Ionic relations and electrophysiology of single cells of *Characeae*

Part V. Investigations on electric resistance of internodal cells of *Characeae*

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INTRODUCTION

The electric resistance of plant cells in the past was studied by various authors (Blinks 1930, 1936; Umrath 1940; Weidman 1940; Williams, Johnston and Dainty 1963; Findlay 1959; Walker 1961; Greenham 1966; Higinbotham, Hope and Walker 1964; Paszewski, Stolarek and Gębal 1968).

Walker (1960) published the results of investigations on the electric resistance of *Chara fragilis* of an unidentified species of *Nitella*. The electric resistance between the interior of the cell and the medium was measured with microelectrodes inserted into the vacuole or the cytoplasm when electric current was passed through the cell. For cells bathed by 1.0 mM NaCl the electric resistance of the plasmalemma of *Nitella* was $9 \text{ k}\Omega \cdot \text{cm}^2$ and of the tonoplast $1 \text{ k}\Omega \cdot \text{cm}^2$. The measurements of the total electric resistance of the tonoplast and of the plasmalemma of cells of *Chara australis* were made by two methods — microelectrodes giving the value of $14.8 \text{ k}\Omega \cdot \text{cm}^2$ and by external electrodes according to Blinks (1930); the latter method giving the value of $15.2 \text{ k}\Omega \cdot \text{cm}^2$. On the other hand respective figure obtained by Blinks for *Nitella flexilis* was $250 \text{ k}\Omega \cdot \text{cm}^2$, this value became established in the literature (Dainty 1962). It should be noted that Blinks measured electric resistance in cells soaked in distilled water which makes his work uncomparable with other measurements. Recently Greenham (1966) found that the electric resistance of the plasmalemma was $3 \text{ k}\Omega \cdot \text{cm}^2$ and of the tonoplast $0.5\text{--}0.6 \text{ k}\Omega \cdot \text{cm}^2$ in root hair cells, thus showing a similar picture of electric resistance in cells of higher plants to that in *Characean* cells.

Hope and Walker (1961) found that the resting electric properties of cell membranes of *Chara australis* can be described by the Goldman equation with the assumption (generally true) that the passive permeability of cells for anions is very small, and therefore may be disregarded in the consideration. The equation for electric resistance obtained from the Goldman equation subject to the condition of zero net current is:

$$r_0 = \frac{RT (1/C_0 - 1/C_p)}{F^2 \ln C_p/C_0} \quad (1)$$

where:

$$C_0 = P_K [K_0 + (P_{Na}/P_K) Na_0]$$

$$C_p = P_K [K_p + (P_{Na}/P_K) Na_p]$$

K_0 , Na_0 concentrations of K^+ and Na^+ in the external medium

K_p , Na_p concentrations of K^+ and Na^+ in the cytoplasm

$P_{Na}/P_K = \alpha (=0.06)$ — ratio of the permeability constant for sodium to that for potassium, obtained from measurements of bioelectric potentials.

With the above equation it is possible to calculate permeability constants P_{Na} and P_K . By using the abbreviated form of "constant field" expression for the influx of the j^{th} ion

$$M_j^i = \frac{FE}{RT} P_j C_i^o \quad (2)$$

(where M_j^i is the influx of ion j , P_j its permeability constant and C_i^o is the concentration of the ion in external medium). It is possible to calculate the influx of the ion from the medium to the cell, which in *Chara australis* is 10^{-12} M.cm $^{-2}$. sec $^{-1}$, for both sodium and potassium. The true values of the influxes measured with radioactive isotopes under the same conditions are, respectively, $M_{Na}^i = 0.1$ and $M_K^i = 0.4$ pM.cm $^{-2}$.sec $^{-1}$. It is thus clear that the values of the influx calculated from the electric resistance and potential measurements are ten times higher as compared to those measured with radioactive isotopes.

