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Ciliary Activity and Electrical Properties of *Opalina*^{1), 2)}

By

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(With 7 Text-figures)

In the parasitic ciliate *Opalina*, Kinoshita (1954) and Ueda (1961a) presented some evidence to show that the magnitude of the membrane potential decreased when a change in the direction or the reversal of the ciliary beating occurred. According to Koshtoyants and Kokina (1958), the rhythmicity is one of the most marked characteristics of the action potential in *Opalina*. On the other hand, Hisada (1957) reported the action potential found in correlation with the spontaneous activity of the tentacle of the luminous flagellate *Noctiluca*, and Chang (1960) found that the action potential was generated by the application of inward current in non-luminous *Noctiluca*.

In a sense, these phenomena in mechanical and electrical activity of protozoa seem to be analogous to those of muscular contraction (e.g., Tamasige, 1953). There are, of course, great differences in many points between the former and the latter. In order to analyse the mechanism of ciliary movement in *Opalina*, it is important to make clear the electrical properties of the cell membrane. The object of this paper is to present electrical measurement on the cell membrane of *Opalina*, in particular the resting and action potential, the resistance, and the response to current applied, and to clarify the linkage between the ciliary movement and the electrical properties of *Opalina*.

Material and Method

Preparation. *Opalina* (*O. obtrigonoides japonica*), obtained from the rectum of frog, *Rana japonica*, were washed completely with frog Ringer's solution, and kept in the solution more than an hour before the experiments.

Microelectrode. A single intracellular microelectrode with a compensation bridge-circuit was used both for potential recording and for application of currents (Araki and Otani, 1955, and Okumura and Yamaguchi, 1962). This method was reasonably useful for the present study, because of the difficulty in impalement of two separate microelectrodes into the moving animal. The microelectrode was filled with 3M KCl solution and had a direct current resistance of 5–30 megohms. It was connected to a cathode follower tube

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(12AU7) through a Ag-AgCl type non-polarizable electrode, and the output of this step was fed into a direct coupled amplifier and read on a cathod ray oscilloscope. In most cases the impaled *Opalina* rotates around the microelectrode but it was ascertained preliminarily that this rotation had no effect on recording potential.

Membrane resistance. The membrane resistance of *Opalina* was measured by means of the above mentioned microelectrode. The rectangular inward current pulses (duration, 200 msec.; frequency, 5c/sec.; intensity, 1×10^{-8} A) were applied into the cell. *Opalina* has an ellipsoidal shape and an equation applicable to the electrotonic potential, V and the applied current, I may be expected to be more complicated than that in the cylinder case of Hodgkin and Rushton (1946). The membrane resistance was, for simplicity, determined from V/I , assuming that the cytoplasm resistance is negligibly small compared with the membrane resistance in *Opalina*.

Observation of ciliary movement. The ciliary movement was ordinarily observed under the light microscope, in parallel with measurement of electric potential. The detailed observation was made by means of a television microscope (number of scanning line, 625; frame feeding, 50/sec.)³⁾. The closer analysis of ciliary movement was made possible by obtaining cinematographic records by a 16 mm cinecamera (25 frames per sec.) synchronized with the television system. Some typical examples of the cinematographic records are shown in figure 1. The cilia of *Opalina* exhibit obvious metachronal waves which normally travel backwards over both dorsal⁴⁾ and ventral surfaces of the cell (Fig. 1A). However, the pattern of the wave is widely variable, and the direction of the metachronal wave changes instantly to give a quite different pattern (Fig. 1B); in some cases the waves disappear completely (Fig. 1C). These observations are in principle identical with the detailed description by Okajima (1953).

Solution and temperature. The frog Ringer's solution used was of the following composition: 120 mM NaCl, 2mM KH_2PO_4 , 1.2 mM CaCl_2 , pH to 7.2 by NaHCO_3 . All experiments were carried out at room temperature of 16°–25°C.

Results

Resting and action potential: When the microelectrode was impaled obliquely from the upper side into the cell, the resting potential was recorded instantly, the inside of the cell being negative. In 50 measurements, the magnitude averaged 32 ± 16 mV at 16°–25°C.

