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# Transmembrane Potentials of Parenchyma Cells and Nematode-Induced Transfer Cells

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With 9 Figures
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## Summary

A comparison of transmembrane potential (pd) properties of parenchyma cells and giant transfer cells induced by a root-knot nematode in the roots of *Impatiens balsamina* has been made. Apart from some differences in rate of response to a few treatments, parenchyma and giant cells had similar pd values; active and passive components of the pd (cyanide, azide); responses to total ion concentration, pH and potassium concentration; responses to protein synthesis inhibitors (puromycin, cycloheximide and actinomycin D) and responses to sugars.

Both parenchyma cells and giant cells are depolarized by puromycin, cycloheximide and actinomycin D. The cells recover from the depolarization in the presence of cycloheximide, suggesting that this presumed protein synthesis inhibitor does not act in a straight-forward manner. The cells do not recover in the presence of puromycin or actinomycin D.

Parenchyma cells and giant cells clearly have different metabolic rates and ion fluxes, but their pd responses are the same. This suggests that the pd does not reflect metabolic activity or ion fluxes of a cell, but is strictly controlled in itself. Part of this control may be via a feedback mechanism acting on an electrogenic pump.

The depolarization caused by glucose is induced by aging the cells after excision. The effect is discussed in terms of an $H^+$ dependent cotransport system and an ATPase permease system.

The apparent normality of pd responses of nematode-induced giant transfer cells suggests that they may be a useful model system for experiments on higher plant cells.

## 1. Introduction

Recent electrophysiological work on the ionic relations of algal, fungal and higher plant cells provides good evidence that the transmembrane potential difference (pd) of plant cells is made up of two components, a diffusion (passive) potential and an electrogenic (active) potential (Kitasato 1968,

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SPANSWICK 1972, SLAYMAN 1965, HIGINBOTHAM, GRAVES, and DAVIS 1970). The electrogenic part of the potential may be collapsed by treatment with respiratory inhibitors such as cyanide or azide, and under dark conditions it appears that the energy required to maintain the asymmetry of ions which causes this potential is provided by ATP and is not directly linked to electron transport (SLAYMAN, LONG, and LU 1973). If the measured internal and external concentrations of the major ions and their permeability coefficients for oat coleoptile cells are inserted into the Goldman constant field equation, the predicted value of the pd resulting from diffusion alone is within experimental limits the same as the value measured after the addition of respiratory inhibitors (HIGINBOTHAM *et al.* 1970).

Which ion or ions contribute most to the electrogenic component of the pd has been indicated by a number of lines of research. In work with giant algal cells a major problem was that the sum of the conductances of $K^+$, $Na^+$, and $Cl^-$ was much too small to account for total membrane conductance. KITASATO (1968) suggested that this discrepancy was made up for by $H^+$ conductance and proposed an $H^+$ efflux pump. There is much evidence that higher plant tissues such as *Avena* coleoptiles actively secrete $H^+$ ions into the medium in response to auxin (HAGER, MENZEL, and KRAUSS 1971, CLELAND 1973). However, the pd response of higher plant cells to pH indicates that passive influxes of $H^+$ ions are not so large as in giant algal cells (HIGINBOTHAM, ETHERTON, and FOSTER 1964).

The control of cytoplasmic pH of a cell is necessary to maintain enzyme activity and metabolism. If $H^+$ ions were in passive flux equilibrium in plant cells, since the cytoplasm is up to 200 mV negative with respect to the bathing solution, from the Nernst equation the cytoplasmic pH would be up to 3.5 units more acid than the medium. Measured cytoplasmic pH values range from 6.0–7.5, and pumping of $H^+$ ions out of the cell (or $OH^-$ into the cell) against a free energy gradient is necessary (RAVEN and SMITH 1973). Assuming the plasmalemma is relatively impermeable to $H^+$ ions this would also make the internal pd more negative. RAVEN and SMITH (1974) suggested that almost half of the respiratory energy could be used in active $H^+$ extrusion in *Nitella* cells, and SLAYMAN *et al.* (1973) estimated that about 30% of the total supply of ATP of *Neurospora* is used by the postulated electrogenic $H^+$ efflux pump. This energy drain would seem more rational if the electrical gradient were used to drive the transport of other substances into the cell via cotransport carriers.

RAVEN and SMITH (1973) estimated that under certain growth conditions the products of metabolism carry a net negative charge, because cell constituents have an excess of free carboxyl groups over free amino acid groups. From this one might expect that cells with different metabolic rates would produce different numbers of $H^+$ ions, resulting in different rates of $H^+$ efflux and consequently different pd values.

From this basis, we have measured pd properties of cells with different metabolic activities—cortical parenchyma (control) and giant cells induced by root-knot nematodes (*Meloidogyne incognita*) in roots of dwarf balsam (*Impatiens balsamina*)—from two viewpoints. 1. To determine whether the pd of a higher plant cell reflects the sum of a number of metabolic activities, or is an important parameter controlled independently of general cell metabolism, 2. to increase understanding of aspects of giant cell physiology.

The structure of giant cells in a variety of host plants has been described at the light and transmission electron microscope levels (DROPKIN and NELSON 1960, JONES and DROPKIN 1974, BIRD 1961, PAULSON and WEBSTER 1970, JONES and NORTHCOTE 1972). Each nematode induces about six giant cells (each ca. 150 × 350 μm) in a complex in the center of the root. These cells have no central vacuole, are filled with cytoplasm and have wall ingrowths typical of transfer cells. The nematode feeds from these cells for one month or longer, during which time it increases in size by about 1,000 times and produces several hundred eggs. All the nutrients required by the nematode must pass through the giant cells. An important point is that mature giant cells are not connected to other cells in the root by plasmodesmata, although there are plasmodesmata between giant cells. JONES and DROPKIN (manuscript in preparation) have studied this point by scanning electron microscopy after digestion of the cell contents to reveal internal walls. Pit fields are present between giant cells and neighbouring cells 3 days after infection, but are sealed off by 6 days after infection.

Our results suggest that the pd is an important parameter controlled independently of general cell metabolism.

## 2. Materials and Methods

Seedlings of dwarf balsam (*Impatiens balsamina*), chosen as host plant since it has translucent roots, were germinated on damp paper in petri dishes until roots were 1–2 cm long. Sand (60–80 mesh) was sprinkled onto the roots, and one drop of a suspension containing 25 larvae of *M. incognita* was added to the sand over each root. The larvae were freshly collected with a mist apparatus from heavily infested tobacco plants. The roots were incubated with the larvae for 24 hours, then transplanted to vermiculite in a growth chamber, watered with Hoagland's solution twice a week and distilled water at other times. The growth chamber was maintained at 27 °C during the 14 hours day period and 23 °C at night. Root segments 1 cm long with 10–15 day old galls containing giant cells ("undissected") were mounted horizontally in a plexiglass golder. In some experiments the cortical cells of the gall were "dissected" from the root with fine forceps and an extra fine scalpel under a dissecting microscope. The next 1 cm root segment on the side of the gall nearest to the stem was used as a control. The differences between "undissected" and "dissected" giant cells are shown in Fig. 1. The swelling in the whole root (Fig. 1 *A*) is the gall, which contains a nematode and about 6 giant cells at the center. Fig. 1 *B* shows a resin-embedded 1 μm thick longitudinal section of two of the giant cells in a similar gall in coleus (JONES and NORTHCOTE 1972). In Fig. 1 *C*, the cortex has been "dissected" away to about the pericycle, and two giant cells are clearly visible. Only a few cell layers

overlay the giant cells, as shown in Fig. 1 *D*, which is a 1 µm longitudinal section of "dissected" giant cells in balsam. Giant cells in live roots can easily be identified since their contents appear granular and yellow-brown in transmitted light, and white in reflected light. Except where indicated, the segments were incubated for 16–20 hours overnight in aerated bathing solution without further handling after excision. Some aspects of the effects of excising tissue (stress and aging) will be described in another publication.