Dainty, Johnstone and Williams (1961) measured the electric resistance of the cell membranes of *Nitella translucens* bathed in the solution called "artificial pond water" or APW of the following composition: 0.1 mM CaCl $_2$, 0.1 mM KCl and 1.0 mM NaCl. They used glass electrolyte filled microelectrodes and rectangular impulses. The authors took into account the fact that these cells possessed cable properties as far as their electric conductivity is concerned. The value of electric resistance thus obtained was 19 k Ω . cm 2 and was considered to be the electric resistance of the plasmalemma. Applying this method the authors obtained an electric capacity of 1 μ f.cm $^{-2}$, which is in the contradiction with the data of Bennett and Rideal (1954) who found a capacity of 10^{-3} μ f.cm $^{-2}$.

MacRobbie (1962) on the basis of the values of the fluxes of sodium and potassium measured, calculated the electric resistance of the plasmalemma of cells of *Nitella translucens* and obtained 360 k Ω .cm 2 which value exceeds many times the measured one. Thus in cells of *Characeae* the measured electric resistance is by one order less than the one calculated on the basis of the Goldman equation.

Besides the enormous discrepancy between the measured value of electric resistance in cells of *Characeae* and the calculated value, it is noteworthy that the data on electric resistance of plant cells given by various authors differ to a great extent. These differences are partly due to the different media in which the cells remained before and during the experiments. A comparison of such data is difficult and necessitates closer investigations which have been undertaken in the present study carried out on cells of three species of *Characeae*: *Nitella translucens*, *Chara*

fragilis and *Lychnothamus barbatus*, the resistance of the cells of two latter species being investigated for the first time.

IAA (β -indolylacetic acid) bringing about the changes in the permeability of the cytoplasm and affecting the electric potential differences in cells of *Characeae* (Stolarek 1968) also affects the membrane resistance. The effect of IAA on electric resistance of cell membranes in three *Characeae* species was also investigated. Such a study was performed for the first time since there are no available data concerning the effect of IAA on electric resistance of plant cells.

MATERIAL and METHODS

The measurements of electric resistance were taken on internodal cells of *Nitella translucens*, *Chara fragilis* and *Lychnothamus barbatus*. The cells cultured in the laboratory, were separated from the stems and placed in APW for two to

Fig. 1. Block-diagram of the apparatus

1 — Galvanometr GES-2A or Oscilloscope; 2 — DC Amplifier

three days before the measurements. In some cases the cells were pretreated with 5 mM NaCl in order to remove exchangeable calcium from the cell walls (Spanswick, Stolarek and Williams 1967).

The diagram of the circuit used for the measurements of electric direct current resistance is shown in Fig. 1. During the experiments the cell was placed in a bath filled with a solution which can be replaced by other solutions if required. Part A of the apparatus injects subthreshold current of 0.5 μ A into the cell through a microelectrode E_{i1} inserted into the vacuole. The second "current" electrode E_{i2} is placed along the cell in its close proximity. A pair of electrodes E_{v1} and a microelectrode inserted into the vacuole and E_{v2} which is a calomel reference electrode

placed in the medium were used for the measurements of resting potential of the cells. When electric current of $0.5 \mu\text{A}$ was passed through the cell the electrodes E_{v1} and E_{v2} recorded the change in the resting potential of the cell. They were connected to an a.c. and d.c. push-pull amplifier (Gładyszewski and Stolarek 1966). In its turn the amplifier is joined to the oscilloscope OK-5. The accuracy of the measurements is about 5 %.

The measurements of electric resistance of cells of *Nitella translucens* were made by another method which is shown in Fig. 2. The impulse of the current of $0.5 \mu\text{A}$ and

Fig. 2. Block-diagram of the apparatus used in experiments on *Nitella translucens*

1 — Generator; 2 — Cathode follower; 3 — Tectronix Oscilloscope

Fig. 3. The equivalent electrical circuit for the half-length of a *Nitella* cell. (Williams, E. J. Johnstom R. J., and Dainty J. 1964). For the explanations see the text

of 0.3 sec. duration was injected through microelectrode E_{i1} inserted into the vacuole at a point at half length of the cell. The passing current was measured by the first scope of a double beam Tectronics oscilloscope connected to the ends of the resistor $R3$. The second scope of the same oscilloscope is connected via a cathode follower to the pair of electrodes E_{v1} and E_{v2} measuring changes in the resting potential.

