Ionic relations and electrophysiology of single cells of *Characeae*

Part III. Comparative investigations on bioelectric potentials in internodal cells of *Characeae*

J. STOLAREK

INTRODUCTION

The electric properties of plant cells have been studied by various authors but only few of them were able to discuss these complicated phenomena in physico-chemical terms. It should be noted that electrophysiology of plant cells is far behind animal electrophysiology, especially as far as muscle and nerve cells are concerned. A considerable progress in plant electrophysiology has been recently made in the research carried out on giant internodal cells of *Characeae* (Dainty and MacRobbie 1958; MacRobbie 1962, 1964, 1966; Hope and Walker 1961; Spanswick 1965; Spanswick, Stolarek and Williams 1967), which constitute a very suitable material for the study of ion transport and bioelectric parameters.

The bioelectric potential difference between the vacuole and the cytoplasm and between the latter and the medium is an important parameter in ion movement across cell membranes. Hodgkin and Horowicz (1959) have studied the effect of changes in the external ionic concentration on bioelectric potentials in muscle and nerve cells. On this basis they were able to measure the relative permeability of the cell membranes to sodium, potassium and chloride ions. As far as plant cells are concerned such studies have been seldom performed because of the difficulties arising owing to the greater structural complexity of plant cells as compared to those of animals.

Many difficulties in plant electrophysiology are due to the existence of at least two plasma membranes — tonoplast and plasmalemma and it is necessary to consider bioelectric potential differences across both these membranes simultaneously. In addition there is a thick cell wall which with its Donnan system (Dainty 1959; Spanswick, Stolarek and Williams 1967) can be considered as an additional source of diffusion potential. The existence of electric potential difference across the tonoplast was until recently neglected or denied and this is the reason why the effect of electrolytes on potential differences across the tonoplast was not studied.

Recent investigation (Spanswick, Stolarek and Williams 1967) has shown the existence of a potential difference across the tonoplast in *Nitella translucens* of

Fig. 1. The general view of the apparatus

the order of 10–20 mV, the cytoplasm being more negative as compared to the vacuole.

The present paper is devoted to the study of the effect of the changes in external sodium, potassium, calcium and chloride ions concentration on electric potential differences across the plasmalemma and tonoplast membranes in cells of three *Characeae* species. However, prior to this study it was necessary to undertake control investigations on the magnitude of bioelectric potential difference between the vacuole and the medium, and between the cytoplasm and the medium in species which have not been studied from the electrophysiological aspect i.e. *Nitella syncarpa*, *Lychnothamnus barbatus* and *Chara fragilis*. Such control measurements are justified from another point of view. The measurements of electric potentials on plant cells are comparable and valuable when obtained in the same conditions and possibly at the same time. For these reasons due attention has been paid in this work to control measurements of electric potentials of three *Characeae* species which have not been studied so far.

MATERIAL AND METHODS

The cells of *Chara fragilis* were grown in the laboratory in a medium consisting of 0.1 mM KCl, 0.1 mM CaCl_2 , and 1.0 mM NaCl with the addition of natural pond water. *Chara fragilis* is a common member of the *Characeae* flora in Poland and was

originally collected in the Lublin district. *Lychnothamus barbatus* and *Nitella syn- carpa* were collected in eutrophic ponds near Lublin and then cultured in the laboratory in diluted pond water. Three to seven days before the experiments the cells were isolated and then placed in deep Petri dishes in a medium of the following composition: KCl 0.1mM, CaCl_2 0.1mM and NaCl 1.0mM, referred to as "artificial pond water" or "APW". Just before the experiments the cells were observed under the microscope, measured and selected for investigations. Only healthy cells with a high turgor pressure and rapid protoplasmic streaming were used.

