, in life, by structures which are not obvious in this fixed and sectioned material. The presence of such structures within the lumen of the plasmodesma would more readily explain the high resistance of the node. Other structures of the peripheral cytoplasm which might occlude part of the lumen of the plasmodesma are the microtubules, seen in large numbers near the origin of the plasmodesmata, but no evidence has yet been found to substantiate this.

Until very recently the only published resistance measurements relating to plasmodesmata were those of Lou (1955). He immersed pairs of *Nitella* cells in paraffin, passed a current along the system, and used external electrodes to measure the potential distribution. Using a modified form of cable theory he calculated a 'constriction factor' for the node. No anatomical data for *Nitella* were available at the time but the constriction factor was of the same order of magnitude as that calculated from anatomical measurements on sieve tubes. Unfortunately, the relative dimensions of sieve tubes are very different from those of *Nitella* and so the agreement was merely fortuitous. In fact, it is possible to deduce from one set of his observations that the specific resistance of the node was approximately $1200 \, \Omega \, \text{cm}^2$ which is well within the range of values obtained in the present work.

Sibaoka (1966) has made a rough estimate of the nodal resistance of *Chara braunii* using a technique basically similar to the one described here. Assuming that $R_A = R_B$, and ignoring the effects of tonoplast resistance, he estimates that the specific resistance of the internodal cell is approximately $30 \, \text{k} \, \Omega \, \text{cm}^2$ while that of the node is only $0.15 \, \text{k} \, \Omega \, \text{cm}^2$. He makes no mention of the possibility that the low resistance is due to the presence of plasmodesmata.

The transmission of the action potential has no obvious function in *Nitella* but the manner in which it is transmitted may be of interest to those studying systems where it may have some function—for example, *Mimosa* (Sibaoka, 1962). The p.d. microelectrodes were placed less than 1 mm from the node and it is apparent from the time delay (Fig. 5A) that the action potential is not propagated directly between the internodal cells. We suggest that the increase in p.d. across the node associated with the first action potential causes a current to flow through the plasmodesmata and hence across the plasmalemma of the neighbouring internodal cell. If this current is of
Fig. 5. Transmission of the action potential between adjacent internodal cells.

Fig. 5A. Top trace: the stimulating pulse, $S$, is followed immediately by an action potential. Bottom trace (adjacent cell): the attenuated component of the stimulating pulse, $S_a$, is followed by the attenuated component of the action potential which triggers off a separate action potential.

Fig. 5B. Top trace as in Fig. 5A. Bottom trace: in this case the attenuated component of the action potential in the first cell did not trigger off a separate action potential in the adjacent cell.
Plasmodesmata in Nitella

sufficient strength and duration it will produce a separate action potential, otherwise it will cause only a small transient depolarization (Fig. 5B). *Nitella* is probably not, however, analogous to phloem tissue in that the small nodal cells which separate the internodal cells may be inexcitable. Sibaoka (1966) reaches a similar conclusion.

It is clear that, in spite of the restricted diffusion of ions, the plasmodesmata provide a vastly more efficient means of intercellular transport than the alternative of secretion and reabsorption with its consequent wastage of energy and materials. It seems likely therefore that plasmodesmata may be of great importance in higher plants, and one of the main objects of this work has been to make a detailed test of a method which will have sufficient spatial resolution to trace intercellular relationships and provide a test of the symplasm theory in higher plants.

APPENDIX

When a current $I_A$ is passed from A to O (Fig. 2), let the changes in p.d. across the resistances $r_A$ and $r_B$ be $V_A$ and $V_B$ respectively. Let the corresponding changes in p.d. produced by a current $I_B$ from B to O be $V_a$ and $V_B$. The current $I_A$ is equal to the sum of the currents passing through $r_A$ and $r_B$; that is:

$$I_A = (V_A/r_A) + (V_B/r_B).$$

(1)

Similarly,

$$I_B = (V_a/r_A) + (V_B/r_B).$$

(2)

Solving for $r_A$ gives

$$r_A = (V_AV_B - V_aV_b)/(I_AV_B - I_BV_B).$$

(3)

Then, using this value for $r_A$,

$$r_B = (r_A(r_B)/(I_Ar_A - V_A).$$

(4)

The change in p.d. across $r_N$, in practice corrected for attenuation, is $(V_A - V_B)$ or $(V_B - V_a)$, and therefore

$$r_N = [(V_A - V_B)/V_B]r_B,$$

(5)

or

$$r_N = [(V_B - V_a)/V_a]r_A.$$  

(6)

The specific resistances are obtained by multiplying by the area of the appropriate component of the cell.

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Fig. 6. Light micrograph of a thick longitudinal section of a node of N. translucens, fixed and embedded for electron microscopy, illustrating the histology of the node and the comparative thicknesses of the outer, o, internodal, l, and nodal/nodal, n, cell walls. (es, external space; l, leaf cell; ic, lower internodal cell; uc, upper internodal cell.)
Fig. 7. Electron micrograph of a bifurcation in an immature cell wall between nodal cells which shows plasmodesmata in both longitudinal and cross-section. We believe that the electron-transparent areas on each side of the cell wall, at the area marked a, and surrounding the plasmodesmata seen in cross-section, are formed as a result of plasmolysis and resultant stretching of the plasmodesmata. A microtubule is seen at m and there are a number of unusual structures, b, which appear to be associated with the origins of the plasmodesmata.
Figs. 8–10. The nodal/nodal cell wall. Sections of the immature wall between nodal cells showing that an extremely high proportion of the area of these walls is occupied by the plasmodesmata. Fig. 10 shows cross-sections of the plasmodesmata at high magnification.
Figs. 11–13. The internodal/nodal cell wall.

Fig. 11. Longitudinal section of a mature wall showing the lower incidence of plasmodesmata in these walls, and the complex sinuses, $c$, formed at their centres.

Fig. 12. Cross-section of an immature wall showing the high proportion of cell wall area occupied by the plasmodesmata, and the simple sinuses, $s$, at the centres of these walls. Arrows indicate where the structure of the membrane of the plasmodesmata is especially clearly seen, and $m$ indicates microtubules.

Fig. 13. Within a cross-section of a plasmodesma the presence of a membranous structure is indicated by the double arrow.
Fig. 14. A section showing an outer wall, o, and a wall dividing two nodal cells, n.

Fig. 15. A section from a preparation in which very little plasmolysis had taken place, in which it is seen that the plasmodesma very nearly fills the aperture in the cell wall (arrows). The cells were fixed in 2% KMnO₄ for 30 sec, then in 0.3 M glutaraldehyde, and post-fixed in 2% OsO₄.

Fig. 16. A section of an immature internodal/nodal cell wall showing anastomoses, d, and simple sinuses, s, formed by the plasmodesmata. Elements of the endoplasmic reticulum (er) are well-preserved in this embedding but we have failed to see any spatial association with the plasmodesmata.