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Membrane Permeability and Response to Light in *Paramecium*^{1), 2)}

By

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(With 5 Text-figures)

It was found in previous study (Okumura, 1963) that a sudden change in direction of ciliary beating was evoked by flashed light in dark-adapted *Paramecium*. Excitability to light was affected by the relative difference in intensity of the stimulating light and background illumination. The maximum value in the spectrum sensitivity curve of *Paramecium* was between 330 and 350 m μ . Moreover, "off-response" was evoked by ordinary light or weak ultraviolet rays, and "on-response" was evoked by strong ultraviolet rays. According to these results, it was suggested that the response to light in *Paramecium* was elicited by a change in membrane permeability due to the change in the molecular structure of cell membrane which had absorbed radiation energy from the stimulating light. In order to analyze the response to light in *Paramecium*, electrical properties of the cell membrane were examined during excitation by means of a microelectrode.

Material and Methods

All of the experiments were made with specimens of *Paramecium caudatum* cultivated in a vegetable powder infusion. In preparation for the experiments, the animals were washed with physiological salts solution³⁾ (Yamaguchi, 1963) and kept in the dark for more than 2 hours. For minimization of swimming velocity of the animals, methylcellulose was dissolved in the experimental solution (6% medium of methylcellulose). This method was useful in overcoming the difficulty of inserting the microelectrode into the moving animal.

The microelectrode was filled with 3M KCl solution and had a direct current resistance of 5–20M Ω . It was connected to a cathode follower tube (12AU7) through an Ag-AgCl type non-polarizable electrode, and the output of this step was fed into a direct coupled amplifier and read on a cathode ray oscilloscope. In order to measure transitional change in the membrane resistance during excitation, a single intracellular microelectrode with a compensation bridge circuit was used for both potential recording and application of currents.

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3) The physiological salts solution used was of the following composition; M/10 KCl (10ml)+M/10 CaCl₂ (1.2ml)+M/10 NaHCO₃ (1.5ml)+redistilled water (287.3ml).

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This method was reasonable and useful for the present study because of the difficulty of inserting two separate microelectrodes into the small moving animal, and was identical in principle to that used in the experiments on *Paramecium* (Okumura and Yamaguchi, 1962) and *Opalina* (Yamaguchi and Okumura, 1962). Weak rectangular hyperpolarizing pulses of 20 msec. duration were applied to *Paramecium* at a frequency of 30 cycles per second.

All of the experiments were done in the dark room (average light intensity was kept at 0.3 luxes), and the animals were transferred to a chamber which was weakly illuminated (10 luxes) with a tungsten lamp for more than an hour, to adapt them to the dark. A tungsten lamp, xenon lamp and high pressure mercury lamp were used for the stimulating light source, and light filters (Canon UV-filter, Toshiba IR-DIB and Corning 7-54 for exclusion of the visible rays) were also employed to select the wave length of the light. In the present experiments, infrared rays were not employed as the stimulating light. It was found in previous study (Okumura, 1963) that *Paramecium* does not respond to infrared rays. The intensity of the stimulating light was measured with a luxmeter (Toshiba No. 4 and No. 5) or phototransistor (OCP-70) against the standard intensity, and the duration of illumination was changed with a camera-shutter. The above-mentioned method was identical in principle to that used in previous experiments on *Paramecium* (Okumura, 1963).

Most of the experiments were carried out at room temperature (19°–25°C).

Results

(1) *Relationship between ciliary reversal and change of membrane resistance evoked by flashed light.*

When the dark-adapted animals were stimulated by flashed light, the direction of their movement changed after a short latent period, resulting from a sudden change in direction of the ciliary beating. The membrane resistance was measured during the excitation evoked by flashed light to analyze the relationships between the ciliary beating and the change of membrane permeability. The intensity of background illumination in the experimental chamber was kept constant at 10 luxes with a tungsten lamp. Flashed light from a stroboscopic photograph xenon lamp (Kako S-2) was employed for stimulation. The light intensity was 3.5×10^5 luxes, and the duration was 4 msec. Visible light was isolated with a UV-filter (Canon), and ultraviolet rays were selected with a Corning 7-54 filter. Immediately after the microelectrode was inserted, the ciliary activity of *Paramecium* which had been previously decreased by dark-adaptation, was confused by the mechanical effect. Flashed light of strong intensity was applied to the animal, after the ciliary beating became normal again. The membrane resistance decreased between 50 and 100 msec. after application of flashed light (Fig. 1), and the change in the membrane resistance was co-ordinated with the ciliary reversal. The membrane resistance decreased sharply, and recovered to the initial level after reaching a minimal value. When ultraviolet rays (260–380m μ) were applied, the latent period of the response was small, and recovering time to the initial level was longer. According to these results, it can be said that ultraviolet rays have a stronger effect on the cell membrane of *Paramecium* than visible light.