The potential difference measured between the vacuole and the medium would be algebraic sum of the two potential differences that across the tonoplast and also across the plasmalemma. The potential difference between the vacuole and the medium was measured with a microelectrode inserted into the vacuole or the cytoplasm connected through a calomel electrode and a d. c. amplifier to the reference electrode placed in the external medium. The general view of the apparatus used for insertion of the microelectrodes into the cells is shown in Fig. 1. The cell lies in a perspex bath with glass walls and is inspected through a horizontally placed microscope. A slide micromanipulator was used for insertion of microelectrodes. In order to change quickly the composition of external media a special multiway tap was incorporated into the apparatus. The tap has a single outlet and ten inlets.

Fig. 2. Block diagram of the apparatus

The outlet is connected to the bath and the inlets are joined to bottles containing different solutions. A constant flow of the solution through the bath with the cell is maintained during the experiment by means of a water pump connected to the outlets of the bath.

The microelectrodes were made of "Pyrex" glass tubings by the use of a micro-electrode puller. The micropipettes prepared by the puller were filled with 3M KCl solution by boiling and keeping them in a dessiccator for several hours. The tips of the microelectrodes were examined under the microscope prior to their use and only those whose external diameter was about $1\ \mu$ and having sharp tips were used.

Measurements of electric potential difference were made:

1) between a microelectrode inserted into the vacuole of the cell and a reference calomel electrode placed in the bathing medium,

2) between a microelectrode inserted into the flowing protoplasm and the reference electrode. The block diagram of the apparatus is given in Fig. 2. The simultaneous measurement of the potential differences across both plasmalemma and tonoplast involves the insertion of a microelectrode into the cytoplasm of the cell. The maximum period for which a microelectrode can be left in the cytoplasm is about 20 min. thereafter the plugging process described by Walker (1956) starts. Thus in experiments of long duration the cytoplasmic electrode was removed and reinserted when it was necessary to measure the potential difference again. This process did not seem to affect either the electrode or the cell. The microelectrodes had tip diameter of about 1μ , consequently little or none of the cell contents oozed out when the electrode was removed. The insertion of the second microelectrode (into the cytoplasm) does not affect protoplasmic streaming, one can, therefore, conclude that the insertion of microelectrodes does not injure the cells.

RESULTS

a) *Electric potential difference between the vacuole cytoplasm and external medium in the control bathing medium (APW).* The results of this series of measurements are listed in Table 1.

Table 1

Potential difference between cytoplasm, vacuole and external medium in cells of four species of *Characeae*

Species	Number of cells	Potential difference (mV)	
		across plasmalemma	across ton.
<i>Nitella translucens</i>	37	-141.2 (± 1.2)	15
<i>Chara fragilis</i>	15	-180.0 (± 1.1)	15
<i>Lychnothamus barbatus</i>	5	-188.0 (± 1.0)	12
<i>Nitelloopsis obtusa</i>	7	-151.0 (± 1.0)	10

It appears from the data of the above Table that the investigated species show a very different potential difference when soaked in an identical external medium.

b) *Effect of sodium and potassium ions on bioelectric potential differences in cells of Characeae* (experiments performed on cells pretreated with 5 mM NaCl before p.d. measurements carried out in Ca^{2+} free media). (Tab. 2).

The effect of sodium and potassium ions on bioelectric potential differences in cells and organisms has been studied by a number of authors but mostly by those working on nerve cells and muscles.

Hope and Walker (1961) have shown that the electric potential differences in *Chara australis* changed significantly in response to a change in external potassium

concentration, when the cells used for the experiments were pretreated with 5 mM NaCl for several hours. Such a procedure is believed to remove Ca^{2+} ions from the cell wall.

The effect of sodium and potassium ions on the bioelectric potentials in cells of three species is shown in Table 3. It is seen from the data of the Table that bioelectric potential difference between cytoplasm and external solution changed in response to the change in potassium concentration in the medium. The change

Table 2
The composition of the solutions of A series
(without Ca^{2+})

Solution	NaCl	KCl
A1	1.05	0.05
A2	1.00	0.10
A3	0.85	0.25
A4	0.70	0.40
A5	0.40	0.80
A6	0.25	0.85
A7	0.10	1.00

Table 3
Effect of potassium concentration in the medium on electric potential difference between the cytoplasm and external solution (the cells pretreated with 5 mM NaCl)
(mV)

