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ELECTRICAL RECORDING FROM THE CONTRACTILE CILIATE *ZOOTHAMNIUM GENICULATUM* AYRTON

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SUMMARY

1. The spherical body (200 μm in diameter) of this ciliate protozoan was easily immobilized on a suction pipette and penetrated with glass microelectrodes.

2. No recordings could be made from the contractile stalk, but in the body a negative potential of $30{\cdot}3 \pm 12{\cdot}5$ mV was observed.

3. Approximately 9 ms after the onset of a mechanical stimulus, a rapid depolarization was observed. This was apparently simultaneous throughout the body surface. The amplitude was $38{\cdot}9 \pm 13{\cdot}7$ mV. Recordings with a photomultiplier showed that contraction of the stalk began 10 ms later.

4. A slower depolarization of complex form was observed during systole of the contractile vacuole.

INTRODUCTION

Many species of ciliated protozoa show rapid contractions which occur apparently spontaneously or in response to mechanical or electrical stimuli. High-speed filming shows that after localized stimulation the contraction spreads rapidly through the cell, which may be as much as 2 mm long (Sugi, 1960; Wood, 1970; Nielson, 1975). This suggests that it is triggered by electrical activity of the cell surface. Recording such activity in freshwater protozoa has proved difficult for several reasons, including movement artefact, tip potentials, encapsulation of the electrode and the necessity for external media of low conductivity (Bingley, 1966; Wood, 1970; Naitoh & Eckert, 1972). Disagreement exists as to the sign and even the existence of an action potential in *Stentor* (Nielsen, 1975), and none has been found in *Spirostomum* (Sleigh, 1970; Ettiene, 1970; Hawkes & Holberton, 1974).

This appears to be the first account of electrical recording from a ciliate of the order Peritrichida, though many studies have been made of the rapid contraction in this group, some reviewed by Amos *et al.* (1976). For these experiments the stage in the development of *Zoothamnium geniculatum* was chosen at which a single spherical cell body is attached to an unbranched contractile stalk (Wesenberg-Lund, 1925; Amos *et al.* 1976). The cell body is large (200 μm in diameter) and covered with a tough pellicle which makes it easy to immobilize by means of a suction tube. Another advantage is that the macrozooid at this stage does not feed and has no food vacuoles in the cytoplasm.

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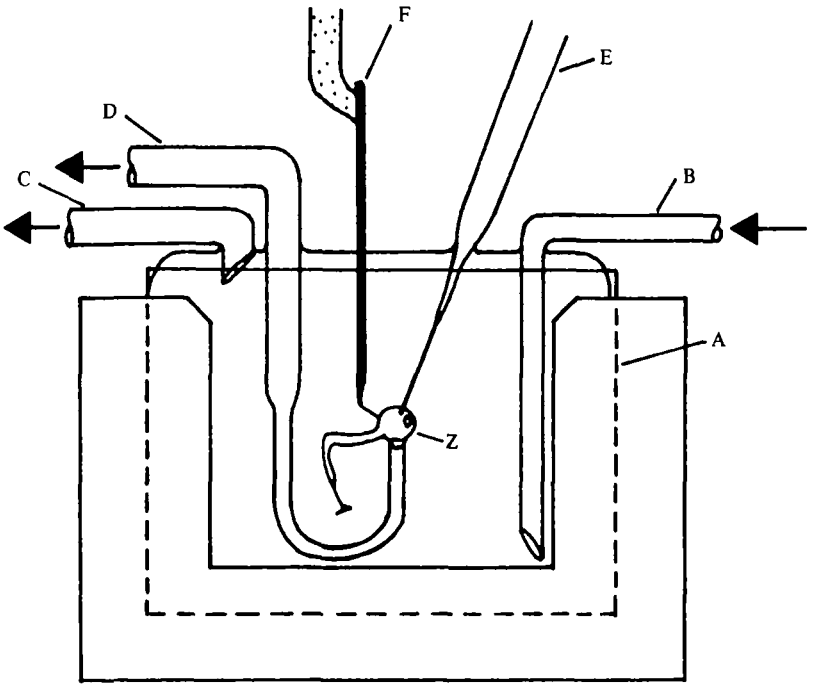

