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Bioelectrical Potentials and Ionic Relations in Cells of *Chara fragilis* Desvaux

Potencjały bioelektryczne i stosunki jonowe w komórkach *Chara fragilis* Desvaux

Биоэлектрические потенциалы и ионные соотношения в гигантских клетках *Chara fragilis* Desvaux

INTRODUCTION

It is generally assumed that the origin of bioelectrical potentials in plant and animal cells can be accounted for by asymmetric distribution of inorganic ions between the cells and their medium. It has also been established that bioelectrical potentials depend upon ionic fluxes especially those of potassium and sodium (2, 4, 6—9, 11).

The giant internodial cells of *Characeae* are an ideal experimental material for the study of both ion uptake and bioelectrical potentials in plants. Much early work was done on these cells (see for example Blinks, 1955) (1) but only recently when the microelectrode and isotopic tracer techniques were developed, it became possible to carry out a proper theoretical consideration of the results (2, 8, 9).

It should be noted that despite the abundance of *Characeae* flora in Poland microelectrode experiments with these cells have been undertaken for the first time in this country.

The present paper is intended to give an account of the distribution of potassium, sodium and chloride ions in the cytoplasm and the vacuole of the cells and the significance of these ions in the maintenance of the observed bioelectrical potential gradients in the cells.

MATERIAL

The experiments were carried out on the cells of *Chara fragilis* Desvaux grown in our laboratory in a medium consisting of 0.1 mM KCl, 0.1 mM $CaCl_2$, 1.0 mM NaCl with the addition of natural pond water. *Chara fragilis* is a common member of *Characeae* family in the Lublin district.

The cells have a simple geometrical form, being regular cylinders whose length varies between one to seven centimeters, the diameter being from 0.8 to 1.3 mm. *Chara fragilis* has fairly stout stems, the internodes are considerably longer than the branchlets.

Cortex is regularly three-ranked and the cells of the two series are about the same breadth. The spine cells and stipulodes are normally extremely small. Branchlets seven or eight are very straight. The oospores are black-coloured, maturing in August.

Only healthy cells having a high turgor pressure and rapid protoplasmic streaming were used in the experiments.

METHODS

The measurements of bioelectrical potentials were carried out by means of the d. c. and a. c. push-pull amplifiers described previously (5). The general view of the apparatus used for the insertions of microelectrodes into the cells is shown in Fig. 1. The cell lies in a Perspex bath with glass sides and is viewed through a horizontally mounted microscope. The magnification of 100 times was obtained by using a 17 X eyepiece and 6 X objective. Two microelectrodes could be inserted into the cell using a Zeiss sliding micromanipulator. The multiway tap made it possible to change the solution in the bath very rapidly. The solution was fed in through the tap from reservoir bottles placed on the rack above the apparatus and was removed from the bath by suction (Fig. 2).

The microelectrodes were made from 2 mm external diameter „Pyrex" glass tubing using a microelectrode puller. The puller consists of a platinum coil heated by the current of about 10 A flowing through it. The coil heats the pyrex glass tubing on which an appropriate weight is suspended. The micropipets obtained in such a way are then filled with 3 M KCl solution by boiling them and keeping in a dessicator for several hours. The tips of the microelectrodes are examined under the microscope and only those whose external diameter does not exceed 1 micron and are not covered with crystals, are used for insertions. As the tips were sharply tapered shanks, the electrodes were able to