Species (number of cells)	Potential difference measured in the solutions containing following concentrations of potassium (mM)							α from the Goldman equation
	0.05	0.10	0.25	0.40	0.70	0.85	1.0	
<i>Nitella translucens</i> (37)	—144.3	—141.2	—136.0	—132.5	—122.5	—118.5	—115.4 ± 1.2	0.27
<i>Chara fragilis</i> (15)	—183	—180	—172	—163	—151	—147	—144 ± 1.1	0.10
<i>Lychnothamus barbatus</i> (5)	—197	—192	—175	—164	—149	—145	—140 ± 1.0	0.07

The standard error of mean given in the Table is the same for the remaining solutions.

in potential differences can be characterised by the coefficient $\alpha = P_{\text{Na}}/P_{\text{K}}$ which is given in the last column of the Table. The coefficient is derived from the Goldman equation. Potential difference as a function of potassium ion concentration in the medium is shown in Fig. 3 and 4. The change in potassium concentration of the medium did not seem to affect the potential difference across the tonoplast.

c) *Effect of chloride ions* (the cells were pretreated with 5 mM NaCl and the measurements were carried out in Ca^{2+} free media). In order to investigate the effect

of anions on bioelectric potentials, experiments consisting in substituting choline for sodium were carried out (Table 4). The effect of anion substitutions on bioelectric potential difference between the vacuole and external medium was also investigated (Table 5).

Fig. 3

Fig. 4

Fig. 3. The electric potential difference across plasmalemma in 15 cells of *Chara fragilis* as a function of potassium concentration in the medium

Fig. 4. The electric potential difference across plasmalemma in 5 cells of *Lychnothamnus barbatus* as a function of potassium concentration in the medium

d) *Effect of Na^+ , K^+ and Cl^- ions in the presence of Ca^{++} ions in the medium.* The effect of calcium ions on the bioelectric potential difference as well as sodium, the effect of potassium and chloride together with calcium were studied by the use of a series of solutions referred to as APW, B1—B7. The composition of the solutions is shown in Table 6, which also gives experimental data obtained on three Characeae species. Here like in the previous experiments the potential difference across tonoplast did not change in response to a change in composition of the bathing solution and therefore the change in potential difference between the vacuole and the bathing solution was investigated instead of the potential difference across plasmalemma. As seen from Table 6 the substitution of solution B4 for B1 corresponds to an 8.5-fold increase in chloride concentration of the medium. The change in potential difference of the cells of *Nitella translucens* is +6 mV, *Nitella syncarpa* +5, *Chara fragilis* +7 and *Lychnothamnus barbatus* +4 mV. Potential difference in the solution B1 was more negative than in B5. A 6.5 fold increase in chloride

Table 4

Effect of the substitution of choline for sodium in external solution on bioelectric potential difference change across plasmalemma when potassium concentration rose from 0.1 to 1.0 mM

Cation concentration in the medium (mM)				Potential difference (mV)		
	K	Na	Choline	<i>Nitella translucens</i> (10 cells)	<i>Nitella syncarpa</i> (10 cells)	<i>Chara fragilis</i> (10 cells)
a	0.1	1.0	—	-145 ± 4.4	-133 ± 0.9	-168 ± 0.6
b	0.1	—	1.0	-160 ± 4.9	-147 ± 1.0	-184 ± 3.1
c	1.0	0.1	—	-113 ± 2.5	-104 ± 0.6	-136 ± 2.1
b — c				-47 ± 2.8	-44	-48

Table 5

Effect of anion substitutions in medium on bioelectric potential differences between vacuole and external solution (mV)