It was found in the previous study (Okumura, 1963) that the latent period of the ciliary reversal evoked by flashed light was 180 ± 30 msec. at 80 luxes, and was 280 ± 30 msec. at 120 luxes background illumination. The latent period of the change in the membrane resistance that was obtained in the previous study was about 100 msec. shorter than that of the ciliary reversal evoked by flashed light. Therefore, it was concluded that the ciliary reversal was due to the change of membrane permeability that was produced by flashed light.

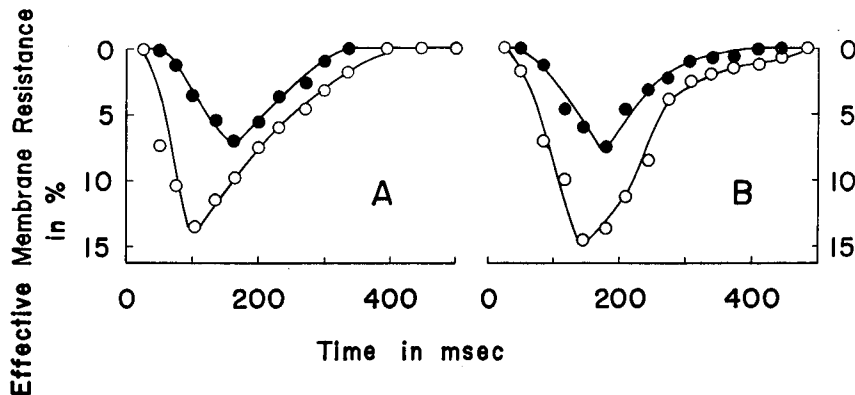


Fig. 1. Change in the membrane resistance during excitation by flashed light of a constant intensity at various intensities of background illumination. A: visible light (intensity: 3.5×10^5 luxes, duration: 4 msec.). B: ultraviolet rays (relative intensity of radiation energy: 100, duration: 4 msec.). The standard intensity (100) of ultraviolet rays was defined to be that intensity having radiation energy equivalent to 1.8×10^4 luxes in the visible light (Okumura, 1963). Hollow circle shows the results obtained at 10 luxes. Solid circle shows the results obtained at 80 luxes.

(2) *Effects of change in the intensity of both the stimulating and the background illumination.*

When stimulating light of constant intensity was applied to the dark-adapted animals at various intensities of background illumination, the excitability decreased in inverse proportion to the intensity of background illumination (Okumura, 1963). The change in the membrane resistance was measured as described above, and the result is shown in Fig. 1. The latent period of the change in membrane resistance became longer together with the increasing intensity of background illumination, but the stronger the background illumination became, the greater the change in the membrane resistance. If the intensity of the background illumination was over 100 luxes, the response was never evoked even by the strongest stimulating light (3.5×10^5 luxes). On the other hand, the latent period of change in the membrane resistance produced by flashed light at 0.3 luxes of background illumination was the same as at 10 luxes. The results obtained with

ultraviolet rays were the same as with visible light (Fig. 1, A and B).

Changes in the membrane resistance produced by flashed light of various intensities were measured at a constant intensity (10 luxes) of background illumination, and they are shown in Fig. 2. The latent period of change in the membrane resistance was not affected by the change in intensity of flashed light, but the stronger the stimulating light, the greater the change in membrane resistance. It was observed in the previous study (Okumura, 1963) that the number of responding animals, e.g. individuals in which the reversal was produced, became larger in proportion to the intensity of the stimulating light under background illumination of a constant intensity. According to these results, it was concluded that the excitability to light of *Paramecium* was more affected by the intensity of the background illumination than of the stimulating light.