In most experiments the bathing solution was that of HIGINBOTHAM *et al.* (1964) and contained in mM: KCl, 1.0; Ca $(NO_3)_2$, 1.0; $MgSO_4$, 0.25; $NaH_2PO_4$, 0.904; $Na_2HPO_4$, 0.048; pH 5.5–5.7, designated 1 X (0.01 X, 0.1 X and 10.0 X solutions are 0.01, 0.1, and 10.0 times the standard concentration). This was modified by addition of 1 mM HCl and adjusted to pH 7.0 with Tris (1 X, 2 mM $Cl^-$, pH 7.0). In experiments with cyanide ($CN^-$), KCl was replaced by KCN, 2 mM HCl was added to keep $K^+$ and $Cl^-$ concentrations constant, and the pH was adjusted to 7.0. In experiments with azide ($N_3^-$), 1 mM sodium azide was added to 1 X, 2 mM $Cl^-$, pH 7.0. In experiments with pH changes, 1 X solution was adjusted to the required pH by addition of NaOH or HCl. In other experiments with sugars (glucose, sucrose, fructose, 3-0-methyl-glucose, 2-deoxy-glucose) and protein synthesis inhibitors (cycloheximide, puromycin, actinomycin D) the compounds were added to 1 X, 2 mM $Cl^-$, pH 7.0 solution.

The equipment used to measure pd values (JONES, NOVACKY, and DROPKIN 1974) was shielded by a Faraday cage. The glass micropipette (tip diameter 0.5 µm) was filled with 3 M KCl, and the reference pipette (diameter 1 mm) was filled with 3 M KCl solidified with 2% agar. Ag/AgCl electrodes connected the pipettes to a Keithley 604 electrometer amplifier, and the electrometer output was continuously recorded on a Gould Brush 220 chart recorder. The micropipette was advanced into the cells by a Leitz micromanipulator, and insertions into cells were observed through a microscope. Root segments in plexiglass holders were mounted in a plexiglass chamber (volume 2.9 ml) with flowing bathing solution (6 ml/min). The resistance of the micropipettes was about 15 MΩ. Tip potentials were between 5 and 15 mV in 10 X solution, and were subtracted from measurements.

Each experiment was repeated 3 times or more.

## 3. Results

pd values from cells of segments 10–15 days old are somewhat more variable than from uniform segments taken 4–5 days after germination 1 cm behind the root tip. Variations between plants and between different roots on the same plant also occur. To reduce such effects control segments were taken from non-galled regions of the roots adjacent to the gall. The giant cells in galls induced by one nematode were measured where possible since giant cells are larger in these galls than when more than one nematode is present in the same gall.

The pd value obtained in higher plant studies is normally considered to be between the vacuole and the external solution (ETHERTON and HIGINBOTHAM 1960, ETHERTON 1963) and is the sum of the differences in potential across the tonoplast and the plasmalemma. Giant cells are basically non-vacuolate, although they do contain some relatively small vacuoles. We estimate that the changes of inserting a microelectrode into a vacuole in giant cells to be less than 1 in 10, and it is reasonable to assume that giant cell pd values are across the plasmalemma alone.

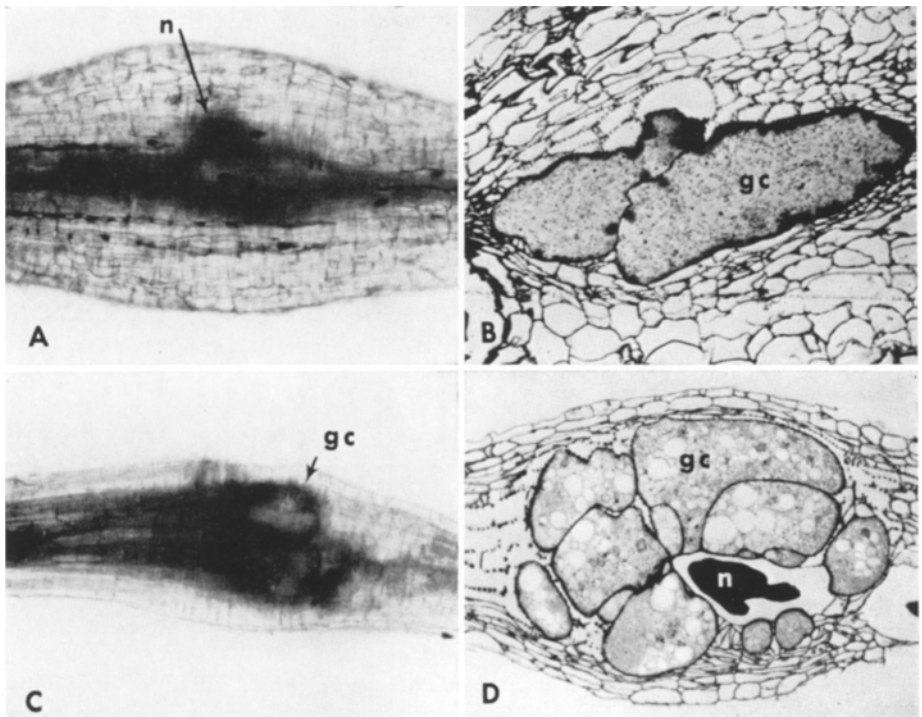



Fig. 1 *A*. Whole gall on root of balsam induced by a root-knot nematode (*n*). The giant cells ("undissected") are in the center of the root (×47). *B*. Longitudinal section through a whole gall in a coleus root. Two giant cells (*gc*) are filled with cytoplasm, some vascular elements occur outside these cells, and vacuolate parenchyma cells of the gall surround the vascular and giant cells. The dark staining areas of the giant cell walls are composed of many fine, branching transfer cell wall ingrowths ×160. *C*. "Dissected" giant cells (*gc*) in the stele of a balsam root from which the cortical and gall parenchyma cells have been removed. ×87. *D*. Longitudinal section through "dissected" giant cells in balsam root. Only a few cells overlay the giant cells (*gc*). The head of the nematode (*n*) has stained much more intensely. ×116

### *3.1. pd Values of Parenchyma and Giant Cells*

A typical trace of pd values when an electrode is advanced through an intact gall into giant cells is shown in Fig. 2. The upper trace (Fig. 2 *A*) shows pd values soon after excising the gall, and the pd values of the parenchyma cells and giant cell increase with time. The lower trace (Fig. 2 *B*) shows pd values after aging for 23 hours; the pd is no longer increasing, and all the cells have similar values. We have repeated the experiment shown in Fig. 2 *B* about 20 times, and the pd values of gall parenchyma and giant cells differed only by a few mV up to about 10 mV. Since the micropipette tip is probably in the vacuole of parenchyma cells and the cytoplasm of giant cells, this further confirms that in higher plant cells the tonoplast contributes