Species	Cell	Chlo- ride	Benze- ne sul- phonate	Chlo- ride	Cell	Chlo- ride	Nitrate	Chlo- ride	Cell	Chlo- ride	Sulph- ate	Chlo- ride
<i>Nitella translucens</i>	1	-103	-104	-98	10	-106	-106	-106	16	-106	-106	-106
	2	-112	-118	-111	11	-111	-111	-111	17	-111	-108	-110
	3	-109	-116	-109	12	-109	-110	-108	18	-102	-102	-104
	4	-111	-112	-108	13	-108	-107	-106	19	-113	-107	-107
	5	-129	-132	-125	14	-117	-113	-113	20	-107	-110	-109
	6	-119	-120	-118	15	-118	-115	-115	21	-106	-106	-106
	7	-127	-131	-127					22	-117	-113	-113
	8	-115	-128	-116								
	9	-132	-143	-137								
<i>Chara fragilis</i>	44	-203	-205	-198		-198	-198	-198		-195	-195	-195
	77	-184	-190	-185		-183	-183	-183		-186	-186	-186
	10	-179	-184	-178		-179	-180	-180		-181	-180	-180
	11	-201	-200	-194		-195	-196	-196		-190	-188	-186
	16	-199	-203	-197		-193	-193	-193		-190	-188	-187
	17	-190	-194	-187		-185	-185	-185		-183	-180	-180
	20	-184	-186	-180		-178	-177	-177		-177	-170	-169
	21	-191	-193	-187		-187	-187	-187		-185	-184	-183

concentration in the medium was achieved when changing the APW solution for B5. The potential difference was more negative in B5 by 5, 4, 7, 3 mV for the respective species. The changes in potential differences brought about by a change in chloride concentration were reversible.

The effect of changes in sodium, potassium and calcium concentration was investigated when changing the solutions B1-APW, APW-B3, B3-B1, B1-B2, B2-B6,

Table 6

Changes in electric potential difference across plasmalemma in response to changes in chloride concentration in the medium

Sequence of the solutions							Changes in concentration				Changes in potential difference across plasmalemma, i.e. between cytoplasm and extl. medium (mV)			
Designation	Concentration (mM)						Na	K	Ca	Cl	<i>Nitella translucens</i>	<i>Nitella syncarpa</i>	<i>Chara fragilis</i>	<i>Lychnothamus barbat.</i>
	Na	K	Ca	Cl	Choline	Benzene sulpho-nate								
B1	0.1	0.1	0.1	0.4	—	—	—	—	—	+8.5x	+6 (20)	+5(15)	+7(10)	+4(5)
B4	0.1	0.1	0.1	3.4	3.0	—	—	—	—	—	—	—	—	—
APW	1.0	0.1	0.1	1.3	—	—	—	—	—	—6.5x	—5.5(20)	—4(15)	—7(10)	—3(5)
D5	1.0	0.1	0.1	0.2	—	1.1	—	—	—	—	—	—	—	—

The figures in brackets correspond to the number of investigated cells.

B7-B2, B2-B1 (Table 7). The corresponding relative values of potential differences for three species are shown in Table 7. It is worth mentioning that the most pronounced effect in cells of all three species may be attributed to calcium ions. All other ions in the presence of Ca^{2+} in the medium exert only a weak influence as compared to that of calcium.

DISCUSSION

Comparison of bioelectric potential differences in cells of various species of *Characeae* leads to the conclusion that there are considerable differences between the individual species. The largest divergences were found between *Lychnothamus* and *Nitella translucens*. It should be noted that a potential difference of the order of 10–20 mV was found across the tonoplast in all the cells investigated. The potential difference across tonoplast was detected by Findley and Hope (1964) and further investigated by Spanswick Stolarek and Williams (1967) on *Nitella translucens*. The present measurements supply fresh evidence for the existence of potential differences across the tonoplast in three species of *Characeae*, two of which have not been studied so far.

