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ELECTROPHYSIOLOGICAL STUDIES OF THE PROTOZOAN, *STENTOR COERULEUS*

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SUMMARY

Transmembrane potentials and membrane characteristics of *Stentor coeruleus* were studied by means of microelectrodes and standard electrophysiological techniques. Intracellular resting potentials were found to be variable and recordable for only a brief span of time owing to the encapsulation of the recording electrodes. During this brief time span of recorded resting potentials were anamolous in that they were generally of positive polarity.

When an extended *Stentor* was stimulated to contract, a 10–60 mv negative-going transient response was recorded from intracellular electrodes. After the electrodes had been encapsulated, a diphasic transient response was observed when the penetrated animal contracted. Simultaneous recordings from intracellular and encapsulated electrodes showed that the diphasic and negative-going transient responses occurred simultaneously.

Contractions of *Stentor* occurred in 7 to 8 msec following a 2 to 3 msec latent period after a suprathreshold shock. Records obtained by use of a photomultiplier and microelectrodes indicated that the contractions began 1.8 msec after the onset of the diphasic response.

Prepotentials were observed prior to mechanically stimulated responses.

INTRODUCTION

Behavioral observations made in the previous study (Wood, 1969) were obtained by direct visual observation of rapid bodily contractions of *Stentor*. Contractions of muscle fibers are activated by action potentials; therefore, it is reasonable to suspect that action potentials might initiate contractions of *Stentor*. On the other hand, Jahn (1966) has theorized that protozoan contractions are not dependent on action potentials. The studies reported here were designed to ascertain the

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relation between transmembrane potentials and contractions of *Stentor* if such a relation exists.

Preliminary observations indicate contractions of *Stentor* exhibit a number of properties analogous to those of muscle fibers. For instance, the contractions appear to be all-or-none whether elicited by mechanical or electrical stimuli. Furthermore, a reproducible threshold for electrical stimulation could readily be defined for individual animals or groups of animals. Employing electrical stimuli, a preliminary study was made of accommodation and the strength-duration curve of *Stentor*. Results from these studies were sufficiently in accord with results predicted on the basis of metazoan excitable tissues to warrant the further investigation reported below.

It should also be noted that action potentials have been recorded from the marine dinoflagellate *Noctiluca*. Chang (1960), and Eckert and Sibaoka (1968) have demonstrated that these hyperpolarizing responses are correlated with a transient decrease in membrane impedance. Also, Eckert (1965a,b) has found these responses to be propagated and coupled with the animal's bioluminescent flash. Transmembrane potential changes have also been correlated with contractile movements determining ciliary reversal in *Paramecium* (Naitoh, 1966) and tentacle movements of *Noctiluca* (Eckert and Sibaoka, 1967; Sibaoka and Eckert, 1967). However, there appears to be no previous report demonstrating a correlation between transient transmembrane potential changes and contractions of the entire protozoan body analogous to what occurs in muscle fibers.

METHODS

Maintenance of Stentor. *Stentor* for these studies were cultured in the manner previously described (Wood, 1969).

During the electrophysiological recording sessions they were maintained in approximately 1 ml of culture medium in a plexiglas dish. A small amount of 10% methyl cellulose was mixed with the culture medium in this dish to slow the swimming of the *Stentor* and to hasten their change to the sessile form. The animals were viewed through a stereomicroscope at 7 to 60 X. Room temperature was maintained at 18–22°C.

Recording apparatus and procedure. Glass microelectrodes filled with either 1.5 *M* KCl or 0.1 *M* KCl produced records of similar time course and voltage. All electrodes had resistances between 5 and 30 megohms and tip potentials of less than 5 mv when measured in the culture medium (Adrian, 1956). Such electrodes also showed minimal (>2mv) shifts in tip potential when their tips were transferred from the culture medium to a medium containing 18.5 mM/1. $CaCl_2$. 18.5 mM/1. is the previously determined total concentration of Ca^{2+} within *Stentor*, through much of this Ca^{2+} may be bound (unpublished results). Hence, changes in tip potential should have been small when the electrode tips were transferred from the culture medium into an animal's cytoplasm (Bingley, 1964a).

The culture medium in the plexiglas dish and the microelectrodes were connected via Ag-AgCl half cells to a D.C. preamplifier which was directly coupled both to an oscilloscope and recording voltmeter. Grid currents were monitored at frequent intervals and never exceeded 10^{-11} amp.