Fig. 1. Schematic view of perspex recording chamber as seen from the usual direction of viewing. Glass slides (A) fit into greased slots to form front and back. Inlet and outlet tubes for saline (B, C) are made of nylon, and the suction electrode (D) on which the animal (Z) is mounted is made from pyrex glass, with the end ground flat. The microelectrode (E) is inserted from above and mechanical stimulation is provided by a sharpened tungsten wire (F), glued to the stylus of a magnetic record-player cartridge. Illumination is from behind, using a light-guide. The chamber is 10 mm square and 8 mm deep, giving a saline capacity of about 1 cm^3, including the meniscus.

METHODS

Organisms

Mature colonies of *Zoothamnium geniculatum* were collected from submerged leaves of the yellow water lily (*Nuphar*) in the rivers Cam and Ouse, Cambridgeshire. They were detached from the leaves and left overnight in the laboratory in shallow dishes filled with filtered river water. During this time the macrozooids detached themselves from the colonies, settled on the sides of the dishes and grew stalks. Individuals at the single cell stage were chosen for the experiments, but development continued in the experimental chamber: the cells often divided while impaled with the microelectrode.

Electrical recording

The chamber for electrical recording was made of perspex (Fig. 1), with front and rear faces of glass to allow viewing by transmitted light, using a binocular microscope. Light was provided by a fibre-optic light-guide, either directly or, for dark-field illumination, via a small plane mirror. The chamber contained about 1 ml of solution, which could be renewed by perfusion from an elevated reservoir, a constant level being maintained by the suction line.

Animals were mounted on a suction pipette made from 1·2 mm diameter glass tubing, drawn out to a diameter of about 150 μm and bent into a U shape in a microflame. The end was cut off and ground flat on a miniature abrasive wheel, before mounting the pipette on a micromanipulator and connecting it by a short length of vinyl tubing to a Hamilton 50 μl syringe. Syringe and tubing were kept full of solution. Animals at the one-cell stage were detached from the substrate by gripping the base of the stalk with fine forceps and transferred to the chamber. By gentle suction the zooid could be captured on the pipette, where it could be kept for periods up to 2 h, with the stalk hanging free to one side (Fig. 1). Contractility of the stalk and regular emptying of the contractile vacuole could be observed throughout this period.

Microelectrodes were made from pyrex glass tubing with internal filament (Clark Electromedical Instruments) and filled with 0·5 M-K_2SO_4 by capillarity, to give tip resistances of 20–30 MΩ. Recordings were made using either one electrode, two electrodes inserted together, or two electrodes inserted separately into different parts of the zooid. Signals were taken via high-impedance amplifiers to a Tektronix 565 oscilloscope with Nihon-Kohden continuous recording camera, and to a Servoscribe 2-channel chart recorder.

Mechanical stimulation was provided by an electrolytically sharpened tungsten wire, glued to the stylus of a magnetic record-player cartridge, which was mounted on a micromanipulator. A 10 msec current pulse from a stimulator was used to move the tip of the wire by about 50 μm. By careful positioning of the wire, stalk contractions could be reliably evoked. The stimulator also triggered the oscilloscope sweep for photographic recording. Optical records of stalk contraction were made by replacing one eyepiece of the binocular microscope with a fibre-optic light-guide leading to a photomultiplier (Custom Instrumentation, Ravena, New York). By offsetting the illumination it was possible to get a dark-field image in this eyepiece, and a bright-field image in the other. Contraction of the stalk produced a change in photomultiplier output which was displayed on the oscilloscope screen simultaneously with the microelectrode recording. To get a smooth trace, the illuminating lamp was powered from an accumulator. In order to determine the response time of the probe, an opaque screen containing a narrow slit was inserted into the experimental chamber in such a position that the probe interrupted the passage of light from the slit into the photomultiplier. The movement of the probe was thus recorded by the photomultiplier and could be related in time to the voltage pulse delivered to the magnetic pickup. The resulting trace showed a delay of approximately 0·5 ms from the beginning of the pulse before movement of the probe commenced.