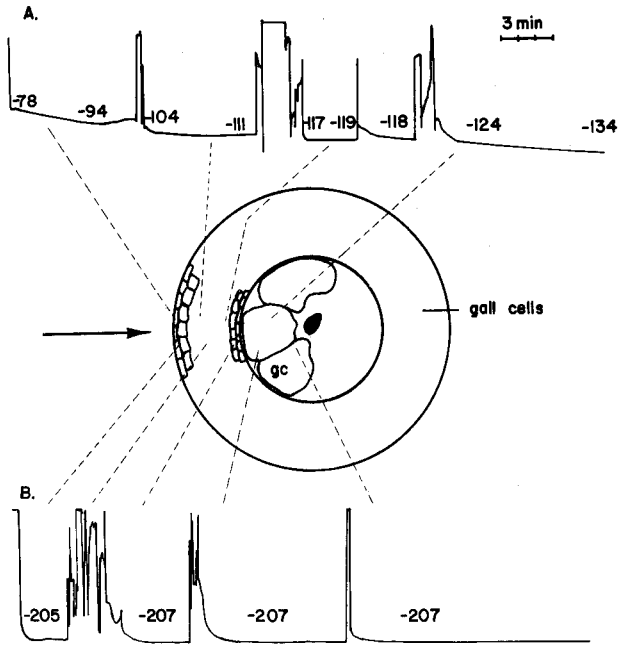



Fig. 2. pd traces on advancing a micropipette through a whole gall from the position marked by the arrow. Dotted lines indicate the regions of the gall from which the recordings were made. *A.* Recording obtained soon after excising the gall—pd values increase. *B.* Recording from root aged for 23 hours after excising—pd values in gall parenchyma and giant cells are stable and similar

little to the measured pd. In a separate experiment we tested the effect of the presence of the microelectrode in a giant cell on the aging rate. Three dissected galls containing giant cells on the same root segment were mounted in a holder. The pd was continuously recorded from one giant cell for 190 minutes, during which time the value increased from —100 to —160 mV. The pd of a giant cell in each of the other two complexes was then measured, and their values were —160 and —152 mV. This indicates that the presence of a recording electrode does not affect the aging rate.

The pd values of cells in the few cell layers outside dissected giant cells and in parenchyma cells in intact galls is shown in Table 1, along with values of the giant cells.

These results indicate that removal of the gall cortex does not significantly affect pd values of giant cells in the gall, and that there is no significant difference in pd values between giant cells and the cortical and vascular parenchyma cells of the gall. The aged pd values of giant cells and cortical parenchyma cells of adjacent control segments are also the same (Table 2 *B*).

During the course of these experiments we have made many pd measurements of the type shown in Fig. 2 from the outside to the center of the root.

Table 1. *pd (Aged) of Parenchyma and Giant Cells (gc) of Undissected and Dissected Roots (N = number of cells)*

| | Cell outside gc | gc |
|---|---|---|
| Dissected galls | 185 ± 9 mV N = 6 | 185 ± 8 mV N = 6 |
| Whole galls | 190 ± 7 N = 6 | 189 ± 10 N = 5 |

Table 2 *A. Immediate Drop in pd (mV) on Changing Total Bathing Solution Concentration 10 Fold*

| | 0.01 – 0.1 X | 0.1 – 1.0 X | 1.0 – 10.0 X |
|---|---|---|---|
| Giant cells in whole galls | 35 ± 10 N = 4 | 29 ± 7 N = 4 | 35 ± 11 N = 4 |
| Giant cells in dissected galls | 24 ± 6 N = 3 | 24 ± 6 N = 3 | 21 ± 4 N = 3 |
| Control cells | 12 ± 4 N = 3 | 19 ± 4 N = 3 | 19 ± 4 N = 3 |

Table 2 *B. Steady pd Values after Incubation Overnight in Total Bathing Solutions with 10 Fold Concentration Differences (mV)*

| | 0.01 X | 0.1 X | 1.0 X | 10.0 X |
|---|---|---|---|---|
| Control cells | −203 ± 3 N = 3 | −192 ± 8 N = 3 | −175 ± 11 N = 3 | −166 ± 10 N = 3 |
| Giant cells | −203 ± 12 N = 7 | −183 ± 6 N = 5 | −168 ± 3 N = 7 | −164 ± 9 N = 7 |

Although we were not specifically looking for a gradient of pd, in agreement with BOWLING (1972) there was no obvious pd gradient. In the aging period after excision, the pd of all the cells in the tissue (except close to the cut surface) increased at about the same rate.

### *3.2. The Effect of KCl, Total Ions, and pH*

#### 3.2.1. KCl

Control and giant cell segments were aged overnight in 1 X solution, then the segments were put in the chamber with 0.01 mM KCl solution flowing through. After about half an hour the pd was measured. When the value in 0.01 mM KCl was steady, the solution was changed for 0.1, 1.0, 3.33, 10.0, and 33.3 mM KCl solutions; each change was made after a steady pd in the previous solution had been recorded. A number of parenchyma cells in each

solution were measured, but single giant cells were continuously monitored through the sequence of KCl concentrations.

The effect of different KCl concentrations on the pd is shown in Fig. 3. The pd of control and giant cells was the same in 0.01 mM KCl, but the control cells depolarized considerably more than the giant cells with increasing KCl concentrations. If the cells were acting as potassium sensitive electrodes (*i.e.*, the pd were a $K^+$ diffusion potential) the theoretical slope of response to KCl concentrations would be 59 mV per log change in $K^+$

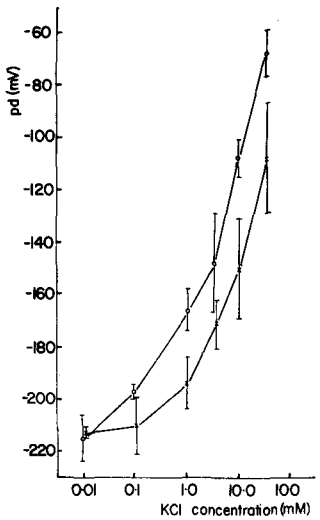



Fig. 3. Effect of KCl concentrations on the pd of parenchyma (*o*) and undissected giant cells (*X*). Vertical bars represent standard deviation

concentration. Over the range from 0.01 to 1.0 mM KCl it is clear that the response is much less than this. Between 1.0 and 10.0 mM KCl control cell pd depolarized by 54 mV and giant cells by 30 mV. At above 10 mM KCl both cell types depolarized by more than the theoretical value.

