Changes of the trans-membrane potential of the leaf cell of *Funaria hygrometrica* under influence of light

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INTRODUCTION

Changes in the value of trans membrane potential of the green plant cells under illumination were reported by some investigators (Bliks, 1939, 1955, Marsh 1939, Nagai and Tazawa 1962, Nishizaki 1963, Volkov 1964, Schilde 1966, Metlicka and Rybova 1967) although not only the range of changes but also their direction do not coincide in all cases. Most of the mentioned studies were done on big cells of *Nitella*, *Chara*, *Valonia* and *Acetabularia*. Only few data concerning small algae assimilating cells are available (Metlicka and Rybova 1967).

In our study of light induced chloroplasts displacements we found that the photo-receptor active in this phenomenon is, at least in *Funaria* leaf cell, situated in the outer plasma membrane. It suggests that the primary photoreaction leading to displacements of chloroplasts may be connected with changes in permeability of plasmalemma and consequently with changes of the transmembrane potential.

The aim of the present study was to investigate the connections between two light dependent reactions of the cell: changes in the transmembrane potential and rearrangements of chloroplasts. Leaves of the moss *Funaria hygrometrica* were chosen for investigation material. The second phenomenon and its dependence on light quantity and quality has been recently reported (Zurzycki 1967 a-c) for this material.

MATERIAL AND METHODS

Plants used for experiments were taken from laboratory cultures of *Funaria* grown from spores.

Leaves were cut off with a sharp razor blade in a drop of artificial pond water. This solution used as medium for all experiments was prepared according to Spanswick et al. (1967) in the following composition: NaCl 1mM, KCl 0,1mM, Ca Cl₂ 0,1mM. Cutting off leaves was performed under a dissection microscope. About 2/3 of the upper part of lamina were cut out and transferred to a small drop of medium on a cover glass. The leaf was pressed down with a small fragment of another cover glass so that about a half of its length including basal part was covered the other

part (tip part) was free. The excess of liquid medium was sucked by blotting paper and a drop of hot vaseline was put on the leaf. After solidification of vaseline the small part of the cover glass was removed and a new drop of liquid medium was put on the free part of the leaf. By this procedure the leaf was fixed stable on the glass which permitted to introduce the microelectrode into the cell without any shifting of the blade. The cover glass with the fixed leaf was used as the top of a humid chamber of a Zeiss gliding micromanipulator.

For potential measurements Ag-AgCl electrodes were used. Micropipettes were made on an automatic needle puller; their tip diameter was $1-2\ \mu$. The tubes for micropipettes the melting point capillaries "Klimax" (A. H. Thomas Co Phila-

Fig. 1. Relative transmission of the filter sets used for experiments *b* — blue, *g* — green, *r* — red, *fr* — far red, *ir* — infra red range. Broken line — filters used for white light.

delphia) 1 mm diameter were used. For filling the micropipettes the procedure of Tasaki at all. (1954) was used. The pipettes were filled with boiling methanol under vacuum and subsequently methanol was replaced by diffusion by a saturated KCl solution. Resistance of the microelectrode was about 100 M Ω .

The potential was measured with electrometer Orion Keti (Budapest) type TR 1501 of impedance 10^{14} and registered by compensating recorder type KBT 3/EN (Messgeräte — und Armaturenwerk, Karl Marx Stadt).

The leaves were illuminated according to Köhler principle; a microscopic lamp 100 W 12V with filters was used for illumination. For white light GG 13/2 + KG 2/5 mm filters were used. Chromatic ranges were selected by following filters sets: BG 1/2, GG 13/4, KG 1/5, BG 23/2 (blue), GG 14/2 VG 8/2 KG 1/5 (green), RG 1/2, KG 1/5 (red), RG 8/2, KG 1/5 (far red), UG 8/2, BG 1/2 mm (infrared). Relative spectral transmission curves of the filter sets is presented in fig. 1. Measurements of radiation intensity were made with thermoelements described elsewhere (Zurzycki 1961).

Plate I

Leaf cells during intracellular potential recordings.

