FAILURE TO FIND INTERCELLULAR PROTOPLASMIC CONTINUITY IN GRIFFITHSIA GLOBULIFERA*

By HOWARD J. CURTIS AND JOHN L. MYERS

(From the Department of Physiology, Vanderbilt University School of Medicine, Nashville, and the Marine Biological Laboratory, Woods Hole)

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It has been claimed by many investigators that there is protoplasmic continuity between adjacent cells in certain botanical forms (3). Characteristic of the organisms which have been claimed to exhibit such connections is the red marine alga *Griffithsia globulifera.* Practically all adjacent cells of *Griffithsia* appear to be connected by broad cytoplasmic strands, the plasmodesmae, which traverse pit cavities in the intercellular junction. It has been proposed that this continuity is important in the transportation of food material and in conduction of stimuli (3). Other authors have suggested that the intercellular pit cavities are closed by a delicate membrane which permits an easier passage of substances from cell to cell, but that unbroken connections are not to be found (2). All such previous studies have been entirely of a morphological nature and were based on microscopic and histological findings.

Recently Curtis and Travis (1) have found positive evidence of a physiological nature indicating protoplasmic continuity between adjacent cells in the Purkinje tissue of the ox heart. The present work is an attempt to obtain direct evidence of protoplasmic continuity in a simpler system, essentially by measurement of the electrical resistance between the protoplasm in adjacent cells of *Griffithsia globulifera.* This is a red marine alga common to the Woods Hole area. Individual cells are approximately 3 mm. long and 0.5 mm. in diameter. It is an unusually favorable form in which to observe the typical intercellular strands because of their large size and simplicity (4).

Experimental Procedure

Measurements were made in a wax block chamber (Fig. 1) consisting of two electrode wells filled with sea water or isotonic KCl (0.53 M). Strands of single cells of *Griffithsia* were isolated and allowed to equilibrate in sea water for several hours before use. Only turgid, freshly collected cells were employed. The strands were placed in the measuring chamber so that they bridged the space between the wells.

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There were usually about three cell junctions between the cells dipping into the solutions. The surface of the chamber was covered with a layer of mineral oil so that the portion of the strand between the wells was immersed in oil. A trace of methylene blue was added to the solutions so that a sharp meniscus could be seen where the cells entered.

The potential difference between the two electrode wells was measured by a D. C. amplifier through silver-silver chloride electrodes. Before and after each potential measurement a zero of potential was established by shorting between the wells with a cotton wick soaked in sea water.

The position of the cells in the chamber was observed by means of a dissecting microscope fitted with a micrometer eyepiece, and manipulations were made with metal forceps. Specifically, for each potential reading, the total length of the cell was measured as well as the fraction of the cell which protruded into the KCl. In this manner it was possible to plot the potential as a function of the per cent of the cell in KCl.

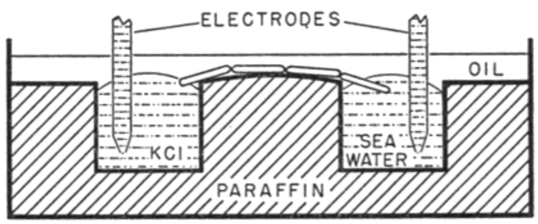

FIG. 1. Diagram of measuring chamber. A strand of single cells of *Griffithsia* is shown bridging the space between the electrode wells.

A few experiments were performed in which both wells were filled with sea water and a mechanical injury produced at the end of the cell. Again the potential was measured as a function of the per cent of the cell protruding into the solution.

RESULTS

The results of one experiment are shown in Fig. 2. It will be seen that as a given cell is pushed further into the KCl solution the potential decreases steadily until it is zero, or practically so, when the KCl meniscus is at the end of the cell. As the meniscus moves over and touches the next cell, the potential immediately increases again to its maximum value for that cell. This general pattern was followed in every experiment except that with KCl it was difficult to obtain a completely zero potential when the meniscus was just short of the cell junction. It is believed that this was a result of the diffusion of KCl a little beyond the actual meniscus. To test this, experiments were performed with sea water in both wells and with the injury produced mechanically, and it was quite clear that in this case the potential did go exactly to zero as the meniscus approached the end of the cell. It was difficult, however, to obtain as complete a curve in this case due to the tendency of the cells to collapse. After the cells had been in isotonic KCl for some minutes, their potential could be quickly restored by soaking in sea water. Maximum demarcation potentials were between 5 and 20 mv., depending on the length of the cell and the amount of sea water which adhered to it. Maximum potentials due to mechanical injury were of the same order of magnitude, except that it is difficult to produce a satisfactory mechanical injury which involves only one end of the cell.

In one experiment with sea water in both wells, a mechanical injury was produced on several cells in a line, and it was found that the individual injury

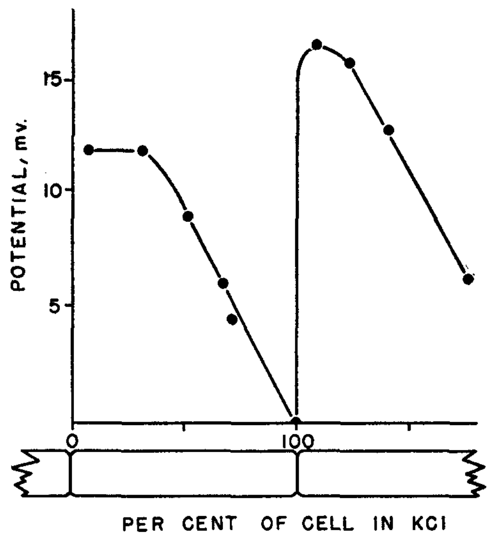

FIG. 2. Demarcation potential plotted as a function of the per cent of the cell in KCl for one experiment. A diagram of the cell is shown below the abscissa.

potentials would add to give a measured potential which was the sum of the individual potentials.

When the cells were soaked in sea water saturated with chloroform for a few seconds, no potential whatever could be measured.

DISCUSSION

The only reasonable explanation for these results would seem to be that there is a very high electrical resistance between the cytoplasm of adjacent cells in this species. If such were not the case then an adjacent cell would contribute to the injury potential so that the potential would not fall as the meniscus approaches the end of the cells. Furthermore the form of the potential *versus* length curve (Fig. 2) is about what one would expect on theoretical grounds if each cell were completely independent of its neighbor. This would strongly deny the existence of protoplasmic bridges in this species.

SUMMARY

A method was devised for estimtaing the electrical resistance between the cytoplasm of adjacent cells of the red marine alga *Griffithsia globulifera*. A very high resistance was found, which argues strongly against the existence of protoplasmic continuity in this species.

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