The presence or absence of $Ca^{2+}$ affects the pd response to $K^+$ concentrations in higher plants, algae, and fungi (HIGINBOTHAM 1973). Normally $Ca^{2+}$ makes the pd much less sensitive to external $K^+$ concentrations. We believe that the difference in response to $K^+$ of control and giant cells in Fig. 3 is due to $Ca^{2+}$. Unbound $Ca^{2+}$ appears to move in and out of cells slowly in a passive downhill direction (HIGINBOTHAM 1973). The reservoir for $Ca^{2+}$ ions in giant cells is much greater than in control cells, and it is probable that the different response is caused by a faster leaching out of $Ca^{2+}$ ions from control cells. In agreement with this explanation the depolarization of dissected giant cells by increasing $K^+$ concentration was greater than for undissected giant cells, and with longer experiments the giant cells gave lower pd values with the high $K^+$ levels. That $Ca^{2+}$ presence affects cell pd response to total ion concentration (including $K^+$) is suggested in the next section.

### 3.2.2. Total Ions

The response to total ion concentration was monitored by changing solutions with a cell continuously impaled. pd values from individual control and giant cells are shown in Fig. 4. On increasing the ion concentration in the bathing solution, the control cell pd rapidly stabilized at a new value. For both control and giant cells a transient hyperpolarization of about 5 mV lasting for 10 seconds occurred before the rapid depolarization. The initial pd drop of giant cells was much greater than the control, but the pd value slowly

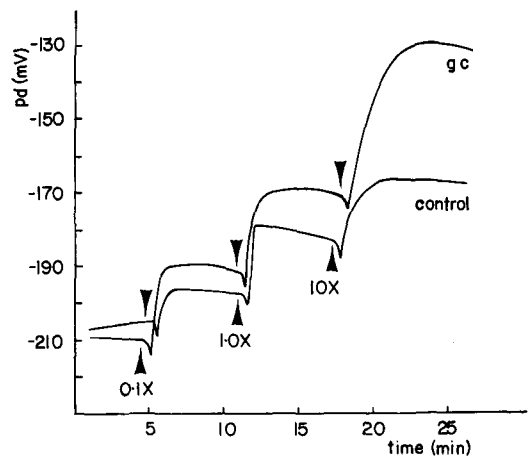



Fig. 4. Effect of changing total ion concentrations on one parenchyma cell (control) and one undissected giant cell (*gc*). Initial readings are for 0.01 X solution, and at the vertical arrows changes in the bathing solution concentration were made to the values marked beneath the lower arrows

increased afterwards and eventually reached the same value as control cells (Table 2 *A*). The difference in the initial depolarization between control and giant cells was reduced by dissecting away the cortex, but dissected giant cell initial depolarization was still greater than that of the control. Control and giant cells incubated in the same solutions overnight had the same pd values (Table 2 *B*).

The total change in pd between the 4 log unit concentration values for total ions (Table 2 *B*) was 37 and 39 mV (control and giant cells respectively), *i.e.*, 13 mV/log unit. The control cells responded to $K^+$ alone over the same 3 log unit change by 108 mV, *i.e.*, an average of 36 mV/log unit, and a change of 59 mV between 1.0 and 10.0 mM $K^+$. Thus the response to total ion concentration was smaller than the response to $K^+$ concentration. This difference is probably caused by the presence of $Ca^{2+}$ ions in the total solution which reduce sensitivity to $K^+$ and $Na^+$ ions.

### 3.2.3. pH

The pd response of control and giant cells to changes in external pH is shown in Fig. 5. Measurements were made on individual cells and the pH changed from 8 to 3. The response is reversible, but takes much longer to reach steady

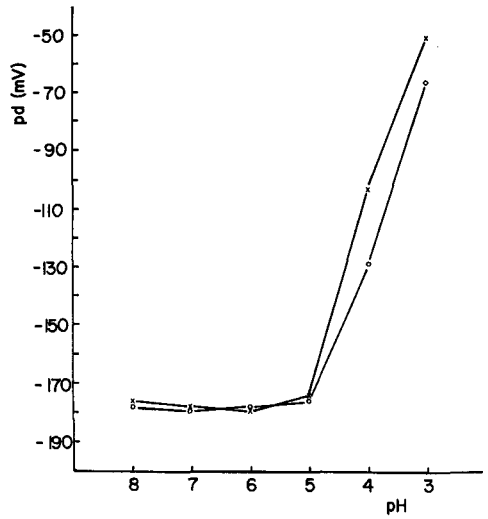



Fig. 5. Effect of external pH on parenchyma cells [average results from 3 cells, (*o*) and dissected giant cells (average from 2 cells, *x*)]

values on changing the pH from 3 to 8. Between pH 8 and 5, the pd of both control and giant cells remains remarkably constant with a variation of only about 5 mV. Between pH 5 and 3 an abrupt depolarization is evident (109 mV for control cells, 122 mV for giant cells). This suggests that the plasmalemma of these cells is not very permeable to $H^+$ ions over the normal physiological pH range of 8 to 5, but at external pH values of less than 5 there is an abrupt increase in the permeability of the plasmalemma to $H^+$ ions.

We have made some initial experiments on the effect of external pH on internal pH of giant cells using an antimony-coated glass pH electrode (type 801, Transidyne General Corporation) (Bowling 1973). Both the pH electrode and a normal micropipette were inserted into the same giant cell and the pH and pd were monitored simultaneously. Between external pH 8 to 5 the internal pH changed from 6.9 to 5.8; with an external pH change of 5 to 3, the internal value dropped to 4.2. The pH electrodes were calibrated in buffer solutions (pH 2 to 8) before and after insertion, and their response was about 50 mV/pH unit. Further experiments to confirm and extend these experiments are planned.

### 3.3. *Effect of Respiratory Inhibitors*

Although the total pd under similar ionic conditions is the same for parenchyma and giant cells, the active and passive components could be different. These components were separated by the action of respiratory inhibitors.

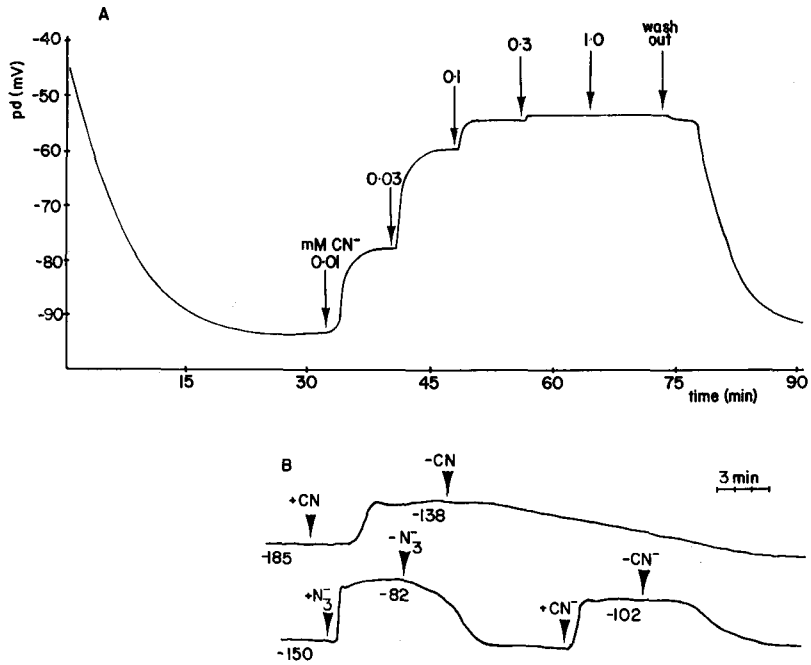



Fig. 6 *A*. Saturating effect of increasing cyanide ($CN^-$) concentrations on the pd of a freshly dissected giant cell. *B*. Typical traces from individual giant cells (upper trace undissected, lower trace dissected) on addition and removal of 1 mM cyanide ($CN^-$) or 1 mM azide ($N_3^-$)