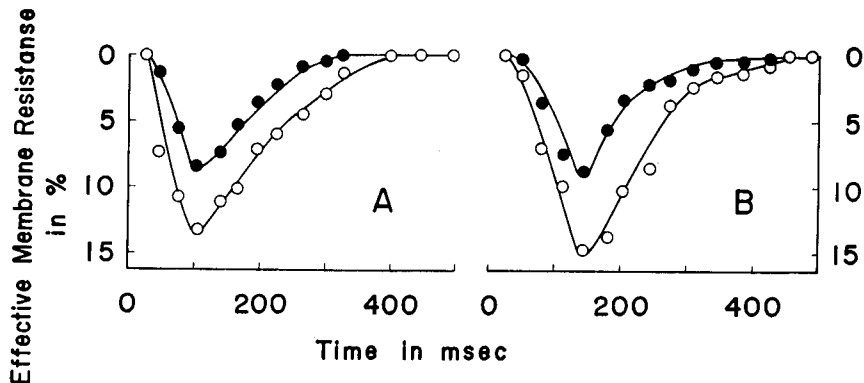


Fig. 2 Change in the membrane resistance during excitation by flashed light of various intensities at a constant intensity of background illumination (10 luxes). A: visible light. B: ultraviolet rays. Hollow circle shows the result obtained with 3.5×10^5 luxes (visible light) or 100 (ultraviolet rays). Solid circle shows the result obtained with 2.0×10^5 luxes (visible light) or 60 (ultraviolet rays).

(3) Effects of repetitive flash light stimulation.

For further analysis of the light effects, changes in the membrane resistance of *Paramecium* stimulated by repetitive flash light at various frequencies were measured. The stimulating light source used was a stroboscopic photography xenon lamp (Toshiba SS-4B), and the frequencies of stimulation were 10, 30 and 50 cycles per second.

The results obtained are shown in Fig. 3. When the repetitive flash light at 10 or 30 c.p.s. was applied, the membrane resistance decreased gradually, as a staircase, at intervals of one step per two or three flashes after stimulation began. The membrane resistance of at 50 c.p.s. decreased suddenly. When the membrane resistance reached a minimum value it showed a tendency to recover the initial

level, but did not recover completely. That is, the membrane resistance was decreased slightly and kept with oscillating between 90 and 95 percent of the normal value during stimulation. The change in membrane resistance of *Paramecium* stimulated by ultraviolet rays was slightly greater than that by visible light. If the visible light was turned off, the membrane resistance recovered slowly to the initial level. When ultraviolet rays were used, the membrane resistance oscillated between two and three times immediately after the stimulating light was turned off, and then recovered very slowly to the initial level. The recovering time to the initial membrane resistance after turning off the stimulation light became longer in proportion to the amount of applied radiation energy, and also the recovering time in the case of ultraviolet rays was longer than that of visible light.

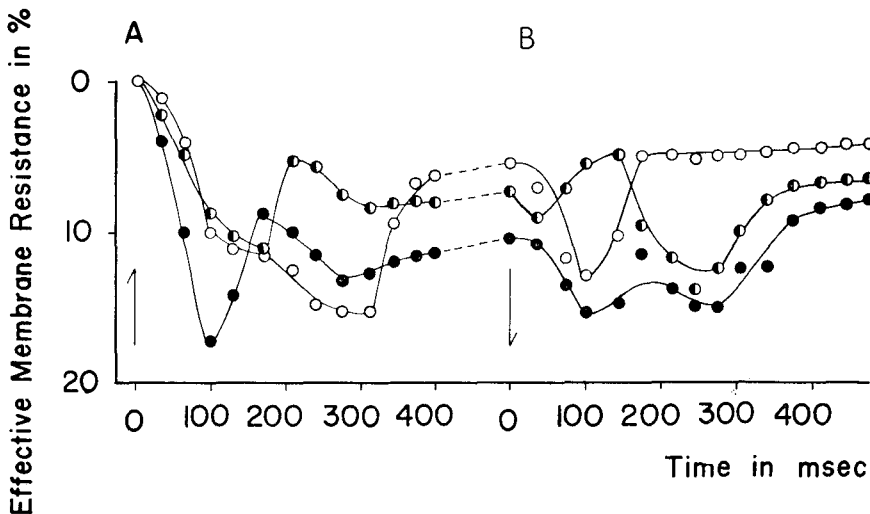


Fig. 3 Graph obtained from the experiment with repetitive flash ultraviolet rays (intensity: 30) at various frequencies. Hollow circle: 10 c.p.s. Half-solid circle: 30 c.p.s. Solid circle: 50 c.p.s. \uparrow : beginning of the stimulation. \downarrow : end of the stimulation.