In a majority of animals, the spontaneous action potential appeared at a constant frequency (6–10 per minute) sooner than 1 minute after successful impalements (Fig. 2). The most remarkable property of this action potential is the membrane hyperpolarization during activity: the degree of negative potential of the cell interior increased during activity, instead of membrane depolarization

3) The details of the television microscope will be published by Prof. M. Tamasige in near future. An oral report and 16 mm cine-film demonstration was given at the 33rd Annual Meeting of the Zoological Society of Japan, in the city of Okayama on Oct. 7th, 1962.

4) The upper side of the animal with its convex side held to the right is called "dorsal" and its lower side "ventral" for the sake of convenience (see Okajima, 1953).

during activity as in the case of muscle and nerve cells. In 50 measurements, the average value of the action potential measured from the resting level was 17 ± 2 mV at 16° – 25° C. Both the resting and action potential remained constant for many repetitions while the electrode stayed inside the cell.

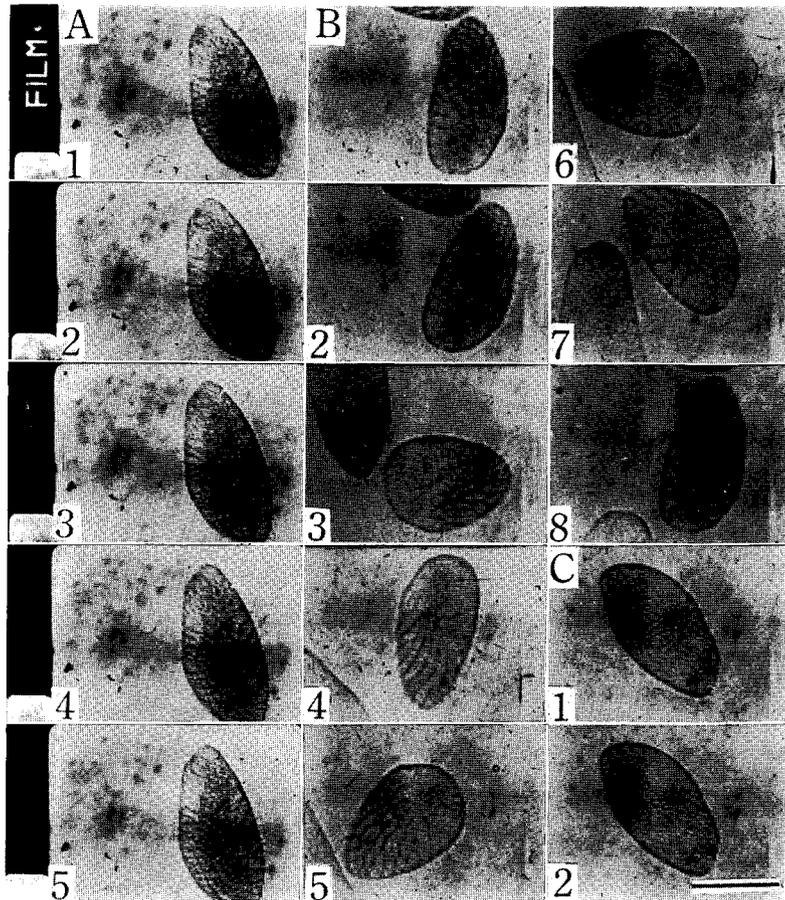


Fig. 1. Successive stages showing the various movement of *Opalina* in frog Ringer's solution. From 16 mm cine-film recorded by means of a cinecamera synchronized with a television microscope. A; forward movement. B; right rotation. C; ciliary reversal. Note the change in the pattern of metachronal waves. All the sequences were taken at 25 frames per second, but in B consecutive photographs were selected at an interval of 5 frames from original film and edited. Scale mark on C 2 represents 200μ . (See the text).

On the other hand, visual observations through the light or the television microscope, in parallel with the potential measurement, revealed that the

spontaneous action potential is closely correlated to the change in the beating direction of effective stroke of cilia. That is, the inclination of the line of metachronal wave to the lateral axis of the animal began to increase at the moment when the action potential was initiated (Fig. 2, 1) and both the inclination and the action potential reached their maximum values synchronously (Fig. 2, 2). The gradual potential recovery from the action potential, was also accompanied with gradual