Treatment of parenchyma cells or giant cells with KCN or $NaN_3$ causes a rapid depolarization which is reversible after removal of the inhibitor. Fig. 6 *A* illustrates that increasing concentrations of $CN^-$ eventually saturate the effect, with 100% depolarization at about 0.3 mM. Similar saturation occurs with $N_3^-$ treatment. To ensure complete depolarization in experiments both inhibitors were used at 1 mM concentration. Typical treatments with $CN^-$ and $N_3^-$ are shown in Fig. 6 *B*, and a comparison of the depolarization produced by these inhibitors is given in Table 3. Inhibition and washing out of $N_3^-$ is faster than for $CN^-$, and typically the level of depolarization with $N_3^-$ exceeds that of $CN^-$ by about 12%. This difference is also found if the same cell is treated sequentially with $CN^-$ then $N_3^-$ (or *vice-versa* Fig. 6 *B*). Although there is a difference in depolarization caused by $CN^-$ and $N_3^-$, there is no significant difference in the level of depolarization of

Table 3. *Effect of Cyanide (1 mM) and Azide (1 mM) on the pd of Giant Cells and Control Cells*

| | Giant Cells | Control |
|---|---|---|
| 1. $CN^-$ | | |
| E (mV) | 167 ± 15 (N = 7) | 161 ± 16 (N = 6) |
| Depolarization (mV) | 59 ± 16 | 62 ± 9 |
| % Change | 35 ± 10 | 38 ± 4 |
| 2. $N_3^-$ | | |
| E (mV) | 159 ± 13 (N = 6) | 156 ± 13 (N = 7) |
| Depolarization (mV) | 81 ± 9 | 75 ± 14 |
| % Change | 51 ± 7 | 48 ± 7 |

parenchyma cells and giant cells with the same inhibitor. Dissected giant cells consistently recovered the original pd faster than parenchyma cells when the inhibitors were washed out.

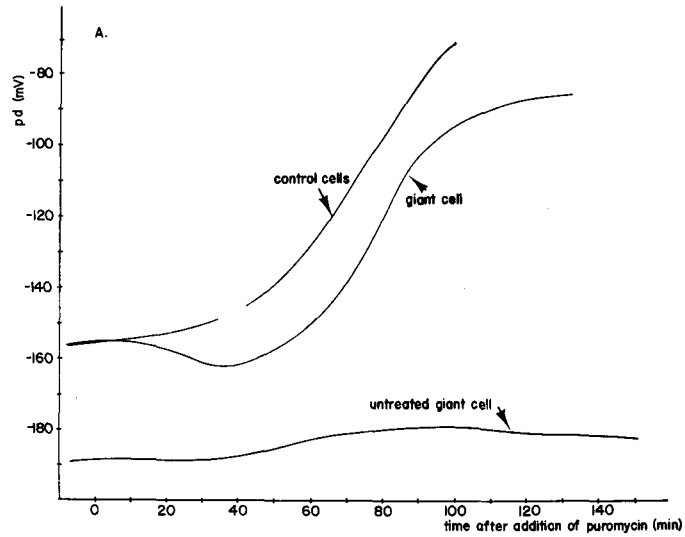
Treatment of cells with the uncoupler dinitrophenol (0.2 mM) (DNP) caused a biphasic depolarization. The first phase was rapid, and the depolarization was slightly greater than with $N_3^-$ treatment. This was followed by a more gradual depolarization over 15–20 minutes to give up to 90% depolarization. Recovery after washing out DNP was also much slower, but the original pd could be obtained. In view of possible effects on the membrane other than uncoupling action, the effects of DNP on pd are difficult to interpret.

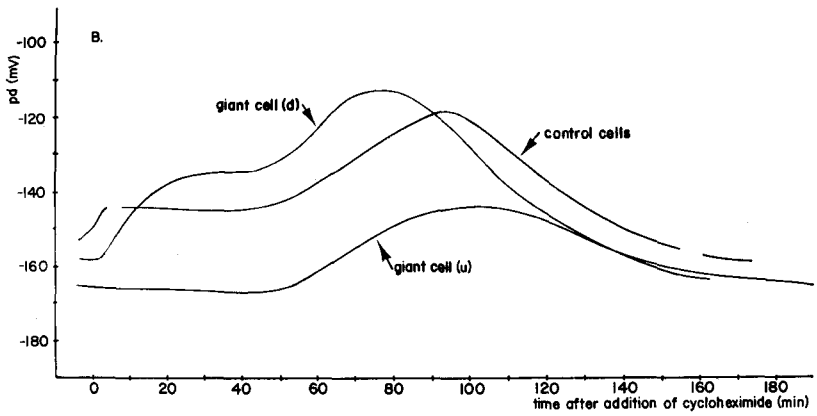
### *3.4. The Effect of Protein Synthesis Inhibitors*

#### 3.4.1. Puromycin

Puromycin, an analogue of the terminal aminoacyl adenosine moiety of tRNA, releases nascent polypeptide chains to prevent protein synthesis (FRANKLIN and SNOW 1971). The effect of $5 \times 10^{-4}$ M puromycin on the pd of individual control, undissected and dissected giant cells is shown in Fig. 7 *A*. (The occasional irregularity in traces of Figs. 7 *A–C* just after

Fig. 7 *A*. Effect of puromycin ($5 \times 10^{-4}$ M) on pd of a control root segment and a dissected giant cell. The untreated dissected giant cell was incubated under similar conditions without addition of inhibitor. *B*. Effect of cycloheximide (25 µg/ml) on pd of a control root segment and dissected (*d*) and undissected (*u*) giant cells. *C*. Effect of actinomycin D (10 µg/ml) on pd of a control root segment and a dissected giant cell. During the dashed part of the giant cell recording a train of Action potential-like fluctuations occurred. The traces from control segments were made from 2 or more parenchyma cells. The blank parts are the time between loss of one cell pd and re-establishment of a recording in another cell of the segment


A.
pd (mV)
-80
-100
-120
-140
-160
-180
control cells
giant cell
untreated giant cell
0
20
40
60
80
100
120
140
time after addition of puromycin (min)


B.
pd (mV)
-100
-120
-140
-160
-180
giant cell (d)
control cells
giant cell (u)
0
20
40
60
80
100
120
140
160
180
time after addition of cycloheximide (min)