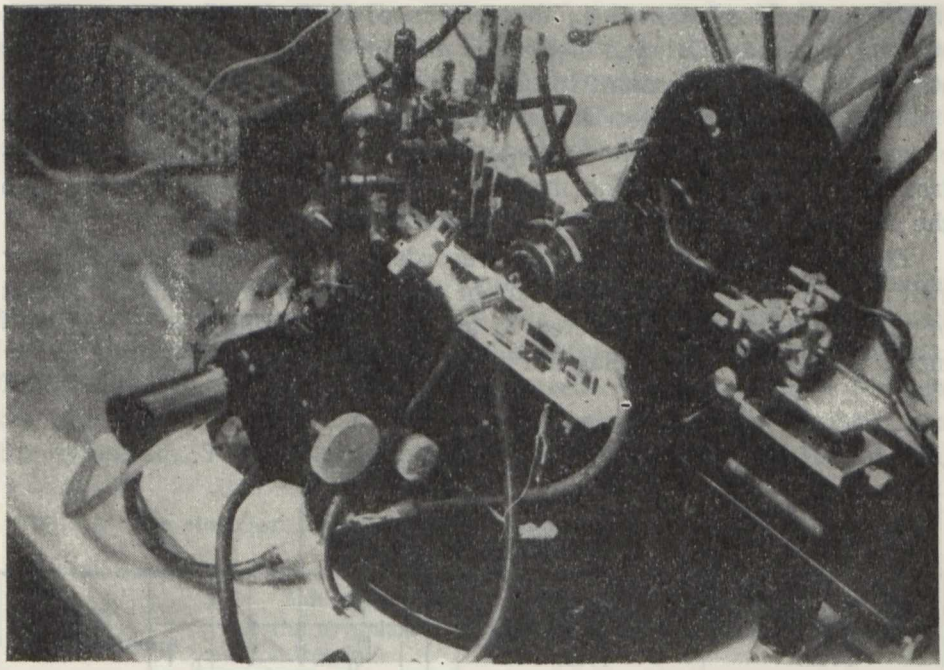

Fig. 1. General view of the apparatus

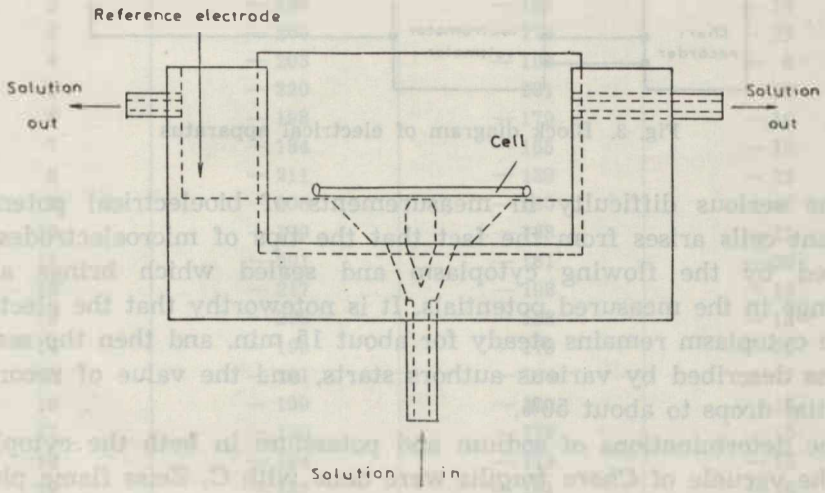

Fig. 2. Bath for the cell

penetrate the cell wall quite easily. The microelectrodes having rather tip potentials or giving unsteady reading were discarded.

Each microelectrode was attached to a "Pye" calomel electrode by means of a piece of rubber tubing filled with 3 M KCl. The electrode

in the external solution, i. e., the reference electrode was also a "Pye" calomel electrode. Calomel electrodes used in the present work are very stable and more convenient in use than Ag-AgCl electrodes.

Measurements of electrical potentials were made between: a) microelectrode inserted into the vacuole of the cell and the reference electrode placed in the bathing medium; b) a microelectrode inserted into the flowing cytoplasm and the same reference electrode.

Each potential difference was recorded by the same amplifier by switching it quickly from one pair of electrodes to another. The output of the amplifier was connected to an oscilloscope OK-5 or a galvanometer GES-2. The block diagram of the apparatus is given in Fig. 3.

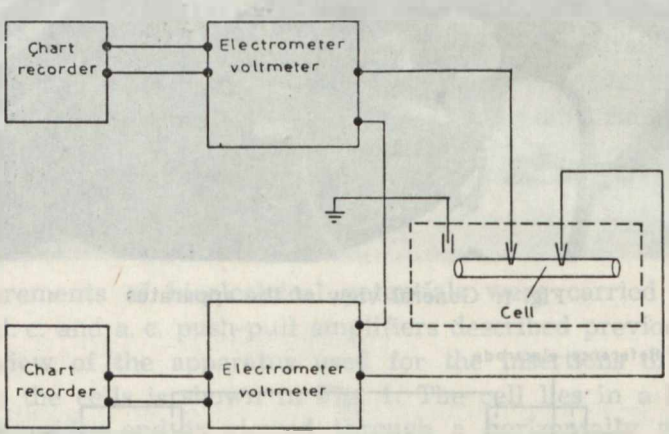

Fig. 3. Block diagram of electrical apparatus

The serious difficulty in measurements of bioelectrical potentials in plant cells arises from the fact that the tips of microelectrodes get covered by the flowing cytoplasm and sealed which brings about a change in the measured potentials. It is noteworthy that the electrode in the cytoplasm remains steady for about 15 min. and then the sealing process described by various authors starts, and the value of recorded, potential drops to about 50%.