A standard bathing solution for all the experiments was based on Carter's (1957) medium for *Spirostomum*, and had the following composition (mM): NaCl 2·0, KCl 0·5, $MgCl_2$ 0·2, $CaCl_2$ 0·5, K_2HPO_4 0·045, KH_2PO_4 0·008 (pH 7·4).

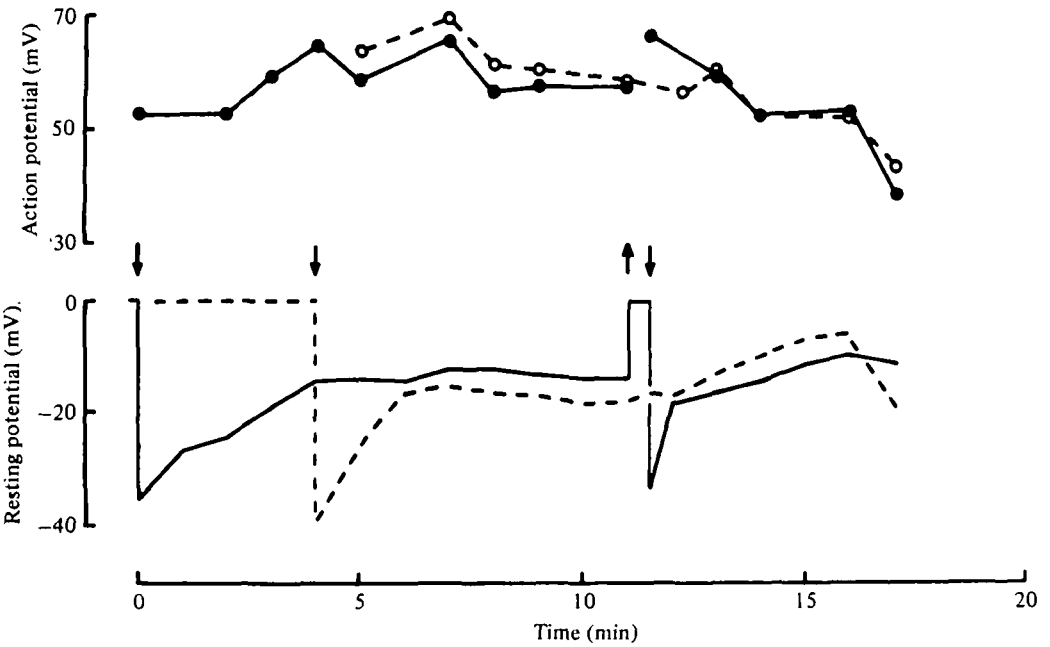

Fig. 2. Record of an experiment using two separate microelectrodes. The lower pair of traces shows the 'resting' potential; the upper pair shows the height of the 'action' potential, as measured from the resting level to the peak of the depolarization. The first electrode (continuous traces) was inserted at time zero, at a position nearly opposite to the stalk. After 4 min the second electrode (broken lines) was inserted, close to the stalk. After a further 8 min the first electrode was withdrawn and re-inserted at an intermediate site, close to the contractile vacuole. As can be seen from the lower traces, the 'resting' potential recorded on each impalement was initially large, but decayed rapidly to a value between −10 and −20 mV. These changes were not accompanied by any systematic change in the amplitude of the 'action' potential, which was evoked in this experiment by tapping on the bench, and was recorded similarly by both electrodes. Comparison of the traces shows that the 'action' potential had a positive 'overshoot' of at least 18 mV, throughout the experiment.

RESULTS

1. *Resting and 'action' potentials*

Typically, impalement was accompanied by a negative-going excursion, averaging (30·3 ± 12·5) mV (1 S.D., N = 20). In seven experiments this was maintained throughout the experiment, although the exact value tended to drift about with time. In the remainder the potential decayed over 5–15 min to a value between zero and −20 mV, where it remained without further systematic change. This decay evidently did not represent a real depolarization of the cell, since subsequent re-impalement with the same or a second microelectrode produced a trace very like that of the first impalement (Fig. 2); it may result from encapsulation of the electrode (Wood, 1970).