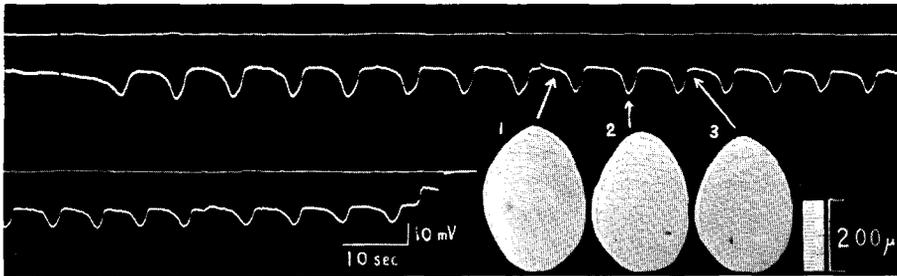


Fig. 2. A typical example of spontaneous action potential and 3 patterns of metachronal waves on the dorsal surface of *Opalina*. Arrows indicate the correlation between the action potential and pattern of metachronal wave. Note the hyperpolarization during the action potential. Temperature: 16°C.

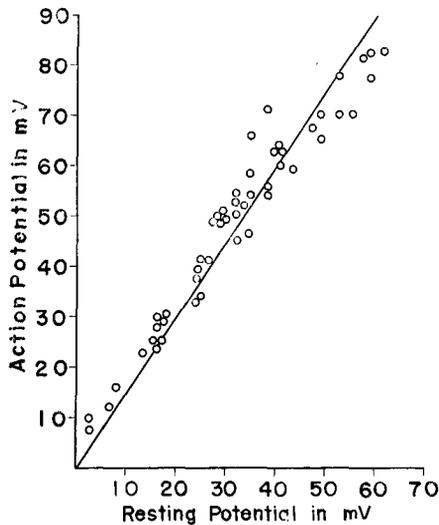


Fig. 3. Relation between the resting potential and the action potential during the spontaneous activity of *Opalina* in the Ringer's solution. Abscissa represents the resting potential and ordinate the maximum amplitude of the action potential. Temp.: 16°–25°C.

decrease in the inclination of wave lines (Fig. 2, 3). It was found that no potential change was observed in correlation with a perfect ciliary reversal produced by such a strong mechanical stimulus as impalement of the microelectrode (Fig. 2).

During the present work great variability was found in the magnitude of resting and action potentials and even in the maximum value of the inclination of wave line (from 10° – 150°) among different individual animals. However, as is shown in figure 3, linear relationship was clearly found between the resting and action potentials. On the contrary, there was no definite relation between the action potential and the inclination of wave lines in different animals, although the magnitude of the action potential was in correspondence to the degree of inclination of the metachronal wave lines in the same animals.

Changes in membrane resistance: A quantitative determination of the change in membrane resistance during spontaneous mechanical and electrical activity in *Opalina* was made by the measurement of amplitude of the anelectrotonic potential produced by application of recurring inward current pulses of constant intensity. A typical result is illustrated in figure 4. At onset of action potential, the membrane resistance begins to decrease and both the action potential and the membrane