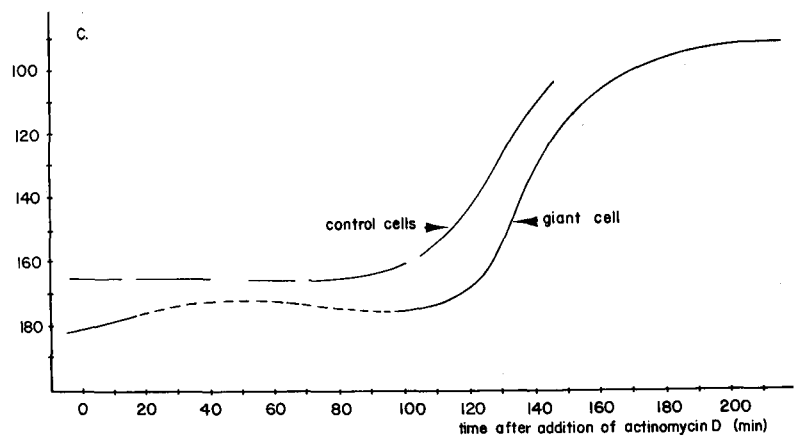

C.
100
120
140
160
180
control cells
giant cell
0
20
40
60
80
100
120
140
160
180
200
time after addition of actinomycin D (min)

Fig. 7

addition of the inhibitors probably results from stopping the solution flow to conserve the inhibitor. It may be caused by KCl leakage from the reference electrode.) The pd depolarized after a lag of 3–40 minutes; this was about the same for the control and both giant cell preparations. The rate of depolarization was greatest for control and dissected giant cells 40–80 minutes after addition of puromycin. The depolarization rate over this time varied between 23 and 68 mV/40 minutes, whereas values of 11 and 14 mV/40 minutes for this time were recorded for undissected giant cells. This implies that the gall cortical cells reduce the rate of access of puromycin to the giant cells. After 80–90 minutes the depolarization rate for control and dissected giant cells reduced to about 1–2 mV/10 minutes. Even after incubation in puromycin for 16–18 hours we could still record pd values of 50 to 60 mV. The stability of these recordings was low.

### 3.4.2. Cycloheximide

Cycloheximide specifically inhibits the function of 80 S ribosomes, and is thought to interfere with translocation of peptidyl-tRNA by acting on the 60 S ribosomal subunit (Franklin and Snow 1971). The effect of 25 μg/ml cycloheximide on the pd of individual control, undissected and dissected giant cells is given in Fig. 7 *B*. The pd of cells depolarized after a lag of about 45 minutes, reaching a minimum value between — 110 and — 140 mV 70 to 90 minutes after addition of the inhibitor, then the pd hyperpolarized again at about the same rate as the previous depolarization to recover the original pd after about 160 minutes. Addition of fresh cycloheximide solution during an experiment did not alter the pattern, so it is clear that inhibitor breakdown was not responsible for the recovery of the pd. In addition segments cut and aged overnight in bathing solution plus excessive concentrations of cycloheximide (100 and 1,000 μg/ml) still maintained pd values in the normal range of — 145 to — 170 mV. Further evidence that this is not an isolated phenomenon is the recording of an identical lag, depolarization and recovery of pd by photosynthetic cells of cotton cotyledons when treated with cycloheximide (Novacky, unpublished results).

### 3.4.3. Actinomycin D

Actinomycin D specifically associates with double stranded DNA to prevent DNA-dependent RNA synthesis (Franklin and Snow 1971). Thus its action in preventing protein synthesis is at the transcriptional level. Fig. 7 *C* illustrates the action of 10 μg/ml actinomycin D on the pd of individual control cells and dissected giant cells. In this case the lag before depolarization is 80–100 minutes after the addition of inhibitor. The pd value dropped by between 48 to 81 mV over 110 minutes then the rate of depolarization reduced after 225 minutes to about 1 mV/10 minutes. Values recorded 5 hours after addition of inhibitor to giant cells were — 70 and — 88 mV.

### *3.5. Effect of Sugars*

Our original interest in the relation of sugars to pd was the possibility that aged cells might be starved of a carbon source. The response to sugars, which are non-charged solutes, on the pd is also important because it may be interpreted in terms of $H^+$ ion-dependent cotransport systems (SLAYMAN and SLAYMAN 1974). All sugars tested were at a concentration of 50 mM. Preliminary experiments with glucose showed that the effect (see below) was saturated at this concentration.

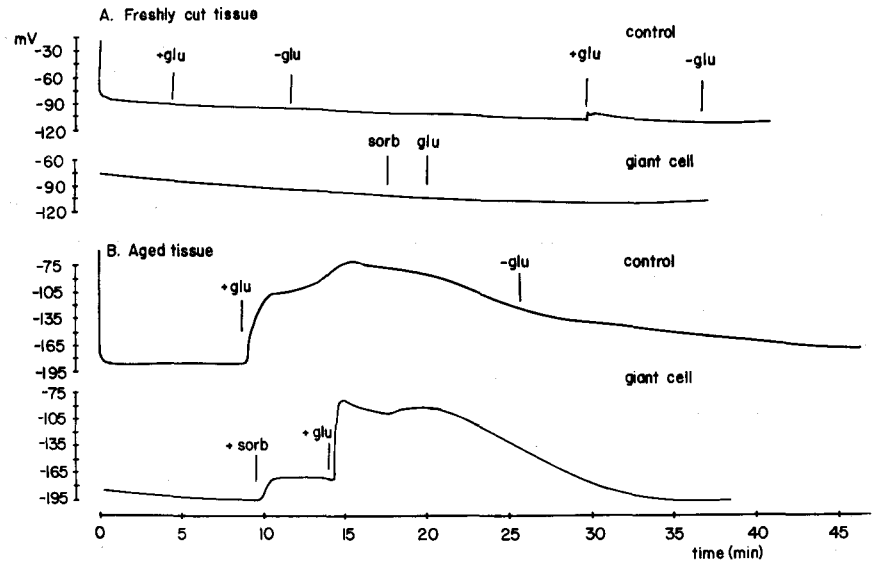



Fig. 8. Effect of glucose (*glu*) and sorbitol (*sorb*) on the pd of individual cells. *A.* Freshly excised tissue, (dissected giant cell). *B.* Aged tissue (dissected giant cell)

The pd response to glucose of control and giant cells, both immediately after excision and after aging overnight was similar, and is shown in Fig. 8. Cells in freshly cut root segments gave no response to glucose. About 35 minutes after cutting a transient depolarization (9 mV, Fig. 8 *A*) occurred on addition of glucose. With increasing aging time this response increased to a maximum of 113 mV. The time course of development and magnitude of depolarization were similar for both control and giant cells. To check that this effect was not an osmotic phenomenon, we added sorbitol (50 mM) before glucose on the assumption that it is taken up by the cells. Fresh cut cells showed no response to sorbitol, but after aging sorbitol caused a transient depolarization of 20–25 mV. We conclude that cutting and aging root segments derepresses a glucose transport system, and that the effect of glucose on the pd is not osmotic. Presumably sugar uptake occurs during the depolarized period.

Recovery of the pd to the original value in the presence of glucose took about 30 minutes; this was usually followed by a hyperpolarization of up to 30—35 mV above the original pd value. When the cells which had been treated once with glucose were incubated in the absence of glucose for a further 20–30 minutes, then addition of glucose a second time gave no pd response at all. When the glucose was washed out while the pd was still depolarized, there was a more rapid repolarization followed by a hyperpolarization.