Fig. 3 shows some examples of the 'action potential', which was associated with contraction of the stalk. The amplitude of this was variable, both between individuals and from time to time in the same animal, but the general form of a fast rise and slow return to base-line was consistent. Mean values of amplitude and rise-time were respectively (38·9 ± 13·7) mV and (33·1 ± 25·2) ms (N = 18). The amplitude was not significantly correlated with the resting potential, and did not show the

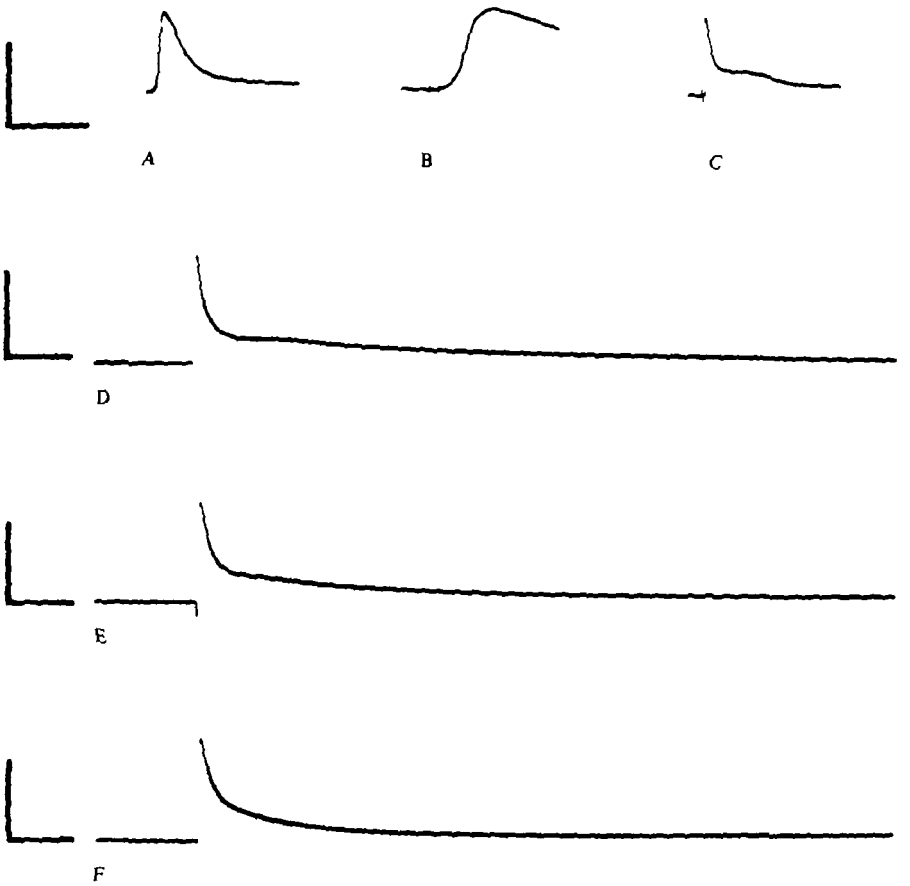

Fig. 3. 'Action' potentials recorded from three different individuals. (A, B) Stimulated with the wire probe, and recorded at two different sweep-speeds; (C) second animal, stimulated with the wire probe; (D–F) third animal, respectively spontaneous, and stimulated with the wire probe, or by tapping the bench. Scale-bars: vertical 50 mV; horizontal 500 ms (A–F), 100 ms (B).

same progressive decay; in many cases it was larger than the maximum recorded resting potential, so that the potential inside the cell became transiently positive with respect to the exterior (cf. Fig. 2).