The determinations of sodium and potassium in both the cytoplasm and the vacuole of *Chara fragilis* were done with C. Zeiss flame photometer. Each reading for a given sample was compared with standard solutions and the results were obtained by proportion. The calibration showed that the galvanometer readings varied linearly with the concentration of the sample at the dilutions used. About 15 µl of cell sap can be collected from each cell. The determinations of sodium and potassium were made in samples consisting of the sap or the cytoplasm

obtained from 75 or 79 cells. "Microcap" micropipets of the volume of 1, 5, 10, 50 µl were used for the isolation of the cell sap or the cytoplasm. Chloride concentrations in the vacuolar sap and in the cytoplasm were determined by potentiometric titration, using the method described by Ramsay, Brown and Croghan (12).

RESULTS

The measurements of electrical potentials were made on young cells about 2 to 3 cm. long. One electrode was inserted into the vacuole and left for the period of 30 min. to one hour before the readings were taken. The position of the tip of this microelectrode was investigated through the microscope. After this, another microelectrode was inserted into the cytoplasm. The values obtained for the potential of the cytoplasm,

Table 1. The electrical potentials of the cytoplasm and the vacuole in young cells of *Chara fragilis*

Nr of experiment	Pot. diff. of the cytoplasm (mV)	Pot. diff. of the vacuole (mV)	Pot. difference across the tonoplast (mV)
1	— 195	— 180	— 15
2	— 199	— 185	— 14
3	— 200	— 178	— 22
4	— 203	— 195	— 8
5	— 220	— 201	— 19
6	— 198	— 179	— 19
7	— 184	— 165	— 19
8	— 211	— 189	— 22
9	— 206	— 190	— 16
10	— 179	— 168	— 11
11	— 201	— 181	— 20
12	— 217	— 198	— 19
13	— 206	— 188	— 18
14	— 195	— 179	— 16
15	— 201	— 184	— 17
16	— 199	— 180	— 19
17	— 190	— 175	— 15
18	— 194	— 179	— 15
19	— 167	— 181	— 16
20	— 184	— 169	— 15
21	— 191	— 173	— 18
22	— 195	— 178	— 17
23	— 198	— 182	— 16
Mean ± s.e.m.	— 198.4 ± 2.1	— 181.7 ± 1.6	— 16.7 ± 0.7

Table 2. The concentrations of K, Na and Cl, in the cytoplasm and the vacuole of cells of *Chara fragilis*

Number of cells	Cytoplasm			Vacuole		
	K	Na	Cl	K	Na	Cl
Sample consisting of 75 cells	101.0	25.0		91.0	151.0	
Sample consisting of 79 cells	92.0	29.0		94.0	155.0	
25 separate measurements			191.6			66.7
Mean value ± s.e m.	96.5 ± 2.0	27.0 ± 2.2	191.6 ± 3.6	92.5 ± 1.5	153.0 ± 2.0	66.7 ± 3.6

Table 3. The concentrations of chloride and the electrical potentials in the cytoplasm and the vacuole

Nr of experiment	υ^E o (mV)	c^E o (mV)	Cl_{cyt} (mM)	Cl_{vac} (mM)
25	−177	−194	90	189
26	−185	−208	95	155
27	−202	−221	54	198
28	−194	−202	45	210
29	−177	−196	49	193
31	−191	−207	82	199
32	−167	−178	99	202
34	−170	−185	70	201
35	−178	−193	85	187
36	−197	−216	58	186
38	−179	−191	62	194
41	−178	−194	47	158
42	−179	−198	53	178
43	−174	−192	51	235
45	−192	−207	96	196
46	−188	−205	89	178
47	−196	−205	59	205
48	−204	−220	57	201
49	−203	−219	49	189
50	−194	−209	63	172
52	−193	−210	72	191
53	−197	−214	41	183
56	−192	−211	79	208
57	−190	−204	82	205
58	—	—	52	179
Mean ± s.e.m.	−178.9 ± 2.7	−202.5 ± 2.3	66.7 ± 3.6	191.6 ± 3.6

vacuole and the potential difference across the tonoplast for 23 cells are given in Table 1. The mean potential difference across the tonoplast is —16.7 mV, the cytoplasm being more negative than the vacuole. The measurements were carried out in "artificial pond water" (A. P. W.) (11) of the composition 0.1 mM KCl, 1.0 mM NaCl and 0.1 mM $CaCl_2$.

In Table 2 the results of the determination of sodium and potassium concentrations in the vacuole and in the cytoplasm are presented.

Table 3 contains the results of the measurements of electrical potentials in a further batch of 24 cells which were then used for the measurements of chloride concentrations. The mean values for Cl concentration in the cytoplasm for 25 cells are 191.6 mM and 66.6 mM in the vacuole.