The depolarization in response to glucose is remarkable because there was virtually no lag, and the rate of depolarization was frequently 100 mV/second or more (*cf.*, Slayman and Slayman 1974). This is a considerably greater rate than the depolarizations induced by the respiratory inhibitors $CN^-$ and $N_3^-$ (see Fig. 9). The depolarization of giant cells with 1 mM $CN^-$ was followed by a further depolarization (average value 23 mV) on addition of 1 mM $CN^-$ plus glucose. With incubation with $CN^-$ plus glucose the pd repolarized to the base $CN^-$ value after about 15 minutes. This observation agrees with other experiments in which separate $CN^-$ and glucose treatments were tested on the same cell, and the initial depolarization with glucose was greater than the depolarization with $CN^-$.

Sucrose is probably taken up by plant cells after being split to glucose and fructose by the action of a cell wall invertase (Sacher, Hatch, and Glasziou 1963), so we also tested the effect of sucrose and fructose on the pd. This system might be useful for estimating cell wall invertase action. We also tested the sugars 3-0-methyl glucose, which is taken up as glucose, but not further metabolized (Gayler and Glasziou 1972), and 2-deoxy-glucose which is taken up, phosphorylated and then not further metabolized (Guy and Reinhold 1974). These sugars were added to non-dissected giant cells; in general the depolarization was less than with dissected giant cells, since the magnitude of the initial depolarization appears partly to depend on the rate of access of the sugars to cells. The effects (Table 4) of 3-0-methyl glucose and 2-deoxy-glucose are both less than that of glucose, but more than sucrose and fructose. Recovery time in the presence of 3-0-methyl glucose and 2-deoxy-glucose was shorter than in the presence of glucose. After recovery from 3-0-methyl glucose treatment there was no additional response to glucose 20–30 minutes later. This implies that intracellular glucose is monitored in some way before it is further metabolized.

### *3.6. Action Potential (AP)-Like Phenomena*

We have already reported the irregular occurrence of trains of AP-like fluctuations of the pd in giant cells (Jones, Novacky, and Dropkin 1974) but we did not record any such trains from control cells. However, in the course of experiments with the respiratory inhibitors $CN^-$ and $N_3^-$, sugars and

external pH change from 4 to 3, we have recorded a number of single AP-like phenomena from both control and giant cells. Examples are given in Fig. 9. In each case the AP's occurred during depolarization phase. They were most frequent after addition of $CN^-$ or $N_3^-$ and sugars. One AP-like fluctuation caused by external pH change was recorded with a pH electrode in a giant cell, and the electrode output indicated that no internal pH change occurred during the fluctuation. (The pH response was fast enough to register any

Table 4. *Average Depolarization (mV) Caused by Sugars (50 mM) on pd of Aged Giant Cells in Galls*

| Sorbitol | Glucose | 3-0-Methyl Glucose | 2-Deoxy Glucose | Sucrose | Fructose |
|---|---|---|---|---|---|
| 21 ± 4 (N = 3) | 66 ± 23 (N = 6) | 40 ± 16 (N = 7) | 39 ± 8 (N = 4) | 29 ± 4 (N = 4) | 31 ± 5 (N = 4) |

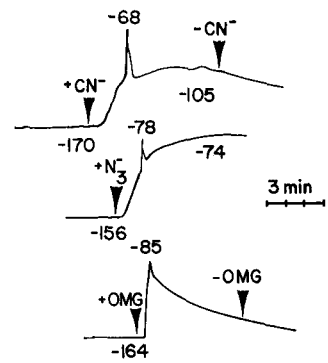



Fig. 9. Action potential-like phenomena during the depolarization phase on the addition of cyanide ($CN^-$) and azide ($N_3^-$) to parenchyma cells and 3-0-methyl-glucose (OMG) to a dissected giant cell

significant pH change during the fluctuation.) AP's occurred in about 50% of the treatments with $CN^-$ and azide, AP depolarization values ranged from 15 to 52 mV (average for cyanide, control 31 mV, giant cells 36 mV; azide control 34 mV, giant cells 25 mV). An interesting point is that the peak of AP depolarization was frequently more positive (up to 25 mV) than the depolarized pd value in cyanide. These AP-like fluctuations on addition of respiratory inhibitors have apparently not been reported before, but they are probably not related to the presence of giant cells or the nematode, since AP-like phenomena have been recorded on a few occasions from normal cells of cotton cotyledons (NOVACKY, unpublished results).

### *3.7. Nematode Feeding*

At no time during experiments on giant cells did we observe any pd phenomena which could be directly attributed to feeding by the nematode.

## 4. Discussion

A general analysis and comparison of galls and non-galled roots by OWENS and SPECHT (1966) and OWENS and RUBINSTEIN (1966) showed a considerable increase in nearly all cell fractions (free amino acids, proteins, nucleotides, RNA, DNA, lipids, minerals, hemicellulose) in galls over non-galled roots, a higher respiratory quotient in galls and a higher incorporation of U-$C^{14}$ glucose after pulse feeding in gall fractions. Cytochemical staining for a series of oxidative and hydrolytic enzymes in fresh tissue sections all showed increased activity in giant cells over parenchyma cells (ENDO and VEECH 1969, VEECH and ENDO 1969, 1970). The ultrastructural appearance of giant cells is also typical of metabolically active cells. These cells have been classified as exaggerated transfer cells (JONES and NORTHCOTE 1972, PATE and GUNNING 1972). Transfer cells occur where intensive short distance transport of solutes is thought to occur (PATE and GUNNING 1972). Nuclei and nucleoli are enlarged, with amoeboid profiles; mitochondria and Golgi bodies are numerous and occasionally are more frequent near wall ingrowth regions, and the plasmalemma surface area next to vascular tissue is amplified by 6 to 10 times more than normal (JONES 1974). We do not know whether per unit volume of cytoplasm, metabolism is the same in giant cells and parenchyma cells, but it is quite clear that per unit volume of parenchyma cell (ca. 90% vacuole) and of a giant cell (ca. 70% cytoplasm) the synthetic, metabolic and transport activities of the latter will be proportionally greater than of the former.

Clear differences between parenchyma and giant cells are the stability of recordings and the occasional trains of AP-like fluctuations in giant cells. Some differences in the rate of responses to treatments (*e.g.*, pd recovery on washing out respiratory inhibitors, initial changes on altering KCl or total ion concentration and responses to protein synthesis inhibitors) may be attributed to the difference in volume of the cells (giant cell volume is 500 to 1,000× greater than parenchyma cells). Within the limits of experimental accuracy the resting pd, active and passive components of the pd, responses to protein synthesis inhibitors, pH and sugars were the same.

In general the results presented here are similar to those reported for other higher plant cells (HIGINBOTHAM 1973). It is clear that the pd remains relatively constant over a wide range of total ion concentrations and external pH (and probably also KCl concentrations in the presence of $Ca^{2+}$; HIGINBOTHAM *et al.* 1964), and it is extremely unlikely that the pd is a simple diffusion potential. Control of the pd must reside at the plasmalemma, since giant cell values are from cytoplasm to the external medium. The tonoplast of parenchyma cells cannot therefore provide more than a few mV to the total pd.