(4) *Effects of continuous light stimulation.*

When *Paramecium* was stimulated by continuous visible light, ciliary reversal did not occur during stimulation, but it was elicited on turning off the stimulation light (off-response) (Okumura, 1963). In order to analyze the change in membrane resistance in this case, the membrane resistance of *Paramecium* stimulated by continuous visible light (10000 luxes) was measured. For illumination a tungsten lamp was used with a direct current of 100V. The results are shown in Fig. 4. The membrane resistance was slightly decreased during stimulation. When the stimulating light was turned off, the membrane resistance decreased and recovered to the initial level after reaching a minimum value. The time course of

the change in membrane resistance was the same as for flashed light stimulation. Repetitive flash ultraviolet rays (260–380 $m\mu$) at a frequency of 250 c.p.s. divided through a Corning 7-54 filter from the light of a stroboscopic photography xenon lamp (Toshiba SS-4B) were used for the stimulation of continuous ultraviolet light. This repetitive ultraviolet flashed light of 250 c.p.s. appeared almost continuous.

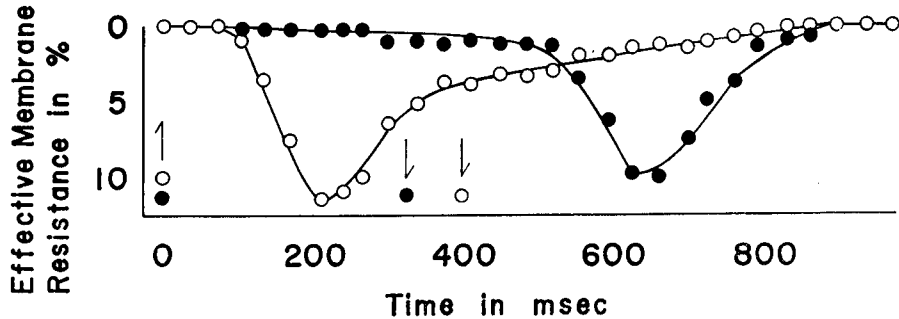


Fig. 4 Change in the membrane resistance during excitation by continuous visible light (A, intensity: 10^4 luxes) or repetitive ultraviolet rays (B, intensity: 10, frequency: 250 c.p.s.). \uparrow : beginning of the stimulation \downarrow : end of the stimulation.

When the ultraviolet rays were applied, the membrane resistance decreased, and reached a minimum value during stimulation. If the duration of stimulation was longer, the membrane resistance began to oscillate. This change corresponded to the "on-response" of ciliary reversal.

(5) *Effect of external Ca^{++} -concentration.*

For the last analysis of the mechanism of light excitement, light stimulation

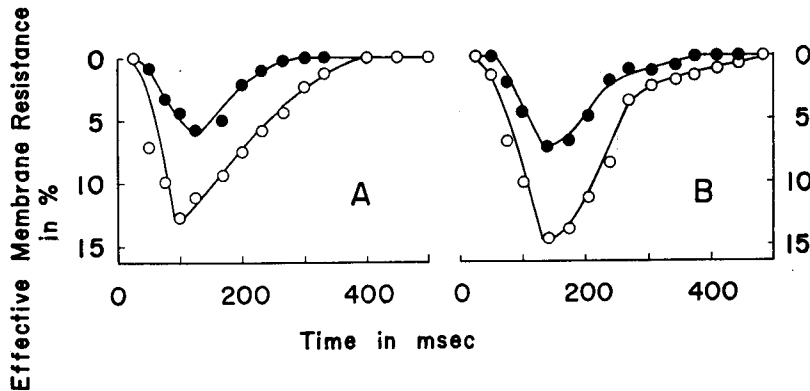


Fig. 5 Change in the membrane resistance during excitation by visible light (A, intensity: 3.5×10^5 luxes) or ultraviolet rays (B, intensity: 100) in the external medium of two Ca^{++} -concentrations. Hollow circle: M/300 $CaCl_2$. Solid circle: M/100 $CaCl_2$.

was compared with electrical stimulation. In the latter case, external rich calcium ions raised the electrical threshold (Tamasige, 1951). Here the experiments of light stimulation were carried out in various Ca^{++} -concentrations of the surrounding medium. The test solutions used had an ionic composition as follows; (1) M/100 KCl+M/150 CaCl_2 in equal volume; (2) M/100 KCl+M/50 CaCl_2 in equal volume; and (3) M/100 KCl+M/25 CaCl_2 in equal volume. A single flash of light was used for stimulation.

The results obtained are shown in Fig. 5. The change in membrane resistance never occurred with solution (3), and ciliary reversal was not evoked. Change in membrane resistance and ciliary reversal were both elicited with solutions (1) and (2); the response evoked with solution (1) being more remarkable than that with solution (2). According to these results, it was concluded that the response to light in *Paramecium* was more inhibited with the increase in Ca^{++} -concentration in the experimental solution.