The value of the resistance was obtained from Ohm's law:

$$R_m = \frac{\Delta V}{\Delta I} \quad (1)$$

where ΔV is the change of the resting potential and ΔI is the amplitude of the current impulse. When measuring electric resistance in internodal cells of *Characeae* the cable properties of the cells should be taken into account. It has been established that in these cells like in nerve fibers, the change in resting potential ΔV brought about by the current passed through the cell diminishes gradually when moving from the electrode injecting the current towards the ends.

Williams, Johnstone and Dainty (1963) applied to the cells of *Characeae* the electric model worked out by Hodgkin and Rushton (1945), shown in Fig. 3,

Fig. 4. The positions of the electrodes used by E. J. Williams, R. J. Johnston and J. Dainty (1964). (Explanations in the text)

Fig. 5. (Hogg J. 1968). Explanations in the text (abbreviated)

which takes into account the loss of current along the cell. Mathematical analysis of this model leads to the following dependance between the voltage V_x measured at distance x from the current electrode and the intensity of current I injected into the cell (Fig. 4).

$$V_x = \frac{I\lambda}{2(r_i + r_o)} \frac{\cosh(l - x)/\lambda}{\sinh l/r} \quad (2)$$

where l is the half length of the cell, $\lambda = \sqrt{\frac{r_m}{r_i + r_o}}$ is the space constant, r_m is the resistance of a unit length of the membrane (plasmalemma) in Ωcm , r_i is the resistance of the cell interior (Ωcm^{-1}) r_o is the resistance of the medium (Ωcm^{-1}).

As the equation (2) contains two unknowns r_m and $(r_i + r_o)$, the value of V_x was measured at two points along the cell at a distances $x_1 = a$ and $x_2 = b$ from the current injecting electrode (Fig. 4). The value of λ in *Nitella translucens* is 2.6 ± 1 cm and the electric resistance $R_m = 21.4 \text{ k}\Omega \cdot \text{cm}^2 \pm 10.6$.

J. Hogg (1968) found a very useful dependence between the distance x , half length of the cell l and the space constant. This analysis will be briefly summarised here.

The electric resistance can be calculated on the basis of Ohm's law $R'_m = \frac{V_x}{I}$ (surface area of the cell in cm^2). Thus R'_m is the measured electric resistance in $\Omega \cdot \text{cm}^2$. However, the true resistance of the cell should be equal to $R_m = \pi d r_m$ (3) where d is the cell diameter. From equation (2)

$$\frac{V_x}{I} \pi d 2l = \frac{r_m \cdot \pi \cdot d \cdot 2l}{2\lambda} \frac{\cosh(l - x)/\lambda}{\sinh(l/\lambda)}$$

and

$$R'_m = R_m \left[\frac{l}{\lambda} \frac{\cosh(l - x)/\lambda}{\sinh(l/\lambda)} \right]$$

It is seen that the measured value of the resistance R'_m is related to the true value R_m in the following way

$$R'_m = k R_m \quad (4)$$

where

$$k = \frac{1}{\lambda} \frac{\cosh(l - x)/\lambda}{\sinh(l/\lambda)}$$

Introducing new symbols

$$X = \frac{x}{\lambda} \quad \text{and} \quad L = \frac{l}{\lambda}$$

we get

$$k = L \frac{\cosh(L - X)}{\sinh L} \quad (5)$$

The value of k at a point $x = 0.42 l$ is one and the changes of λ do not affect k at this point (Fig. 5). This is an important point, as due to the above conclusion it was possible to use only one pair of electrodes for recording the change in the membrane potential brought about by injected current with the microelectrode inserted at a distance of $0.42 l$ from the middle point of the cell (Fig. 1 and 2). In this case $k = 1$ and $R'_m = R_m$. It was shown that for cell lengths up to 6 cm $\lambda = 1.6 - 4.0$ cm and $k = 1$. Such cells were used in this work.