Since these cells have different metabolic activities yet the same pd values and responses to a variety of external conditions, it appears that the pd is

strictly controlled by a cell despite variations in metabolic activity and presumably $H^+$ (or $OH^-$) production. This is all the more remarkable considering the unusual nature of the nematode induced giant transfer cells, and suggests that the pd of normal transfer cells (PATE and GUNNING 1972) and other cells (*e.g.*, guard cells or pulvini) where considerable ion fluxes occur will not reflect different rates of ion transport. In agreement with this, although considerable movement of $K^+$ occurs into and out of guard cells, PENNY and BOWLING (1974) have found no change in pd. The movement of $K^+$ must therefore be balanced by movement of equal numbers of cations ($H^+$, RASCHKE and HUMBLE 1973) in the reverse direction (or anions in the same direction) to maintain electrical neutrality. Since cells with different metabolic and ion transport activities maintain similar pd values, it seems unlikely that cell pd's simply reflect the result of active $H^+$ efflux from control of cytoplasmic pH. Maintenance of cytoplasmic pH and pd are probably closely related, but our results clearly indicate that cell pd is in itself an important parameter which is controlled independently of general cell metabolism and the rate of ion movements across membranes.

The control of total pd may be via a feedback mechanism acting on the electrogenic pump (ANDERSON, HENDRIX, and HIGINBOTHAM 1974). This may be described by the equation:

$$E_M = E_G + iR,$$

where $E_M$ is the total pd, $E_G$ is the potential from the Goldman diffusion equation, $i$ and $R$ are respectively the current through and resistance of the electrogenic pump. Thus a lowered total pd may stimulate the pump to work faster, increasing $i$ and raising total pd. Some evidence for such a feedback mechanism is seen from traces after addition of $CN^-$, $N_3^-$ or glucose—after the initial depolarization a small repolarization frequently occurs before the inhibitor is removed (HIGINBOTHAM *et al.* 1970). This is also well illustrated by the pd response of higher plant green cells to light. When the light is switched off there is a transient hyperpolarization of 15–20 mV, then the pd returns within 2–3 minutes to the original value. When the light is switched on, a similar transient depolarization occurs, but the original pd is again quickly restored (*Mnium, Attriplex, Chenopodium;* LÜTTGE and PALLAGHY 1969, Cotton, NOVACKY, unpublished observations). Thus the steady resting pd in one cell is the same if photosynthesis is or is not occurring.

Why do cells need to maintain a particular pd so strictly? One reason may be to maintain the correct electrostatic conditions on the plasmalemma for its integrity and functioning. Since a considerable amount of energy appears to be required for maintenance of the pd, another possibility (based on MITCHELL's chemiosmotic hypothesis, MITCHELL 1966) is that the pd is a device for transferring energy. Thus the energy used in the necessary

extrusion of $H^+$ ions to maintain the correct internal pH is not wasted, but the $H^+$ electrochemical potential gradient from the outside to the inside of a cell may be linked to a cotransport system in which $H^+$ movement down the potential gradient provides the energy to move an ion or solute against its potential gradient into the cell.

SLAYMAN and SLAYMAN (1974) believe that the depolarization in *Neurospora* caused by glucose is evidence for just such an $H^+$ dependent cotransport system. They found little change in intracellular ATP concentration and a pulse of alkalinization of the medium during the depolarization. These results with *Neurospora* and higher plant cells (a similar depolarization occurs on addition of amino acids also, ETHERTON and NUOVO 1974), are however, equally compatible with a classical model based on an ATPase permease. In this model, a certain fraction of ATP is available for moving solutes and ions at the plasmalemma. On addition of glucose to a starved cell ATP which has been used previously to pump $H^+$ ions is switched to glucose uptake. Thus an alkalization of the medium may result from diffusion of $H^+$ ions back into the cell and a depolarization will occur since $H^+$ pumping out has been stopped for pumping in non-charged glucose. No change in ATP concentration is required. When enough glucose has been pumped in the ATP is switched back to $H^+$ pumping, and a hyperpolarization occurs since the cell cytoplasm will have become more acid during glucose uptake. An important difference between these models is that a decrease in membrane resistance is predicted by the cotransport model, but the reverse is predicted by a permease model. SLAYMAN and SLAYMAN (1974) recorded a slight and perhaps significant decrease in resistance which tends to support the co-transport model. An increase in the $H^+$ gradient across the plasmalemma by lowering external pH over the stable pd range (to pH 5) should increase the sugar induced depolarization and sugar uptake according to the co-transport model. This might be interpreted as a pH optimum effect with the permease model. However, HILL and HILL (1973) believe that exudation of NaCl from the *Limonium* salt gland cannot be coupled to $H^+$ extrusion or exchanged for other ions, and have some evidence for a $Cl^-$ stimulated ATPase. Certainly any $H^+$ cotransport carrier would be more difficult to identify than an ATPase permease carrier.

The response to the protein synthesis inhibitors are difficult to interpret without further evidence of their effect on the rate of protein synthesis in this system. Both puromycin and actinomycin D probably acted as expected in inhibiting protein synthesis. The increased time lag before depolarization with actinomycin D is consistent with its action at the transcriptional rather than directly at the translational level. We cannot say whether the depolarization results from decreased synthetic activities producing less $H^+$ ($OH^-$) ions in the cytoplasm to pump out, or whether transport proteins turned over without replacement or whether a general decrease in membrane integrity

occurred. The effect of cycloheximide possibly reflects some successful feedback or recovery by the cell. Surprisingly few reports on the effect of cycloheximide on higher plant ribosomes exist. It does for example affect beet, peanut and pea, but not ribosomes from castor bean, tobacco, and wheat. ELLIS and MACDONALD (1970) have studied this point and conclude that cycloheximide does not inhibit all 80 S ribosomes. Apparently ribosomes of balsam are not affected. An effect of cycloheximide is to stimulate oxygen uptake (ELLIS and MACDONALD 1970), and this may be related in some way to the pd response we observed. It would be interesting to measure the active transport component of the pd during the cycloheximide response. Our observations with cycloheximide further emphasize the danger of assuming that it acts similarly in all higher plant systems.

This study did not reveal as much new on giant cell physiology as we had expected, except how normal the pd responses are. This is significant, since it lends support to their classification as exaggerated transfer cells (JONES and NORTHCOTE 1972) and the idea that once formed their metabolism is an automatic plant cell response to the source-sink situation and removal of solutes. Nematode "control" of giant cell metabolism (*e.g.*, by injection of nucleic acids) appears less probable. It also emphasizes the well balanced nature of the host-parasite interaction.

Since the giant cells' pd properties so far measured are normal, the cells may be regarded as a useful model system for higher plant cells. The stability of recordings allows long measurements on the effect, for example, of toxins on one cell; and the cells are large enough for the insertion of more than one microelectrode at once.

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*Note added in proof:*

In the scanning electron microscope we observed no plasmodesmata in walls between giant cells and normal cells. We have since made a detailed study of giant cell walls by transmission electron microscopy and have found that isolated plasmodesmata do occur infrequently in walls between giant cells and parenchyma cells, but are absent in walls next to xylem and sieve elements. This contrasts strongly with the abundant plasmodesmata in large pit fields in walls between neighbouring giant cells. This suggests that giant cells are only very weakly coupled to other cells, but are very well coupled to each other. These observations do not affect our basic conclusions: that pd values alone do not reflect metabolic activities of higher plant cells, and that pd values are strictly controlled despite variations in external solutes.

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