The average value for the membrane potential across the two membranes, i. e. between the vacuole and the external solution for 47 cells, is 180.3 mV. The mean potential difference between the cytoplasm and the external solution for the same 47 cells amounted to −200.4 m mV.

DISCUSSION

The figures of the potential difference across the membrane and the ionic concentrations inside the cell and in the surrounding solution enable us to determine the direction and the site of active ion transport in cells of *Chara fragilis*. If we compare the observed potential difference calculated from the Nernst equation, taking into account the concentration ratio, we can get information about the driving forces acting on the ions. According to Dainty (2) such an approach can be only applied to those ions which are in flux equilibrium. Mac Robbie (9) has shown that in *Nitella translucens* both Na and K are in flux equilibrium.

Table 4. The measured potential difference between the cytoplasm and external solution ($_cE_o$) and the concentration potentials for Na(E_{Na}), K (E_K and Cl (E_{Ci}) across plasmalemma (in millivolts)

$_cE_o$	E_{Na}	E_K	$_cE_o$	E_{Cl}
(47 cells)			(24 cells)	
− 200.4	− 83.0	− 101.9	− 202.5	+ 125.7

In Table 4 the values of the observed potential differences and those calculated from the Nernst equation are presented for each ion across the plasmalemma. The corresponding values for the tonoplast membrane are presented in Table 5.

Table 6 gives the difference between the observed potential (E_m) and the Nernst potential (E_{conc}) across both membranes. The size of this dif-

Table 5. The measured potential difference between the vacuole and the cytoplasm ($_vE_c$) and the concentration potentials for Na, K, Cl across the tonoplast membrane for 47 cells

$_vE_c$	E_{Na}	E_K	E_{Cl}
— 20.1 (47 cells)	— 43.7	— 8.9	—
— 23.6 (24 cells)			+ 27.4

ference ($\triangle E$) gives an indication of the relative importance of active and passive ion movements in maintaining the observed concentration and potential gradients in cells. The driving force acting on an ion moving across a plant cell membrane is proportional to the difference $\triangle E$. The sign of $\triangle E$ taking into account the charge of the ion gives the direction of the driving force. The values of $\triangle E$ for sodium, potassium and chloride at the plasmalemma show that each ion is subject to a great driving force. For Na and K ions the driving force is directed from the outside solution to the cytoplasm, and for Cl from the cytoplasm to the outside solution. The cell has to maintain the concentration of these ions at an approximately constant level, so an active transport must be involved in order to remove the excess of sodium and potassium from the cytoplasm and to "pump in" chloride ions from the outside solution to the cytoplasm.

Table 6. The values of the difference between the measured potentials and the calculated ones from Nernst equation for plasmalemma and tonoplast membranes. E_m is the measured potential difference across a given membrane, E_{conc} is the potential difference calculated from Nernst equation for particular ions

Ion	$\Delta E = E_m - E_{conc}$ (mV)	
	Plasmalemma	Tonoplast
K	— 98.8	— 11.1
Na	— 117.4	+ 23.5
Cl	— 326.0	+ 46.5

From the data described in this paper it appears that there is active transport of potassium and sodium from the cytoplasm to the outside solution and of chloride from the outside solution into the cytoplasm (see Fig. 4).

As far as the tonoplast membrane is concerned there is active transport of sodium from the cytoplasm into the vacuole and active transport

of chloride from the vacuole to the cytoplasm. Potassium at the tonoplast is close to equilibrium.

The results of the present work can be compared with those of Spanswick and Williams (11) on the ionic concentration and bioelectrical potentials in the cells of *Nitella translucens*.

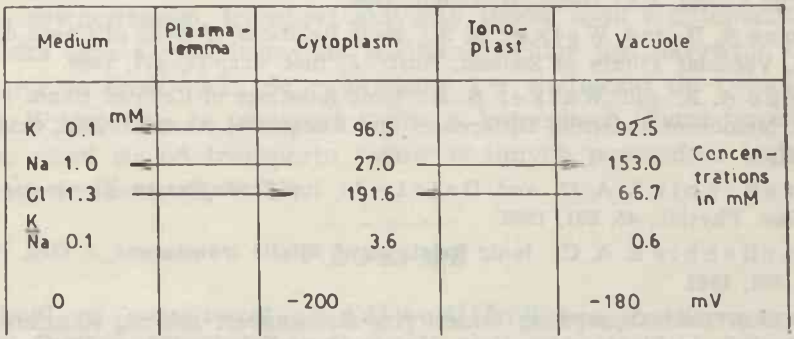

Fig. 4. The distribution of concentration and electrical potentials gradients in the cells of *Chara fragilis*. The arrows indicate the proposed direction of active transport