RESULTS

a) Electric resistance in cells of *Chara fragilis*, *Lychnothamus barbatus* and *Nitella translucens*

Isolated internodal cells were stored before the experiments in a medium of the following composition: 0.1 mM KCl, 0.1 mM CaCl_2 and 1.0 mM NaCl further referred to as APW. The measurements of electric resistance were performed on cells kept in this medium. The experiments were carried under daylight in the day time and at night the room was illuminated with fluorescent tubes. The results of the measurements of electric resistance for the cells of three species are compiled in Table 1. The mean values are 15.6, 35.2 and 45.3 $\text{k}\Omega\cdot\text{cm}^2$ for *Chara fragilis*, *Lychnothamus barbatus* and *Nitella translucens* respectively. In some cells the electric resistance was measured 1, 3 and 17 hours after the insertion of the microelectrodes. It should be noted that the resistance showed a rather steady value during this period.

b) Effect of IAA on electric resistance in cells of *Chara fragilis*, *Lychnothamus barbatus* and *Nitella translucens*

Electric resistance was measured first in cells soaked in APW for one half hour after insertion of the microelectrodes. Then the new solution containing APW and IAA at a concentration of $2\cdot 10^{-5}$ was given and the electric resistance measured

Fig. 6. The effect of IAA on electric resistance measured between vacuole and external medium in a cell of *Nitella translucens*

after a half hours exposure was significantly less as compared to the control. The resistance was also measured after 1, 2, 3, 5, 10 and 20 hours (Table 2, Fig. 6).

It appears that IAA causes a large drop in electric d.c. resistance. The electric resistance of cells of *Nitella translucens* after 3 hours' exposure in IAA solution is only 45 % of the control value i.e. it diminished more than twice. In some cells the drop in resistance was even greater e.g. cell 5 in Table 2. The resistance after 3 hours in this cell was only 25 % of the initial value. The fall in electric resistance

Table 1

Electric d.c. resistance measured between the vacuole and external solution in cells of three *Characean* species in control solution (A.P.W.)

Species	Cell	Electric resistance ($k\Omega cm^2$) measured after a given period after insertion of microelectrodes		
		1 hour	3 hours	17 hours
<i>Nitella translucens</i>	1	30	30	31
	2	35	36	
	3	52	52	
	4	40	40	39
	5	29	29	27
	6	59	58	
	7	28	28	29
	8	76	77	
	9	37	37	
	10	45	45	
	11	48	49	50
	12	65	36	35
	Mean	45.3 ± 3.4		
<i>Chara fragilis</i>	1	15	15	15
	2	10	10	12
	3	12	12	11
	4	22	21	23
	5	14	14	15
	6	13	13	14
	7	16	16	16
	8	18		
	9	17		
	10	19		
	Mean	15.6 ± 1.1		
<i>Lychnothamus barbatus</i>	1	45	44	
	2	49	48	
	3	37	35	
	4	25	26	
	5	38	39	
	6	41	42	
	7	27	28	
	8	30		
	9	31		
	10	29		
	Mean	35.2 ± 2.4		

Table 2

Effect of IAA on electric resistance of cells of three species of *Characeae*

Electrical d.c. resistance measured between the vacuole and the external solution of the following composition:

A.P.W. + auxin (conc.: 2.10^{-5} M) after a given time of soaking

SPECIES	Nr. of experiments	A.P.W. + auxin (conc.: 2.10^{-5} M) after a given time of soaking									
		30 min.		1 hr.		2 hrs.		3 hrs.		5 hrs.	
		k Ω .cm ²	% of the control	k Ω .cm ²	% of the control	k Ω .cm ²	% of the control	k Ω .cm ²	% of the control	k Ω .cm ²	% of the control
<i>Nitella translucens</i>	"Artificial pond water" (A.P.W.) CONTROL.										
	1	55	92,0	45	89,0	35	63,6	30	55,4	31	56,3
	2	75	73,3	52	69,0	48	61,0	45	60,0	45	60,0
	3	151	86,0	105	69,5	80	46,3	62	41,0	58	38,4
	4	120	80,0	84	70,0	70	58,3	63	52,5	62	51,6
	5	180	55,5	80	44,4	65	36,1	55	25,0	55	30,5
	6	80	62,5	40	50,0	35	43,7	30	37,5	25	31,2
	7	80	82,5	60	75,0	50	62,5	45	56,2	45	56,2
	8	54	92,6	43	79,6	32	59,2	28	51,8	28	51,8
	9	85	59,2	40	47,0	35	41,1	35	41,1	36	42,3
<i>Chara fragilis</i>	Mean $\pm$ s.e.m.	82,4	75,2	61,0	65,9	53,3	53,3	46,4 $\pm$ 5,1	47,2 $\pm$ 3,7	43,2	46,6
	1	15	66,6	9	60,0	9	60,0	9	69,0	8	53,3
	2	12	83,3	8	66,6	7	58,3	7	58,3	7	58,3
	3	16	75,0	9	56,2	8	50,0	8	50,0	8	50,0
	4	23	86,9	18	78,2	17	73,9	16	69,5	16	69,5
	5	15	66,6	10	66,6	9	60,0	9	60,0	9	60,0
	6	19	63,1	11	57,9	10	59,6	10	52,6	9	47,4
	7	16	60,0	7	43,7	7	43,7	6	37,5	6	37,5
	8	18	66,6	10	55,5	9	50,0	9	50,0	9	50,0
	9	24	75,0	13	54,1	12	50,0	11	45,8	10	41,6
<i>Lychnorhannus barbatus</i>	10	19	68,4	11	57,9	10	52,6	9	47,4	10	52,6
	Mean $\pm$ s.e.m.	17,7 $\pm$ 1,2	77,1	10,6	74,6	9,9	55,7	9,3 $\pm$ 0,27	53,4 $\pm$ 2,78	9,2	52,0
	1	44	90,9	36	81,8	35	79,5	35	79,5	36	81,8
	2	48	85,4	35	72,9	30	62,3	26	54,1	25	50,2
	3	35	85,7	24	68,5	17	48,5	15	42,8	14	40,0
	4	26	73,0	15	57,7	13	50,0	13	50,0	13	50,0
	5	39	76,9	23	58,9	19	48,2	19	48,2	19	48,2
	6	42	78,6	26	61,9	22	52,4	20	47,6	21	50,0
	7	28	71,4	15	53,6	14	50,0	14	50,0	14	50,0
	Mean $\pm$ s.e.m.	37,3 $\pm$ 3,5	80,2	24,8	65,0	21,4	55,8	20,3 $\pm$ 3,6	53,1 $\pm$ 4,5	20,3	52,8

20 hrs.
k Ω .cm²
% of the control

10 hrs.
k Ω .cm²
% of the control

5 hrs.
k Ω .cm²
% of the control

3 hrs.
k Ω .cm²
% of the control

2 hrs.
k Ω .cm²
% of the control

1 hr.
k Ω .cm²
% of the control

30 min.
k Ω .cm²
% of the control

started just after the addition of IAA and after the lapse of 30 min. it diminished by 25 % in *Nitella translucens*, 23 % in *Chara* and 20 % in *Lychnothamus*.

c) Effect of IAA and ouabain on electric resistance in cells of *Chara fragilis*

The effect of ouabain — selective inhibitor of active potassium transport — on electric resistance of cells of *Chara fragilis* was investigated when ouabain had been added to the following media in which the cells were soaked: APW and APW+IAA ($2 \cdot 10^{-5}$ M).

It was found that ouabain inhibited the stimulatory effect of IAA on potassium uptake by *Nitella translucens* and it also diminished the hyperpolarisation caused by IAA in cells of *Characeae* (J. Stolarek 1968). Thus it seemed worthwhile to investigate the effect of ouabain on control and IAA treated cells. The results of such measurements are listed in Table 3. The resistance was measured first in cells bathed by APW (control medium) and then in the same cells bathed by the next solution: APW+IAA ($2 \cdot 10^{-5}$ M) and in three hours' time the resistance was

Table 3

Effect of auxin and ouabain on electrical d.c. resistance measured between vacuole and external medium in cells of *Nitella translucens* and *Chara fragilis*