Above: normal cells, the electrode tip in the vacuole; below: centrifugated cells, the electrode tip in the cytoplasm.

Various radiation intensities were obtained by using neutral glass filters for the visible range and a wire screen filter for infrared.

In one series of experiments potentials were measured on centrifugated cells. The moss stem with leaves was fixed in a proper position (Zurzycka and Zurzycki 1951) and centrifugated by 1600 xg (3500 rpm) for 20 min. Cutting off leaves mounting and potential measurements were performed immediately after centrifugation.

Plate I shows normal and centrifugated leaves after introduction of micro-electrodes into the cell.

Registration of the position of chloroplasts was performed according to the formerly described method (Zurzycki 1962).

RESULTS

The thickness of the cytoplasmic layer in a *Funaria* cell is very small. When the microelectrode goes through the cell wall it inserts immediately through the cytoplasmic layer to the vacuole. The introduced electrode becomes negative in respect

Fig. 2. Histogram displaying the results of the potential measurements made on the not pretreated — — — and pretreated — leaves.

The Y axis describes the % of readings obtained at a particular potential difference interval.

to the medium. The value of the potential difference rises after introduction of the electrode at first quickly then slower and slower and reaches a stable value after 1–3 min (fig. 3). The registered “intracellular potential” represents essentially the sum of potential differences across the plasmalemma and the tonoplast. As it will

be shown later on, the potential difference on the tonoplasts is negligible so that the intracellular potential represents practically the plasmalemma transmembrane potential. When the tip diameter of the electrode was $3-5\ \mu$ or more, the potential begins to drop slowly after some minutes of steady level, sometimes (with bigger

Fig. 3. Examples of the recordings. Upper curve: cells in darkness, electrode introduced at 0 time. middle and lower curves: effects of white light on the potential difference. ↓ light on, ↑ light off

electrodes) just immediately after reaching the maximal value. Some time later visible changes in the chloroplast structure appeared — and the cell began to die. When the tip of the electrodes is between $1-2\ \mu$ the measured cells live 20–30 min. or more and during this time the potential value is essentially stable (fig. 3). From time to time very small variations of the order $1-5\ \text{mV}$ appeared (fig. 3) — similar to those found by Slayman (1965) in *Neurospora*.

The absolute value of the stable potential depends to some extent on the pretreatment of the material. When the measurements were made in short time ($1/2-3$ hours) after mounting of the leaves variability of the measured value is bigger (fig. 2) and the mean potential is $102.21 \pm 2.10\ \text{mV}$. When leaves after mounting on the cover glass were kept for 24 hours in a humid dark chamber the measured potential values are higher (mean $132.02 \pm 1.79\ \text{mV}$) and their dispersion is smaller (fig. 2). Most of the investigations of the influence of light on the potential changes were carried out on dark pretreated leaves, although the reaction to light is in both materials principally the same.

The measured values constituted the potential difference between vacuole and the outer medium. It was impossible to situate the tip of the electrode into the

cytoplasm because of the very thin layer of it. Some measurements were made on the cells in which the thickness of cytoplasm layer was artificially increased. These were centrifuged cells in which a thick layer of chloroplasts and protoplasm is accumulated on one cell side (Plate I). In such cells it was possible to introduce the

Fig. 4. Examples of recordings of potential measurements in centrifugated cells. *C* — the electrode tip in the cytoplasm *V* — the electrode tip in the vacuole.

tip of electrode into the cytoplasm, to measure the potential difference (cytoplasm — medium) after its stabilisation and then to pull in the electrode some microns deeper and to measure the potential difference vacuole — medium in the same cell (fig. 4).

Some results are present in table 1.

Table 1

The value of transmembrane potentials in centrifugated cells

Cell No	Potential difference mV	
	cytoplasm-medium	vacuole-medium
1	99	93
2	90	90
3	118	109
4	125	119
5	105	102
mean	107,4	102,6

Although the number of measurements was not great enough to ascribe the cytoplasm — vacuole potential difference a significant value it may be stated that this difference is very small and that plasmalemma is the main potential barrier in the *Funaria* cells.