Action potentials of a similar form accompanied all stalk contractions whether spontaneous, or evoked by movement of the tungsten probe, or by tapping the bench. Attempts to evoke either action potentials or stalk contractions by pulsed or d.c. depolarizations of the cell body, using a second internal microelectrode, were totally unsuccessful. Contraction could be evoked by applying a high-voltage pulse (*ca.* 200 V) to external wire electrodes, but this produced a large-scale electrical disturbance in which the action potential could not be identified.

A propagation velocity for the action potential could not be measured, since depolarization of all regions of the cell body appeared simultaneous, as shown by Fig. 4, which was obtained by inserting two electrodes separately. The first electrode was placed near the stalk and the second in a diametrically opposite position.

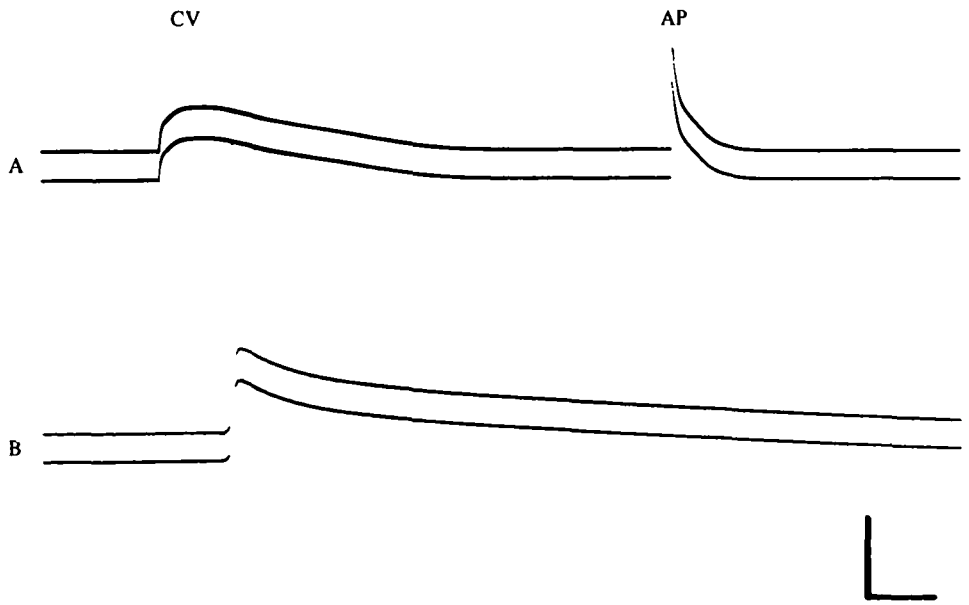

Fig. 4. (A) Simultaneous recording from two electrodes placed at opposite ends of the animal: upper trace, near the stalk; lower trace, furthest from the stalk. The trace shows a contractile vacuole signal (CV) and an action potential (AP) evoked by tapping the branch. (B) High-speed recording of an action potential, showing synchrony of the two traces. Note that both action potential and contractile vacuole signal appear with similar amplitude on both traces, despite the different positions of the two electrodes. Scale-bar: vertical 50 mV; horizontal 2 s (A), 100 ms (B).

It was not possible to record directly from the stalk itself to see whether the action potential is propagated along the stalk, as the extracellular sheath proved too tough to penetrate. No signal could be detected with electrodes placed outside the sheath.

When contraction was elicited with the tungsten wire, allowing for the measured delay of 0·5 ms between the voltage pulse to the mechanical stimulator and the movement of the probe, the average delay between the movement of the probe and the 'foot' of the action potential was $8{\cdot}9 \pm 5{\cdot}3$ ms (S.E.M.).

2. *Relation between the action potential and stalk contraction*

Fig. 5 shows simultaneous microelectrode and photomultiplier recordings. The rising phase of the action potential clearly begins before the onset of contraction, in this case by some 12 ms. This was found to be so in six out of seven experiments, the average delay between the 'foot' of the action potential and the onset of contraction being $9{\cdot}8 \pm 3{\cdot}5$ ms (S.E.M.), suggesting that the action potential could be the event responsible for initiating the contraction.