Species	Cell	"Artificial pond water" (A.P.W.) CONTROL k Ω .cm ²	A.P.W.+auxin ($2 \cdot 10^{-5}$ M), 3 hrs. of soaking		A.P.W.+($2 \cdot 10^{-5}$ M) of auxin+ouabain (10^{-5} M)			
			k Ω .cm ²	percentage of the control	30 min. of soaking		1 hr. of soaking	
					k Ω .cm ²	% of the control	k Ω .cm ²	% of the control
<i>Nitella translucens</i>	1	75	30	40,0	46	61,3	46	61,3
	2	45	25	55,5	32	71,1	33	73,3
	3	52	21	40,3	30	57,7	31	59,6
	4	89	43	48,3	55	62,9	58	65,1
	5	68	25	36,7	34	50,0	36	52,9
	6	90	38	42,2	51	57,7	53	58,8
	Mean $\pm$ s.e.m.	69,5 $\pm$ 7,47	33,3 $\pm$ 3,71	43,8 $\pm$ 2,84	41,3 $\pm$ 4,33	61,6 $\pm$ 2,02	42,8 $\pm$ 4,5	61,8 $\pm$ 2,8
<i>Chara fragilis</i>	1	15	8	53,3	12	80,0	12	80,0
	2	12	8	66,6	14	116,6	14	116,6
	3	16	8	50,0	14	75,0	10	62,5
	4	23	16	69,5	19	82,5	21	91,3
	5	15	10	66,6	13	86,6	14	93,3
	6	19	10	52,6	14	73,6	14	73,6
	7	16	7	43,7	10	62,5	10	62,5
	8	18	10	55,5	13	72,2	12	66,6
	Mean $\pm$ s.e.m.	17,7 $\pm$ 1,9	9,9 $\pm$ 0,8	56,1 $\pm$ 1,2	13,8 $\pm$ 0,2	79,0 $\pm$ 4,8	13,9 $\pm$ 0,3	78,7 $\pm$ 5,4

Fig. 7. The effect of auxin and ouabain on electric resistance measured between vacuole and external medium in nine cells of *Chara fragilis*

measured again, showing a drop of 44 % as compared to the initial value. Then another solution was given: APW+IAA ($2 \cdot 10^{-5} \text{ M}$)+ouabain ($5 \cdot 10^{-5} \text{ M}$) and the resistance was measured again after 30 min. and 1 hour. Ouabain when added to the medium containing IAA brought about a 23 % increase of electric resistance (Tab. 3 and Fig. 7). The electric resistance of control cells i.e. not pretreated with IAA was not affected by ouabain (Tab. 4).

Table 4

Effect of ouabain and Mg^{2+} ions on electrical resistance of cells of *Chara fragilis*. The measurements were done after 30 min. of soaking in each solution ($k\Omega \cdot \text{cm}^2$)

Cell	Electrical resistance in the solution of the following composition:			
	"Artificial pond water" (A.P.W.) CONTROL	A.P.W. + MgCl_2 (5mM)	A.P.W. + ouabain ($5 \cdot 10^{-5} \text{ M}$)	A.P.W. + ouabain ($5 \cdot 10^{-5} \text{ M}$) + MgCl_2 (5 mM)
1	20	20	20	20
2	15	16	15	14
3	17	17	16	16
4	21	20	19	19
5	25	24	24	23
Mean $\pm$ s.e.m.	19,5 $\pm 1,3$	19,9 $\pm 1,2$	19,0 $\pm 1,3$	18,3 $\pm 1,2$

Fig. 8. The effect of auxin, ouabain and Mg^{2+} on electric resistance in 10 cells of *Chara fragilis*

Table 5

Effect of IAA, ouabain and Mg^{2+} ions on electrical d.c. resistance recorded between vacuole and external medium in cells of *Chara fragilis*

Electrical resistance measured in cells bathed by the following solutions:							
Cell	"Artificial pond water" (A.P.W.) CONTROL kΩ.cm ²	A.P.W.+IAA (2·10 ⁻⁵ M) after 20 hours of soaking		A.P.W.+IAA (2·10 ⁻⁵ M)+ouabain (5·10 ⁻⁵ M), after 30 min. of soaking		A.P.W.+IAA (2·10 ⁻⁵ M)+ouabain (5·10 ⁻⁵ M)+Mg ²⁺ (5m N), after 30 min. of soaking.	
		kΩ.cm ²	% of the control	kΩ.cm ²	% of the control	kΩ.cm ²	% of the control
1	15	8	53,3	12	80,0	10	66,6
2	12	8	66,6	14	117,0	9	75,0
3	16	8	50,0	12	75,0	8	50,0
4	23	16	69,5	19	82,6	15	60,9
5	15	10	66,6	13	86,6	9	60,0
6	19	10	52,6	14	76,3	10	52,1
7	16	7	43,7	10	62,4	8	50,0
8	18	10	55,5	13	72,2	12	66,6
9	24	11	41,6	15	62,5	10	31,6
10	19	11	57,8	15	78,9	12	63,2
Mean ± s.e.m.	17,7 1,0	9,9 0,5	66,7	16,7 4,7	79,3 0,2	10,3 4,9	56,0 0,6