The stable potential value reached in 1–3 min following introducing of the electrode into the vacuole does not change when the cells are transferred from dark-

Fig. 5. Upper curve: changes in the potential difference under influence of white light of different intensity. The intensity 100 is equal 50 klx. Lower curve: changes in the potential difference under influence of coloured light of the same intensity (10^5 ergs/cm² sec), *b* — blue, *g* — green, *r* — red.

ness to weak light or reverse. Illumination of the leaf with strong white light induces changes of the measured potential difference. Changes taking place in the course of illumination consist of three phases (fig. 6).

I. A decrease of potential difference. It begins immediately after illumination and lasts 1–2 min. During this time the potential difference drops to the new value, which depends on light intensity. In very high intensity the change of potential may reach 25–35% of the former value (fig. 3).

II. A steady state. In continuous illumination the decreased potential value remains stable for some time. Very often this time is rather short so that the potential curve begins to rise almost immediately after reaching minimum value. But sometimes the steady state lasts till 2–5 min. (fig. 3).

III. A slow rise of the potential value. During the next 5–10 min the potential value rises rather slowly and becomes stable at the level very close to the former value (before illumination). Only in some cases an increase of the potential to a higher level than before illumination was found during this period. Switching off the light

induces in each phase reverse changes of potential. The potential difference rises to the former value i.e. that before illumination. These changes occur at a much faster speed in comparison to that under continuous illumination. After switching off the light in phase I or II new potential values are obtained in a direct way. When illumination lasts longer (phase III especially its end), the break of illumination

Fig. 6. Scheme of the changes of potential difference in the dark — — —, and in the light — — —.

induces the rise of potential value above the steady state and then its slow drop. The described phenomena are schematically shown in fig. 6 and fig. 3 presents some examples of the recordings.

In the subsequent part of our study special attention was paid to phase I — especially the dependence of the magnitude of potential changes (Δ — fig. 6) on light intensity and quality.

As it was mentioned the decrease of the potential value under illumination depends on the intensity of light.

Because of the variability of the absolute potential values between different cells it seemed reasonable to take as a quantitative measure of potential changes induced by light not its absolute value in mV but its relative value (in percent of the dark potential).

The dependence of mean potential decrease on white light intensity is shown in fig. 5 and fig. 7. As it follows from the graphs the light of intensity lower than 10^3 lx does not induce measurable changes in the potential. The higher light intensity the bigger the drop of potential. The saturation of the reaction takes place at about 5×10^4 lx. In the same figure the dependence of chloroplasts arrangements on light intensity was plotted. The position of chloroplasts was established after 1.5 h. of illumination i.e. after adaptation to the new light intensity. Both curves show some coincidence. Significant changes in potential value are induced at this intensities in which high light displacement of chloroplasts begins. Saturation of the potential

Fig. 7. Dependence of light induced potential change (Δ) on white light intensity. Every point represents the mean of 10—20 measurements, rectangles mark the mean errors, vertical lines — the extreme dispersion of the measurements. Dependence of chloroplast arrangements ($\%E$) on light intensity.

Fig. 8. Dependence of the mean potential change (Δ) on radiation intensity for different spectral ranges.

changes curve coincide with the light intensity inducing half displacement of chloroplasts.

Experiments with selected spectral regions of radiation showed that the described effect of light on the membrane potential is induced by the whole visible range of the spectrum.

The dependence of potential decrease on radiation intensity for the investigated spectral regions is shown in fig. 5 and fig. 8. Taking as a criterion of activity the intensity of radiation causing mean decrease of potential of 12% of the former

Fig. 9. Spectral activity of radiation for changes of the potential difference. As the measure of spectral activity the reversal of the radiation intensity D (in $\text{erg/cm}^2 \text{ sec}$) causing 12% of potential change was plotted.

value — the relative spectral activity may be calculated (fig. 9). The highest activity was found for blue, lower for green and medium for red. Activity of far red is very low and infrared shows no activity at all. The relative activity of the mentioned spectral ranges is 1:0.45:0.66:0.07:0 respectively.