From the experiments carried out in Ca^{2+} free media it appears that, in all the investigated species potassium ions determine to the largest extent potential difference between the cytoplasm and external solution. The strongest effect observed was that of potassium ions on the bioelectric potential of the cells of *Lychnothamus barbatus*. A relative measure of the effect of potassium ions is the coefficient $\alpha (=P_{\text{Na}}/P_{\text{K}})$, derived from the Goldman equation

$$E = \frac{RT}{ZF} \ln \frac{K_0 + \alpha Na_0}{K_i + \alpha Na_i}, \text{ mV}$$

Table 7

Relative changes in electric potential difference between the cytoplasm and external solution, corresponding to changes in ionic concentrations of the medium

The sequence of the solutions				Changes in concentration				Changes in potential difference across plasmalemma (mV)			
Designation	Concentration (mM)				Na	K	Ca	Cl	<i>Nitella translucens</i>	<i>Chara fragilis</i>	<i>Lychnothamus barbatus</i>
	Na	K	Ca	Cl							
B1 APW	0.1	0.1	0.1	0.4	+10x	—	—	+3.25x	+7.3±1.2 (20)	+6.0±1.5 (10)	+8.0±0.5 (10)
	1.0	0.1	0.1	1.3	—	—	—	—	—	—	—
APW D3	1.0	0.1	0.1	1.3	—10x	+10x	—	—	+4.0±1.5 (20)	+5.0±1.5 (10)	+3.0±1.5 (10)
	0.1	1.0	0.1	1.3	—	—	—	—	—	—	—
B3 B1	0.1	1.0	0.1	1.3	—	—10x	—	—3.25	—9.3±1.9 (20)	—10.0±0.6 (10)	—8.0±0.7 (9)
	0.1	0.1	0.1	0.4	—	—	—	—	—	—	—
B1 B2	0.1	0.1	0.1	0.4	—	—	+10x	+5.5x	+14.6±2.0 (19)	+13.0±1.0 (10)	+5.0±0.6 (10)
	0.1	0.1	1.0	2.2	—	—	—	—	—	—	—
B2 B6	0.1	0.1	1.0	2.2	+10x	—	—	+1.4	+0.7±1.8 (9)	0 (5)	0 (6)
	1.0	0.1	1.0	3.1	—	—	—	—	—	—	—
B6 B7	1.0	0.1	1.0	3.1	—10x	+10x	—	—	—3.2±2.5 (12)	—4.0±0.5 (10)	—3.0±0.4 (10)
	0.1	1.0	1.0	3.1	—	—	—	—	—	—	—
B7 B2	0.1	1.0	1.0	3.1	—	—10x	—	—1.4	+2.2±0.5 (8)	+3.0±0.5 (10)	+4.0±0.5 (10)
	0.1	0.1	1.0	2.2	—	—	—	—	—	—	—
B2 B1	0.1	0.1	1.0	2.2	—	—	—10x	—5.5x	—13.6±1.9 (11)	—12.0±0.7 (8)	—3.0±0.3 (9)
	0.1	0.1	0.1	0.4	—	—	—	—	—	—	—

In columns 10—12 the results are given together with standard error of mean.
The figures in brackets express the number of cells, which corresponds to the number of measurements.

where K_0 , Na_0 , K_i , Na_i are the concentrations of ions in the external medium and in the cytoplasm respectively, E is the measured potential difference. By measuring E in two solutions corresponding to different potassium concentrations, it is possible to calculate α from the above equation, without taking into account internal potassium and sodium concentrations. It should be noted that the applicability of this form of the Goldman equation was considered in experiments on *Nitella translucens* (Spanswick, Stolarek and Williams 1967). The investigated species differ in a substantial way as far as the value of α , i.e. as far as their permeability for sodium and potassium is concerned. The value of α for *Chara fragilis* is 0.1 i.e. it exceeds 2.5 times the corresponding value obtained by Hope and Walker (0.04) for *Chara australis*. It should be pointed out that the value of potential differences together with that of the coefficient α can be regarded as a feature specific for a species.

The effect of chloride on bioelectric potential was studied by substituting choline for sodium and then increasing concentration of potassium ions ten times. These experiments performed on three species showed that chloride ions have certain effect on bioelectric potentials, though very insignificant. If chloride ions would have no effect, the change in potential difference corresponding to a tenfold increase of potassium ions in the medium, where choline was substituted for sodium, would be equal to +58 mV, i.e. the value predicted by the equation, when Na_0 is zero. In fact only for some cells this was the case, whereas for the majority the potential difference change was equal to 37–58 mV. (As regards Table 4 it must be noted that choline chloride should stand for sodium chloride in row c but was not necessary to do it owing to a very small value of $K_0 + \alpha Na_0$ in the Goldman equation). On the other hand the effect of chloride ions on bioelectric potentials was investigated when substituting other anions for chloride i.e. benzenesulphonate, nitrate and sulphate. In all cases the effect of substitution was very small indicating that chloride ions are not very important in electrogenesis.