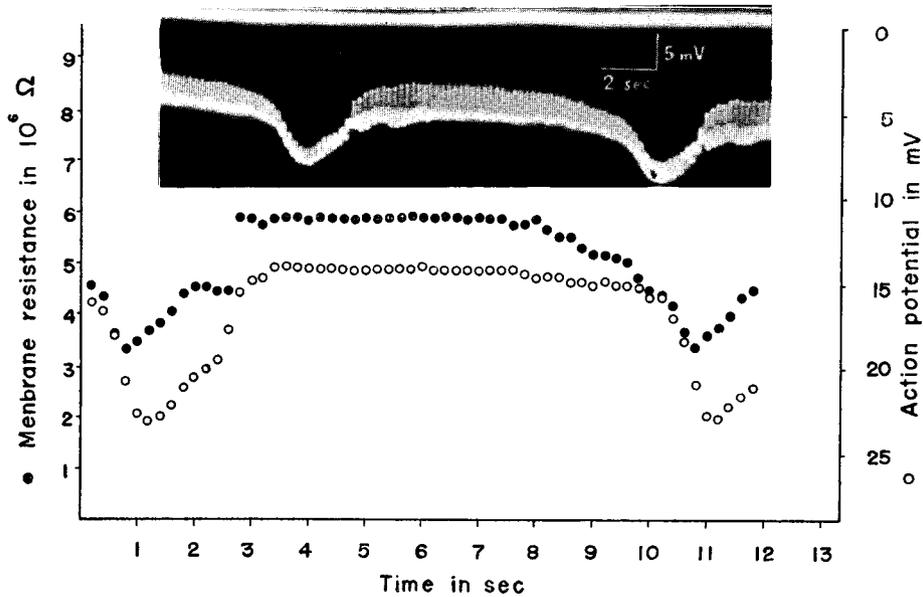


Fig. 4. Changes in the membrane resistance during the spontaneous activity of *Opalina*. Upper record shows the action potential on which the anelectrotonic potentials are produced by recurring constant square pulse of 100 msec. in duration. Lower graph shows the time courses of both the height of action potentials (hollow circles) and the value of membrane resistances (solid circles) determined by oscillographic record. Temp.; 20°C .

resistance reach their minimum values simultaneously. They both have similar time course during the recovery process. It is obvious that the action potential has direct correlation with the change in the membrane resistance. The minimum value of the membrane resistance was 20–50 per cent of the initial value.

On the other hand, no appreciable change in membrane resistance was observed at any stage during the perfect ciliary reversal. It is suggested that the ionic permeability of the cell membrane of *Opalina* markedly increases only during the change in the pattern of the metachronal waves, in company with the action potential.

The influence of depolarizing and hyperpolarizing currents of the action potential: Increasing or decreasing effects in the membrane potential, which were produced by application of polarizing or depolarizing current across the cell membrane, on the excitability and the spontaneous repetitive action potential of *Opalina* were investigated.

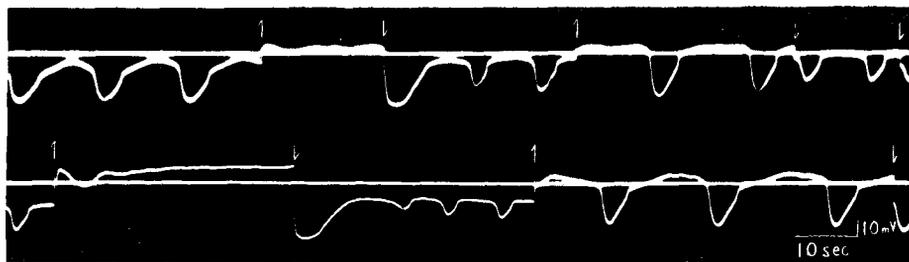


Fig. 5. The effect of change in the resting potential on the action potential. The resting potential is altered by application of the outward current across the cell membrane. Upward deflection indicates the membrane depolarization. Direction of arrows indicates "make" or "break" of the current respectively. Temp.; 19°C.

The results obtained by application of the depolarizing currents were as follows (Fig. 5):

1. When the depolarizing current was applied across the cell membrane, provided that the current was of superthreshold intensity, the action potential was evoked on break of applied current. It was also observed that the evoked action potential was accompanied with change in the beating direction of the cilia.

2. Even if the cell membrane was completely depolarized by the application of outward current, the spontaneous repetitive action potential was still seen. The magnitude of the action potential remained constant although the frequency of it decreased. When the applied current was broken, the action potential was evoked with which the ciliary response was associated.

3. If the depolarizing current was increased, the action potential was evoked on make or break of applied current, while the spontaneous repetitive

action potential was perfectly inhibited as well as the change in ciliary pattern was. Even if the current was broken, the inhibition effect remained for some time before the original active rhythm was re-established.

The results obtained from application of the hyperpolarizing currents across the cell membrane were as follows (Fig. 6):

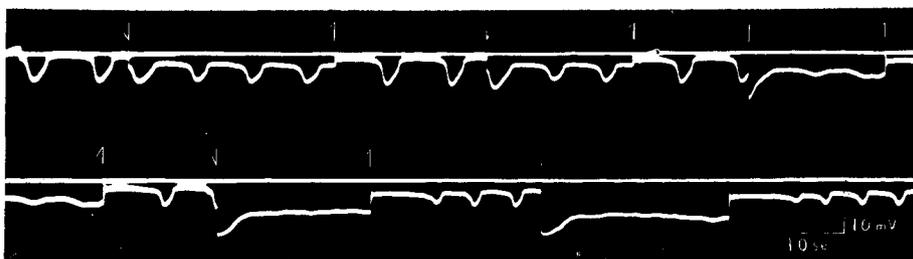


Fig. 6. The effect of change in the resting potential on the action potential. The resting potential is altered by application of the current across the cell membrane. Downward deflection indicates the membrane hyperpolarization. Direction of arrows indicates "make" or "break" of the current respectively. Temp.: 19°C.