DISCUSSION

The effect of light on the electrical phenomena appearing in green leaves have been reported several times. In these studies the method of extracellular recordings by means of electrodes put in contact with the illuminated and darkened parts of leaf was applied (Haake 1892, Waller 1900, Waller 1925, Glass 1933, Uspenskaja 1951). In most cases the appearance of positive potential in the illuminated part of the leaf was stated. An increase of the potential difference between both electrodes during 1—10 minutes after application of light and of its value up till 100 mV were registrated (Waller 1925, Glass 1933, Uspenskaja 1951). Waller (1925) however found that in leaves of some species i.e. *Rosa*, *Rhododendron*, *Pelargonium*, *Quercus* a reverse effect takes place i.e. appearance of minus potential in the illumina-

ted part. Glass (1933) found that the sign of the potential change is related with the topography of the leaf. In *Elodea* leaves illumination induces a positive potential in the apical and a negative in the basal part of the leaf. Some experiments made with coloured light (Waller 1925) showed that, as well blue as yellow and red light can produce potential changes. This fact together with the significant influence of CO₂ concentration on light induced potential changes suggest that the phenomenon in question is related with photosynthesis (Waller 1925). A close correlation between the range of potential changes and photosynthetic activity found by Uspenskaja (1951) also support the last conclusion.

For intracellular recordings of the electric potentials among green plants big algae cells are usually used. There are only scarce data about the internal potential values of the tissue cells. The results of the present measurements showing the resting potential of *Funaria* cells in the range 65–160 (mean 102 mV) for not pretreated cells and 95–185 (mean 132 mV) for pretreated cells lay in the range of potential values reported for tissue cells (Umrath 1956; Etherton and Higinbotham 1960). Recently Sinclair (1967) published the results of potential measurements in the leaf cells of the moss *Hookeria lucens*. His results are very similar to ours for pretreated cells: mean potential 145 mV for *Hookeria*, 132 for *Funaria*, range of distribution 110–230 for *Hookeria*, 95–185 for *Funaria*, normal pattern of distribution around 110–180 and a group of results around 200 for *Hookeria*, normal distribution at the range 95–160 and a group of results around 170 for *Funaria*.

Preliminary measurements of the potentials in vacuole and in cytoplasm showed no significant difference between these two elements. The main if not all the potential difference seems to be localised across plasmalemma. This behaviour is very similar to the phenom found in *Nitella* (Walker 1955, Spanswick at all 1967) and does not agree with the supposition of Sinclair assuming a considerable tonoplast potential difference in moss cells.

The effect of light on the intracellular potential was studied exclusively on big algae cells. Blinks (1939) used for his experiments *Halicystis*, Blinks (1955), Nagai and Tazawa (1962), Volkov (1964) — *Nitella*, Nishizaki (1963), Hope (1965) — *Chara*, Marsh (1939), Blinks (1955) — *Valonia*, Schilde (1966) — *Acetabularia* Retlicka and Rybova (1967) — *Hydrodictyon*. Up till now no effect of light on the internal potential of green tissue cells has been studied.

The results obtained on the algae cells have shown that light induces reversible changes in the internal potential values. But the reported signs of this changes are different in different materials — quite similar as the above discussed results of extracellular recordings. In most cases an increase in the potential difference between the inside of the cell and the medium was found after illumination (Nagai and Tazawa 1962; Schilde 1966; Retlicka and Rybova 1967). Blinks (1955) stated a decrease of potential difference following illumination and Volkov (1964) a decrease at the beginning and a subsequent increase in prolonged illumination. Nishizaki (1963) found that not only the range but also the direction of potential changes depends on the pretreatment of the material.

In our study a decrease of the potential difference after illumination always was

found. The range of changes (average 30, maximal 50 mV for strong light) lays in the limit found for other objects (30—80 mV). In prolonged illumination the decreased potential value is not stable and slowly rises to the dark value, only in exceptional cases a higher value than that before illumination is reached.