d) Effect of magnesium ions on electric resistance of cells of *Chara fragilis*

It was found that ouabain inhibited the stimulatory effect of IAA on potassium uptake and that magnesium ions partly prevented this inhibitory effect of ouabain (J. Stolarek 1968). The measurements of electric resistance described in this section were carried out on the same group of cells which were used in the experiments discussed in the previous section (Tab. 3). The electric resistance of these cells was measured first in APW to obtain control value. The next measurements of the resistance were made in cells soaked in APW+IAA. The third measurement was made when cells were kept in the solution: APW+IAA+ouabain. The data obtained (Table 5) showed that IAA caused a 43 % decrease of electric resistance and when ouabain was added the resistance increased from 9.9 to 14 $\text{k}\Omega\cdot\text{cm}^2$. The fourth measurement made after substitution of the previous medium by APW+IAA+ouabain+ Mg^{2+} (5 mM) gave a resistance value of 10.3 $\text{k}\Omega\cdot\text{cm}^2$ i.e. 56 % of the control). It appears that magnesium ions counteracted the effect of ouabain on IAA treated cells. It should be noted that magnesium ions did not affect electric resistance of cells when added to APW or to APW+ouabain (i.e. without IAA) (Table 4, 5 and Fig. 8).

DISCUSSION

In spite of the difficulties associated with the measurements and interpretation of electric resistance in plant cells the above described experiments resulted in some interesting findings. Though the electric resistance across the cytoplasm in cells of *Chara fragilis* and *Lychnothamnus barbatus* was measured for the first time in the present work, the results obtained on cells of *Nitella translucens* are of more significant interest here owing to the fact that ionic relations of this species were subjected to detailed study (MacRobbie 1962, 1964; Stolarek 1968).

The mean resistance for 12 cells of *Nitella translucens* is 45 $\text{k}\Omega\cdot\text{cm}^2$ (Tab. 1) but the values obtained for some cells are significantly higher: 76, 65, 59. In another control series of measurements accomplished on 9 cells (Tab. 2) the average value is 97.7 $\text{k}\Omega\cdot\text{cm}^2$, but there were cells with 180, 151 and 120. The similar cells of *Nitella translucens* kept in identical conditions show very divergent values of the membrane resistance. Owing to this in the experiments value of the resistance measured in each cell after a given treatment was compared with the resistance of the same cell in control conditions i.e. each cell provided a control for itself.

MacRobbie (1962) calculated the electric resistance from the "constant field theory" (equation 1) for *Nitella translucens* and obtained 360 $\text{k}\Omega\cdot\text{cm}^2$. The d.c. resistance in *Nitella translucens* measured in the present work was 45 and 98 $\text{k}\Omega\cdot\text{cm}^2$ for two groups of cells respectively. It is noteworthy that the figure 98 $\text{k}\Omega$ is only 3.6 times lower than the theoretical value whereas the common opinion is that the theoretical value is 10 times higher than the measured one (MacRobbie, 1962; Dainty 1961).

IAA brings about a large decrease of electric resistance in cells of *Characeae*. In some cells the resistance dropped by 70 % (Table 2). Such a large decrease

cannot be accounted for by taking solely into account the increase in the permeability of the cytoplasm for potassium ions found in IAA treated cells (Stolarek, 1968). The IAA induced decrease of the resistance is far larger than that calculated from equation 1 when taking a twofold increase of P_K (the permeability constant for potassium).

However, the present experiments revealed that ouabain — an active potassium transport inhibitor — when added to the control medium did not affect electric resistance whereas in the medium containing IAA it caused an increase of electric resistance. This leads to the conclusion that ouabain has an opposite effect as compared to IAA. Ouabain added to the external media without IAA did not affect bioelectric potential, whereas it decreased the hyperpolarisation brought about by IAA.