1. When the hyperpolarizing current over the threshold intensity was applied across the cell membrane, both the action potential and the change in the beating direction of effective stroke were produced on make of the current. But the magnitude of the spontaneous repetitive action potential underwent no change during the current flow.

2. When the cell membrane was hyperpolarized to more than the maximum value of the spontaneous repetitive action potential, the action potential was evoked on current make. Subsequently to this event, the spontaneous repetitive action potential was completely inhibited during the current flow. Even if the applied current was broken, the inhibition effect remained for a time. In this case, the strength of such an after-effect on the spontaneous repetitive action potential was proportional to the intensity of applied hyperpolarizing current.

Intracellular stimulation: When the rectangular pulse (duration, 50 msec.) was intracellularly applied during the course of the spontaneous action potential by means of the microelectrode, *Opalina* showed peculiar response according to the direction and intensity of the current applied. Some of the results are shown in figure 7.

In the upper record (A) of figure 7 the spontaneous repetitive action potentials are superimposed by the action potentials evoked by weak cathodic stimuli (5×10^{-8} A). Whenever the action potential was evoked, a correlated change in the beating direction of effective stroke of cilia was recognized. The degree of the change increased with the height of the evoked action potential and the strength

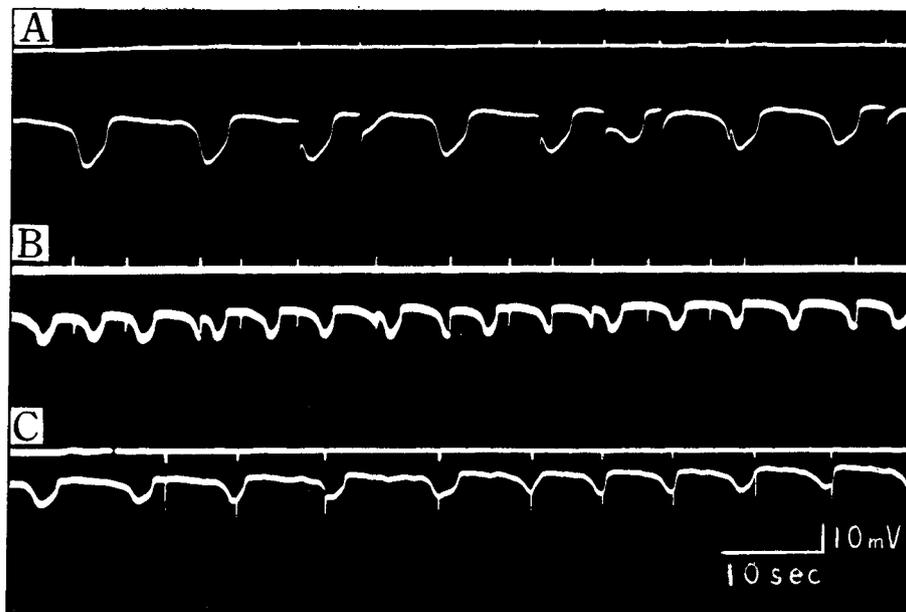


Fig. 7. Stimulation of *Opalina* during spontaneous activity, with rectangular pulse of 80 msec. in duration through the inserted microelectrode. Records A and B show the responses evoked by outward currents and record C by inward currents. The intensity of stimulating current is 5×10^{-8} amperes in record A and 2×10^{-7} amperes in record B and C. The stimulation signal is indicated on the zero-level (upper line of each record). Temp.: 25°C.

of the stimulus. The nearer in time of the valley bottom of the spontaneous action potential the stimulation was applied, the larger was the action potential evoked.

Contrary to this, the strong cathodic stimulation (more than $2 \times 10^{-7}A$) induced the perfect ciliary reversal. Upward sudden deflections in the middle record (B) of figure 7 correspond to the ciliary reversal. The occurrence of ciliary reversal results in the blockage of the spontaneous action potential. But if the stimulation was applied near in time of the valley bottom of the spontaneous action potential, the change in the beating direction of effective stroke of cilia, accompanied with the action potential, occurred immediately after the ciliary reversal.