3. *The contractile vacuole*

Discharge, or systole, of the contractile vacuole was regularly accompanied by an abrupt depolarization, 5–20 mV in magnitude: an example is shown in Fig. 6. The depolarization, which was sometimes preceded by a brief, hyperpolarizing phase, lasted throughout the period of vacuolar systole, after which the membrane potential returned promptly to the resting level. Examination of film records taken at a higher

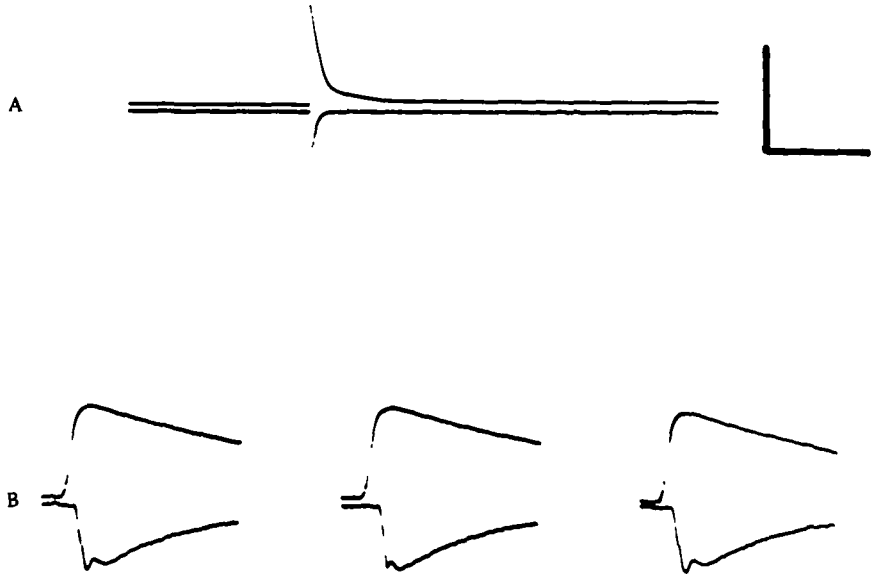

Fig. 5. (A) Simultaneous microelectrode recording (upper trace) and photomultiplier signal from the contracting stalk, taken at a sensitivity of 10^{-7} A, and with the same voltage gain as the microelectrode trace. (B) Single-sweep records to show delay between onset of the action potential and the contraction. Stimulation by wire probe. Scale-bar: vertical 50 mV; horizontal 2·5 s (A), 100 ms (B).