The aim of the experiments with selected spectral regions of radiation was to decide if the stated phenomena in *Funaria* cells are controlled by absorbing in blue region or by a photosynthetic pigment system. If the first possibility suggested by Brauner and Brauner (1937) is true — the electrical phenomena may be closely connected with the mechanism of chloroplasts displacements controlled by a short wave length pigment system. On the other hand, the results obtained on big algae cells (the cells with no chloroplasts displacements) speak in favor of the other possibility. Nagai and Tazawa (1962) showed that in *Nitella* cells blue and red light produce a significant potential difference, whereas, green is deprived of effect or the effects are negligible. The action spectrum reported by Schilde (1966) for *Acetabularia* showed close correlation between the absorption spectrum of photosynthetic pigments and the spectral activity of light in producing potential changes.

The results of the present study are in agreement with the last statements. In spite of using broad spectral regions we were able to show two maximum spectral responses (blue and red); this suggests that in *Funaria* cells the photosynthetic pigment system is the main absorbing system controlling light induced changes of the internal potential. This results do not permit to answer the question if the photosynthetic system is the only system responsible for these changes, or if there is in the short wave length range of the spectrum an accessory activity of blue absorbing not photosynthetic pigments.

The correlation between the effect of white light on potential changes and on photodisplacements of chloroplasts (fig. 7) is in this situation not very important for explanation of the displacements mechanism. Potential changes produced by light seem not to be any sign of primary effect of light on the displacements of chloroplasts but rather secondary metabolic effects of the photosynthetic process. The mentioned correlation is comprehensible because as it was found earlier (Zurzycki 1955) the intensity range of white light inducing high intensity displacements of chloroplasts is usually related with the transition of the light curve of photosynthesis to the saturation range.

SUMMARY

The intracellular potential for *Funaria* leaf cells was measured by means of Ag/Ag Cl electrodes and KCl filled micropipetes of about 1 μ tip diameter. As the mean value of the resting potential $102,21 \pm 2,10$ mV for not pretreated cells and $132,02 \pm 1,79$ mV for pretreated cells were found.

The main potential barrier is plasmalemma. Potential difference across tonoplast is not significant.

Illumination of the cells with white light produces changes in the potential difference consisting of three phases: I — decrease, II — steady state, III — slow increase. After switching off the light the potential value returns to the former dark level. The magnitude of the potential change increases with light intensity getting saturated by 30—50 klx of white light.

The action of selected spectral regions of light showed two peaks of spectral activity in blue and red. Potential changes induced by light seem to be connected with metabolic processes of photosynthesis and controlled by the photosynthetic pigment system.

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Zmiany potencjału membranowego komórki liścia Funaria hygrometrica pod wpływem światła

Streszczenie

Potencjał wnętrza komórki liścia *Funaria* mierzono za pomocą elektrod Ag/AgCl stosując mikropipety wypełnione roztworem KCl, o średnicy wierzchołka około 1 μ .

Stwierdzono że wartość potencjału spoczynkowego komórek mieści się w granicach 65—160 mV (średnia $102,21 \pm 2,1$ mV) dla materiału nieadaptowanego, natomiast w granicach 95—185 mV (średnia $132,02 \pm 1,79$ mV) dla liści przetrzymywanych 24 godz. w środowisku wodnym. Główną barierę potencjału stanowi plazmalemma.

Potencjał rejestrowany w ciemności lub w słabym świetle wykazuje stałą wartość w ciągu 15—25 minut. Oświetlenie komórki światłem białym wywołuje zmiany potencjału, w których można wyróżnić trzy fazy: I — zmniejszenie różnicy potencjałów między wnętrzem komórki a środowiskiem, II — stan stacjonarny, III — powolny wzrost. Wielkość zmian potencjałów wywołanych światłem zależy od intensywności światła, osiągając wartości maksymalne przy 30—50 klx.

Wpływ światła barwnego o wybranych zakresach spektralnych wykazuje istnienie dwóch maksimów aktywności: w zakresie niebieskim i czerwonym. Zmiany potencjału wywołane oświetleniem są prawdopodobnie związane z metabolicznymi procesami fotosyntezy i kontrolowane przez fotosyntetyczny system barwnikowy.