On the other hand, if the anodic stimulation of adequate intensity was applied during the depolarizing phase of the spontaneous action potential, the effective stroke of cilia was strengthened temporarily in normal beating direction. As is shown in the figure 7(C) the spontaneous action potential showed a spike fall with a result of the increased strength of normal stroke.

From these results it may be concluded that the amplitude or the duration

of action potential depends directly on the degree or the time course of the change in the beating direction of the cilia respectively.

Discussion

The results of the present study definitely indicate that the action potential of *Opalina* is related with the ciliary movement, that is, the electric potential varies in exact accordance with the change in the inclination of the line of metachronal wave to the lateral axis of the animal. The action potential of *Opalina* has a polarity opposite to that of nerve or muscle cells, but the membrane resistance of the cell decreases during activity as in the cases of other excitable cells (e.g., Cole and Curtis, 1938, 1939). Contrary to these observations, Kinoshita (1954) and Ueda (1961a) reported that the increase in the degree of ciliary reversal occurs in close association with the decrease in inside-negative potential. According to Koshtoyants and Kokina (1958), the action potential of *Opalina* has some similarities in general character to other excitable cells (especially, in its polarity and its ionic kinetic of the potential). The discrepancy between the present findings and those of the two Russian workers may be ascribed to the differences in the method of measuring or recording potential. In another protozoa, *Noctiluca scientillans*, Hisada (1957) found that the action potential correlates with spontaneous activity of the tentacle. More recently, Chang (1960) reported that in *Noctiluca miliaris*, the action potential is elicited and the impedance decreases when a sufficient inward current flows across the cell membrane. It is a fact of great interest that these action potentials have polarity similar to that of *Opalina*; the cell interior becomes more negative during activity. Such action potentials are not entirely unknown in the other excitable cells. However, this sort of action potential has been reported to be elicited only under abnormal conditions. For instance, the hyperpolarizing response can be elicited only on prior depolarization of squid (Segal, 1958; Tasaki, 1959a), frog (Mueller, 1958; Stämpfli, 1959; Lüttgau, 1960), or toad (Tasaki, 1959a) axons. Although quantitative data on the kinetics of their electrogenetic processes are not as yet available, Grundfest (1960) suggested that these responses may develop as a result of reinstated "K-inactivation" when the K-conductance is first increased under experimental conditions. It is not easily conceivable that Grundfest's suggestion entirely accounts for the generation of the action potential of *Opalina*. Further studies of this point are necessary.

The ciliary activity may be divided broadly into the ciliary reversal, the change in direction of the effective beat, and the ciliary metachronism. Concerning the mechanism of ciliary reversal in *Paramecium*, there are only a few assumptions; Oliphant (1938, 1942) suggested that ciliary reversal arises from the change in viscosity of the cytoplasm, and Kamada (1940) assumed that ciliary reversal is induced by the intracellular liberation of free anions from Ca-salts previously

accumulated within a cytoplasm as a result of the diminution of intracellular free Ca-ions. On the other hand, many investigators have tried to picture the sequence of events during the ciliary reversal in *Opalina* (Okajima, 1953; Kinosita, 1954; Ueda, 1956, 1961a, b; Kanno, 1958; Naitoh, 1958, 1961). According to Kinosita (1954) and Ueda (1961a), the inside-negative potential of the membrane of *Opalina* decreases in close association with the ciliary reversal or the change in beating direction of the cilia. In the present study, no indication of the action potential was observed when the ciliary reversal occurred on application of mechanical stimulation (Fig. 2). Moreover, the ciliary reversal did not appear even if the membrane potential was reduced to the zero level (Fig. 5). In the case of *Paramecium*, the authors also found that ciliary reversal is not directly associated with the change in the membrane potential (Okumura and Yamaguchi, 1962). These facts seem to suggest that the reduction of membrane potential may be not a necessary condition to induce the ciliary reversal. It is considerable from the above results that in *Opalina* the ciliary reversal and the change in the direction of ciliary beating arise from different mechanisms in the animal, and that the action potential is directly related with only the latter. The discrepancy between the present result and those of Kinosita (1954) and of Ueda (1961a) may be attributed to the difference of observation method. Kinosita (1954) used a valve-voltmeter and the potential measurements were not continuous in time. Ueda (1961a) recorded potentials with a cathode ray oscilloscope, but the time sweep was too rapid to record the true action potential.