Consideration of the form of the Goldman equation which contains terms for chloride involves some difficulties as the knowledge of the coefficient α ($=P_{Cl}/P_k$) is required. Chloride fluxes in *Nitella translucens* have been measured by MacRobbie (1962). The significance of chloride ions for *Nitella translucens* has been discussed in detail by Spanswick, Stolarek and Williams (1967); the data presented here and obtained on other *Characeae* species confirm the views of these authors.

Investigations on the effect of calcium ions on bioelectric potential in all investigated species lead to the conclusion that in the media containing both calcium and other ions, calcium plays a major rôle in the maintenance of potential difference between the cytoplasm and the external medium.

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SUMMARY

The electric potential difference between the vacuole and external medium (i.e. across plasmalemma) and between the vacuole and external medium (i.e. across both plasmalemma and tonoplast) in cells of four *Characeae* species: *Nitella translucens*, *Chara fragilis*, *Lychnothamus barbatus* and *Nitellopsis obtusa* was investigated. Significant differences were found between the electric potential of the separate species, the largest being between *Nitella translucens* and *Lychnothamus barbatus*. Potential difference across the tonoplast was also found in all cells ranging from 10–20 mV. The experiments on the effect of ion concentration in the medium on electric potentials have shown that the potential difference across plasmalemma in all species is affected by sodium, potassium and chloride ions. In the absence of calcium ions in the medium the largest effect was produced by potassium. The effect of potassium ions was different in particular species and can be characterised by the coefficient $\alpha = P_{Na}/P_K$ from the Goldman equation where P_{Na} and P_K are the permeability coefficient for sodium and potassium ions respectively. Calcium ions added to the medium changed the electric reaction of the cells in response to sodium and potassium changes in the medium, in such a way that the effect of calcium was the largest of all other ions present in the medium.

Biophysical Laboratory
Department of Plant Physiology
M. Curie-Skłodowska University
Lublin, Akademicka 12, Poland

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Stosunki jonowe i elektrofizjologia pojedynczych komórek Characeae

Cz. III. Badania porównawcze nad potencjałami elektrycznymi internodialnych komórek ramienic

Streszczenie

Badano różnicę potencjałów elektrycznych między cytoplazmą a środowiskiem (tj. przez plazmalemę) oraz między wodniczką a środowiskiem (tj. przez tonoplast i plazmalemę) w komórkach czterech gatunków ramienic: *Nitella translucens*, *Chara fragilis*, *Lychnothamus barbatus*, *Nitellopsis obtusa*. Stwierdzono znaczne różnice między tymi gatunkami jeśli chodzi o wielkość różnicy potencjałów, przy czym największe różnice stwierdzono między *Nitella translucens* i *Lychnothamus barbatus*. Stwierdzono także występowanie różnicy potencjałów przez tonoplast we wszystkich badanych komórkach od 10 do 20 mV. Różnica potencjałów przez plazmalemę zmienia się w zależności od stężeń jonów potasu, sodu, chloru i wapnia; przy braku wapnia w pożywce największy wpływ posiadają jony potasu. Wpływ ten jest różny w poszczególnych gatunkach i charakteryzuje się wartością współczynnika $\alpha = P_{Na}/P_K$ (gdzie P_{Na} i P_K odpowiednio współczynnik przepuszczalności dla jonów sodu i potasu). Wielkość α wchodzi w skład równania Goldmana. Jony wapnia zmieniają reakcję komórek na sód i potas, ograniczając ich wpływ na korzyść jonów wapnia, które w tym wypadku określają w największym stopniu różnicę potencjału elektrycznego przez plazmalemę.