Discussion

It was confirmed in the present experiments that ciliary reversal was evoked, and a decrease in membrane resistance was produced by flashed light in the dark-adapted *Paramecium*. According to these facts, it appears that the ciliary reversal occurred in consequence of the change in ionic permeability of the cell membrane. The change in ionic permeability is due to the reversible change in molecular structure of the cell membrane which absorbed the radiation energy of the stimulating light. It is well known that ultraviolet rays are absorbed in great quantities by protein and lipoid in the cell membrane (Giese, 1945). Therefore, it was expected that the change in membrane resistance would be produced more effectively by ultraviolet rays than visible rays. Indeed it was observed that the change of ionic permeability of the cell membrane became greater when ultraviolet rays were applied. When a stimulating light of constant intensity was applied to the animal under background illumination of various intensities, the latent period of the change in membrane resistance became longer in proportion to the intensity of the background illumination. But the latent period of the change in membrane resistance was not altered when the animal was stimulated by light of various intensities at a constant intensity of background illumination. Therefore, it is concluded that the excitability to light of *Paramecium* is more affected by the intensity of background illumination. It was reported that the ionic permeability (especially to NH_4^+) of light-adapted *Paramecium* was larger than that of the dark-adapted animals (Packard, 1925). In the present experiment, the light-adapted animal did not respond to flashed light, but the dark-adapted animal did. It appears that *Paramecium* responds to light, when the relative differences of radiation energy between the stimulating light and the background illumination reach a certain level.

From the result of the experiment on repetitive flash light stimulation, it

also appears that the change in the molecular structure of the cell membrane becomes greater in proportion to the quantity of radiation energy absorbed from the flashed light, but there was no further change in the structure if the amount of radiation energy absorbed was over a certain level. When the stimulating light was turned off, the molecular structure of the cell membrane recovered to the initial level. It was reported by Giese (1945) that the rate of cell division of *Paramecium* was certainly decreased by application of a large quantity of ultraviolet rays. The reason for the phenomenon was the irreversible change in the molecular structure of the cell membrane which absorbed a large quantity of ultraviolet rays. "Off-response" was evoked by weak ultraviolet rays, but "on-response" was evoked by strong ultraviolet rays. According to these results, it appears that if the absorbed radiation energy increases sharply, the molecular structure of the cell membrane changes transiently, and therefore the ionic permeability of the cell membrane is increased reversibly during stimulation. If the amount of absorbed radiation energy is small, the molecular structure does not change during stimulation though it becomes unstable. But when the supply of radiation energy is suddenly stopped, the molecular structure and therefore the membrane permeability rapidly changes. The "off-response" evoked by visible light is of the same mechanism as that evoked by weak ultraviolet rays.

The ciliary reversal and the change in membrane resistance by light was remarkably inhibited when *Paramecium* was stimulated in the external high Ca^{++} medium. According to the result, Ca-ions in the outer medium bind excessively to the cell membrane, and the excitability to light decreases. It has been reported that the membrane resistance and the threshold of the muscle fibre are raised by increase in Ca^{++} -concentration of the outer solution (Tamasige, 1958). Sanders and Giese (1958) reported that the ionic permeability to K or Na in a yeast cell to which ultraviolet rays were applied, was affected by Ca-ions in the outer solution, that is, the ionic permeability was decreased by increase in Ca^{++} -concentration. They also concluded that Ca-ions in the external solution bound to the cell membrane that was charged negatively by application of ultraviolet rays, and the ionic permeability decreased. It seems that Ca-ions in the external solution bind to the molecular structure of the cell membrane of *Paramecium*, the cell membrane becomes stable, and the excitability to light decreases in a high Ca^{++} -concentration. A common mechanism is conceivable for light and electrical excitement.

Summary

- 1) The relationship between the ciliary reversal and the change in membrane resistance of *Paramecium* stimulated by visible light or ultraviolet rays was investigated by means of a microelectrode.
- 2) When the stimulating light was applied, the membrane resistance decreased, and then ciliary reversal occurred.
- 3) The latent period of the change in membrane resistance was affected by

the intensity of background illumination. The excitability to light was more affected by the intensity of the background illumination.

4) Change in membrane resistance and ciliary reversal were never produced in an external high Ca^{++} -concentration medium. Excitement to light was inhibited by Ca-ions.

5) It is concluded that the ciliary reversal is elicited by the change in ionic permeability of the cell membrane, and also that the change in ionic permeability is due to the reversible change in the molecular structure of the cell membrane which absorbed radiation energy from the stimulating light.

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