Taking into account the above arguments it can be supposed that IAA causes not only changes in potassium permeability but also increases the electron conductivity through the membrane (plasmalemma). This supports the author's hypotheses that in IAA treated cells an active electrogenic transport takes place (Stolarek 1968).

The results of the measurements of electric resistance in *Characeae* cells treated with IAA and ouabain indicate that in this case the electric resistance directly depend not only on passive potassium and sodium transport but also on active transport of potassium and "the constant field equation" is thus inadequate for the description of the changes in electric resistance.

IAA increases the permeability of the plasmalemma for potassium and both active and passive potassium influxes are stimulated (Stolarek 1968). Inhibition of active IAA induced potassium influx by ouabain affected both membrane potential and resistance, the change in the membrane potential being abrupt. It seems probable that IAA can act as an electron carrier and such a behaviour of IAA would account for the large drop in resistance it induces. Such a view is in agreement with Overbeek's (1951) hypotheses suggesting that IAA is built into the plasmalemma of the cells treated with it. If we accept the well known Skou's hypotheses (1963) of active potassium and sodium transport across plasma membrane, it is probable that IAA creates charge transfer complexes which transfer electrons from the $ATP.Mg_2$ complex to the sites in the membrane responsible for vectorial ion transport. According to Eisenman's (1961) theory the affinity of the membrane's negatively charged groups for potassium or sodium ions depends on the field strength near those charges. Such a movement of electrons across the membrane as postulated here from $ATP.Mg_2$ via IAA can be involved and even responsible for the electrogenicity of active potassium IAA induced transport. According to A. Szent-Györgyi (1960) IAA can create charge transfer complexes with common electron donors and acceptors. A similar suggestion was recently advanced by Kulicki and Lorenc (1967) as far as the possibility of the occurrence of charge transfer complexes between cytochromes is concerned.

It should be noted that the electrogenic active transport induced by IAA can also be easily interpreted in terms of recently postulated mechanisms of vectorial

active cation transport across outer plasma membrane (Lehninger 1966; Neifach 1967). Another relevant point is due to Slayman (1965) who has found in *Neurospora crassa* the existence of a metabolic component of bioelectric conductivity.

The above discussed facts suggest that it would be interesting in the future work to take into account the properties of IAA as electron conductor in plant cells. The relationship between active potassium transport, electric potential and conductivity across the membrane found in the present work corroborate the author's hypotheses that active potassium transport in plant cells is electrogenic in certain conditions.

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SUMMARY

The electric resistance for direct current of the cytoplasm of internodal cells of *Nitella translucens*, *Chara fragilis* and *Lychnothamnus barbatus* was 45, 35 and 15 k Ω .cm², respectively. The measurements were made with the use of glass 3 M KCl filled microelectrodes and calomel electrodes. The rectangular current impulses were passed through the cell by means of a microelectrode inserted into the vacuole and the change the pulse produced in the membrane potential was recorded with another pair of microelectrodes measuring potential difference between the vacuole and the external medium.

IAA at a concentration of $2 \cdot 10^{-5}$ M caused a large decrease of the resistance in all investigated cells. The effect of ouabain and magnesium ions was also tested. The discussion of observed phenomena is given.

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Stosunki jonowe i elektrofizjologia pojedynczych komórek Characeae

Część V. Badania nad oporem elektrycznym internodialnych komórek ramienic

Streszczenie

Badano opór elektryczny dla prądu stałego pojedynczych komórek *Nitella translucens*, *Chara fragilis* i *Lychnothamnus barbatus*; otrzymano następujące wartości oporu: 45, 35 i 15 k Ω .cm² odpowiednio dla wymienionych gatunków.

Stosowano mikroelektrody szklane napełniane 3M KCl i elektrody kalomelowe. Do wnętrza komórek podawano impulsy prostokątne o amplitudzie 0.5 μ A i obserwowano zmianę potencjału spoczynkowego, skąd obliczano opór elektryczny między wodniczką a środowiskiem. Kwas β -indoliloctowy (IAA) w stężeniu $2 \cdot 10^{-5}$ M powoduje duży spadek oporu elektrycznego we wszystkich badanych komórkach. Obserwowano także zmiany oporu elektrycznego pod działaniem ouabainy i jonów magnezu. Przeprowadzono dyskusję otrzymanych wyników.

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