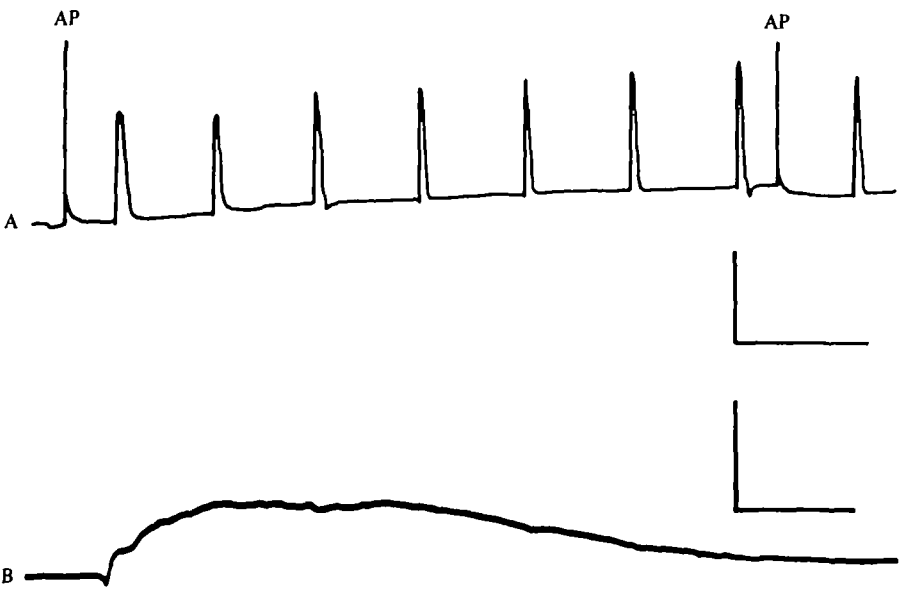

Fig. 6. (A) Chart recording, showing regular discharges of the contractile vacuole. Also shown are two action potentials (AP), much attenuated by the poor frequency-response of the recorder. Scale-bars: vertical 10 mV; horizontal 1 min. (B) Oscilloscope recording of the first contractile vacuole signal in (A), showing a small initial hyperpolarizing phase and fluctuation of the membrane potential during the depolarizing phase. Another example of an oscilloscope recording appears in Fig. 4(A). Scale-bars: vertical 20 mV; horizontal 1 s.

speed (Fig. 6B) shows that the depolarizing phase is characterized by apparently random fluctuations of the membrane potential, with an amplitude of the order of 1–2 mV, which are not seen at other times.

DISCUSSION

The macrozooid of *Zoothamnium* appears to have a negative resting potential and shows a fast depolarization when stimulated mechanically. The resting and transient potentials are therefore of the same sign as those observed in neurones and muscle fibres. In another ciliate, *Stentor*, transient hyperpolarizing potentials were observed in association with contraction by Wood (1970) though not by Nielsen and her colleagues (Nielsen, 1975). Potentials of apparently unorthodox sign are also observed in the flagellate protozoan *Noctiluca*. In this case it has been shown by Eckert & Sibaoka (1968) that the apparent inversion of the action potential is due to the existence of an internal excitable membrane limiting a non-cytoplasmic space. The result obtained by Wood may perhaps be due to the presence of such a membrane in the oral region of *Stentor*. The cytoplasm of the macrozooid of *Zoothamnium* is remarkably free of food vacuoles and other internal compartments (unpublished electron microscope observations by W. B. Amos).

Because of variability in resting potential it is not possible to decide from our results whether the 'action potential' in *Zoothamnium* is an all-or-nothing event. The observed fluctuations in amplitude may be due to defects in the method of recording. On the other hand the electrical responses of *Paramecium* to mechanical stimuli are graded and only weakly regenerative (Eckert, Naitoh & Machemer, 1976).

Presumably the depolarization that we have observed in the cell body invades the stalk and triggers contraction there.

Sugi (1960) observed that in the peritrich ciliate *Carchesium* a contraction wave started at the cell body and passed down the stalk at a velocity of 0·2–0·5 m s^{-1}. The delay of 9·8 ± 3·5 ms between the foot of the action potential and the onset of contraction in *Zoothamnium* allows ample time for the passage of an impulse down the stalk at the same velocity as Sugi's contraction wave: only 2–5 ms are required for a stalk 1 mm long. Our failure to detect such an impulse in the stalk with external electrodes is presumably due to attenuation by the layer of extracellular fibrous material which clothes the stalk in vorticellids (Amos, 1972). This layer varies in thickness from 5 to 20 μm from point to point in the *Zoothamnium* stalk.

The depolarization of the cell during the systole of the contractile vacuole has apparently not been observed elsewhere in the protozoa. Wesenberg-Lund (1925) described the contractile vacuole of the macrozooid of *Zoothamnium* as having a main vacuole which communicates with the exterior by a fine canal during systole. Presumably the depolarization is due to this temporary connexion of the main vacuole to the exterior, while the small bumps on the voltage record may be associated with the fusion of smaller vacuoles with the main one. According to Wesenberg-Lund such fusion may continue even during systole. The contractile vacuole of this species has not been examined in the electron microscope, but that of *Paramecium* consists essentially of a space communicating with the exterior, from which cytoplasm is separated by only a single membrane (Schneider, 1960). The complex

structure of three membranes which covers the majority of the surface in ciliates may well have a higher resistance than the contractile vacuole membrane. If so, the depolarization at systole may be due to a short-circuiting of the cell surface through the vacuolar membrane.

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