Concerning the mechanism of ciliary metachronism, Sleigh (1957) maintains an advanced hypothesis: he attributes the ciliary metachronism in *Stentor* to a "pace-maker system" in the cell. This hypothesis may be further supported by the fact that the cilia along the anterior right edge of *Opalina* are more excitable than the other ones (Okajima, 1953). But, from the present study, problems concerning the mechanism of ciliary metachronism of *Opalina* can not be discussed further until they are investigated in more detail.

On the other hand, the effect of electric current on *Opalina* has been studied by a few authors (Okajima, 1953; Kanno, 1958; Naitoh, 1958, 1961). In the present study, the polar excitation induced by outward or inward current was observed by means of oscillographic devices and some information can be appended to the analytical study of excitability of *Opalina*. Naitoh (1958) has shown that the excitability of *Opalina*, with regard to the ciliary reversal produced by outward current, is kept almost unaltered by the change of membrane potential induced by external application of potassium ions. On the contrary, as is shown in figures 5 and 6, strong depolarization results in perfect inhibition of the spontaneous action potential as well as of the ciliary activity during application of current. An analogous effect was observed in *Noctiluca* when the membrane potential was hyperpolarized (Hisada, 1957). Consequently, it is conceivable that the excitability of *Opalina* is related with the membrane potential in respect to the true action

potential or the change in metachronal wave pattern.

Furthermore, the present observations clearly demonstrate that the excitability of *Opalina* increases gradually during hyperpolarizing phase of action potential (Fig. 7). This is easily explained by the fact that the threshold decreases when the inclination of the line of metachronal wave to the lateral axis of the animal increases (Okajima, 1953). But the fact that on break of the outward current or on make of the inward current the action potential develops, is not in agreement with the results obtained by Naitoh (1958). This discrepancy may be due in part to the difference between conditions of the ciliary wave pattern during application of stimulating current. In the present study, the stimulation current was applied to the animal exhibiting the spontaneous change in direction of the ciliary stroke at regular intervals. In Naitoh's results (1958), the stimulating current was applied at various phases, even without the spontaneous change of ciliary pattern.

Summary

A detailed study on the electrical properties of the parasitic protozoa, *Opalina* has been carried out using a single intracellular microelectrode for potential recording and application of polarizing current. At the same time the ciliary movement was observed, and the following results were obtained:

1. The average resting potential was 32 ± 16 mV, inside negative. Within 1 minute after each successful impalement, there appeared spontaneous action potentials at a constant frequency. The most striking property of these action potentials is the hyperpolarization from the resting level. Oscillographic records show that the magnitude of the action potential from the resting level is 17 ± 2 mV negative to the outside medium. The action potential was in correlation with the ciliary movement, i.e., the electric potential varies in exact accordance with the change in the inclination of the line of metachronal wave to the lateral axis of the animal. When the perfect ciliary reversal occurred, no indication of action potential was observed.

2. There was a strict correlation between the time courses of the action potential and of the membrane resistance: the membrane resistance reaches its minimum at the valley bottom of the action potential.

3. When a sufficient current was applied across the cell membrane in outward direction, an action potential could be evoked on break of the current. With strong current the action potential was evoked on make of the current and, thereafter, the spontaneous action potential as well as ciliary activity was perfectly inhibited. Moreover, after return to the normal potential, the inhibition effect remained for a time.

4. When a sufficient hyperpolarizing current was applied across the cell membrane, an action potential could be evoked on make of the current. When the resting potential was hyperpolarized more than the amplitude of the action potential, both the spontaneous action potential and the change in ciliary wave pattern were inhibited perfectly. On return to the normal potential, the inhibition

effect remained for a time.

5. When single shock stimulation was applied through an inserted microelectrode during the course of action potential, *Opalina* showed peculiar response according to the direction and intensity of the stimulation.

Outward current of adequate intensity induced a change in the beating direction of cilia. With strong outward current, the ciliary reversal occurred. Another remarkable effect of outward current stimulation was that when the stimulation is applied near in time of valley bottom of action potential, the ciliary reversal occurs followed by change in beating direction of the cilia. On the contrary, inward current induced the augmentation of effective stroke in normal direction. There was a strict correlation between the ciliary response and the change in the electric potential.

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