It is noteworthy that the ionic state of *Nitella translucens* is considerably different from that of *Chara fragilis*. There is active transport of potassium from the outside solution to the cytoplasm whereas the reverse is true for the cells of *Chara fragilis*. Sodium and chloride ions are far from equilibrium across the tonoplast membrane. Here again there is a difference between the cells of *Nitella translucens* and those of *Chara fragilis*. In the first case potassium is in equilibrium across tonoplast whereas from our results it appears that there is active transport of sodium from the cytoplasm to the vacuole and of chloride from the vacuole to the cytoplasm.

Experiments with selective inhibitors of ionic pumps are being undertaken in order to establish whether these pumps are electrogenic; they will be reported elsewhere.

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STRESZCZENIE

Praca niniejsza poświęcona jest zbadaniu rozmieszczenia jonów potasu, sodu i chloru w komórkach *Chara fragilis* i roli tych jonów w powstawaniu gradientów potencjału elektrycznego oraz roli potencjału elektrycznego w transporcie jonów w badanych komórkach.

Pomiary różnic potencjałów elektrycznych przeprowadzono za pomocą wewnątrzkomórkowych mikroelektrod szklanych dołączonych do wzmacniacza prądu stałego. Kationy oznaczano za pomocą fotometru płomieniowego, chlor — miareczkowaniem potencjometrycznym (12).

Stwierdzono występowanie znacznego skoku potencjału na granicy cytoplazmy i wodniczki (17 mV). Różnica potencjałów między cytoplazmą a środowiskiem zewnętrznym wynosi —200,4 mV, a między wodniczką a środowiskiem —180,3 (średnia wartość dla 47 komórek). Stężenia jonów sodu, potasu i chloru w cytoplazmie wynoszą odpowiednio: 27,0 mM, 96,5 mM, 191,6 mM. Stężenia tychże jonów w wodniczce były następujące: 153,0 mM Na, 92,5 mM K, 66,7 mM Cl.

Znajomość stężeń jonów i różnicy potencjału elektrycznego między środowiskiem a cytoplazmą i wodniczką pozwala na ustalenie lokalizacji oraz kierunku transportu aktywnego tych jonów (2).

Wyniki niniejszej pracy świadczą o tym, że w komórkach *Chara fragilis* znajdujących się w stanie równowagi stacjonarnej — ang. flux equilibrium (2) — istnieje transport aktywny jonów potasu z cytoplazmy do środowiska zewnętrznego, transport aktywny jonów sodu z cytoplazmy do środowiska oraz z cytoplazmy do wodniczki; chlor jest aktywnie transportowany ze środowiska do cytoplazmy i z wodniczki do cytoplazmy (ryc. 4). Wydaje się, że transport jonów w komórkach *Chara fragilis* zasadniczo różni się od transportu jonów w innych poprzednio badanych gatunkach ramienic (8, 9, 11).

РЕЗЮМЕ

Настоящая работа посвящена изучению распределения ионов калия, натрия и хлора в клетках *Chara fragilis* и роли этих ионов в генерировании градиентов биоэлектрических потенциалов, а также роли этих градиентов в транспорте ионов в растительных клетках.

Измерение биоэлектрических потенциалов проводилось с помощью стеклянных микроэлектродов и усилителя постоянного тока. Концентрации калия и натрия определялись с помощью пламенного фотометра, хлора — потенциометрическим титрованием (12).

Установлено существование значительного скачка потенциала через тонопласт (17 mV). Разница электрических потенциалов между цитоплазмой и средой составляла — 200,4 mV, между вакуолью и средой — 180,3 mV (средние для 47 клеток). Концентрации ионов натрия, калия и хлора в цитоплазме составляют соответственно 27,0 mV, 96,5 mV и 191,6 mV. Концентрации этих же ионов в клеточном соку равны: 153,0 mM Na, 92,5 mM K, 66,7 mM Cl.

Зная концентрацию ионов в цитоплазме и вакуоли и величину биоэлектрических потенциалов можно определить направление и локализацию активного транспорта этих ионов (2).

На основании результатов проведенных исследований можно заключить, что в клетках *Chara fragilis* существует активный перенос ионов калия из цитоплазмы в среду, ионов натрия из цитоплазмы в окружающую среду а также из цитоплазмы в вакуоль. Хлор активно переносится из окружающей среды в цитоплазму и из вакуоли в цитоплазму (см. рис. 4).

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