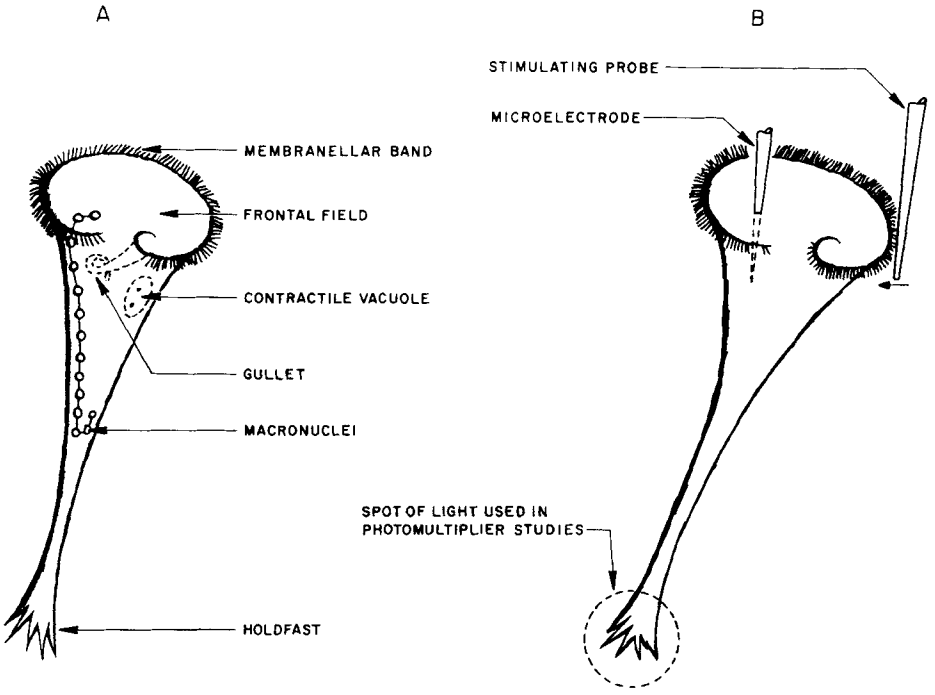

Figure 1A. An illustration of the gross anatomical features of *Stentor* labelling the principal structures discussed.

Figure 1B. An illustration showing the location of microelectrode insertion and localized mechanical stimulation. The area illuminated during the photomultiplier studies of contracting *Stentor* is also shown.

In preparation for an experimental session, 4 to 10 animals were introduced into the test chamber 15 to 30 min prior to experimentation. This interval was sufficient for most of the animals to become sessile and extended in the viscous methyl cellulose suspension. The problem of proper electrode penetration was considerably complicated by the fact that *Stentor* can simply swim away from a penetrating electrode. Holding devices such as a suction pipette or "pinning" electrode were found to aggravate the animals' swimming behavior and damage their pellicles, and therefore were not used. Successful and lasting penetrations were obtained by penetrating the animals only through the frontal field (see Fig. 1). With 1 or 2 microelectrodes in this position, animals in swimming drove themselves further onto the electrodes rather than away from them. About $\frac{1}{3}$ of the attempted penetrations produced usable records.

Stimuli. Monophasic electrical stimuli of calibrated amplitude and duration were obtained from a Grass S4 stimulator through a stimulus isolation unit and a 10^9 ohm series resistor.

Mechanical stimuli were delivered by means of a blunted microelectrode approximately 8 cm long. Movements of this probe were obtained by applying an exponentially rising 60 to 100 V monophasic pulse with a time constant of 2.2 msec across the ceramic element of a phonographic cartridge. The excursion of the stimulating probe tip produced by this pulse reached a maximum displacement after 6.2 msec as indicated by photocell recordings.

Photographic records. For pictures of *Stentor* contracting, a 1 × 1.4 cm rectangular chamber was constructed of plexiglas and mounted on a microscope slide. Both ends

of this chamber were fitted with stainless steel plates which served as stimulating electrodes. This experimental chamber was illuminated obliquely from below by the Xenon bulb of a Grass PS-2D photostimulator which produced a 10 μsec flash. The camera was mounted on a stereomicroscope above the experimental chamber. During picture taking the room was darkened and the camera shutter opened allowing the photostimulator to expose the high speed film. Variable intervals between the suprathreshold shock applied to the animals in the test chamber and the photostimulator flash were obtained by driving both a stimulator and the photostimulator with a second stimulator which had a variable delay. Shock onset—flash intervals of 1–10, 15, and 20 msec were randomly used with each of 13 animals. Temporal values for these intervals were monitored on the oscilloscope and varied less than 0.2 msec from those listed. A picture was taken before each stimulus-flash sequence to control for variations in the degree of extension of the animals and their orientation. From these pictures the time course of contractions in *Stentor* was determined.

Photomultiplier records. The temporal correlation between potential changes recorded from *Stentor* and their contractions was obtained by monitoring these contractions with a photomultiplier tube (IP 21) whose output was displayed on one channel of the oscilloscope. The photomultiplier and its housing were mounted directly on the stereomicroscope. Constant intense illumination was provided from below by a 6 V automobile headlight. Baffles were positioned so that a spot of light illuminating only the tail of a previously impaled *Stentor* was focused on the photomultiplier surface (Fig. 1). When the animal was mechanically or electrically stimulated, the tail was withdrawn from the spot of light leading to a recordable trace deflection on the oscilloscope. In this case the tail was withdrawn to the anterior end of the animal because the anterior end was affixed to the microelectrode while the holdfast was not attached to any solid object. The blunted microelectrode used for mechanical stimulation was kept out of the illuminated spot to avoid artifact due to its movement.

RESULTS

Resting potential. Typically, a 20 to 35 mv positive D.C. potential was observed immediately after microelectrodes were inserted into a *Stentor*. However, many records indicated a smaller positive potential and in some cases the initial deflection was in the negative direction. Within 1 or 2 min this initial potential deflection was lost and the recorded potential returned abruptly to near the 0 mv baseline. Several erratic excursions to positive potentials sometimes occurred within the ensuing several minutes, but eventually the recorded potential returned to near the baseline if there was no change in electrode resistance.

During the period of the initial potential deflection the records were characterized by a low (<1mv) amplitude ripple similar to that reported by Naitoh (1966). This ripple was comprised mostly of high frequency components. The presence of such irregular ripples in the oscilloscope trace and photographic records was readily discernible.

During the later stages of a microelectrode penetration, when the recorded steady potentials were several millivolts or less, visual inspection indicated that the electrodes were still inserted within the animal. Passing current down such electrodes regularly caused the *Stentor* to contract, confirming the close proximity of the electrode to the cell, Nevertheless, since two such electrodes had different steady potentials.

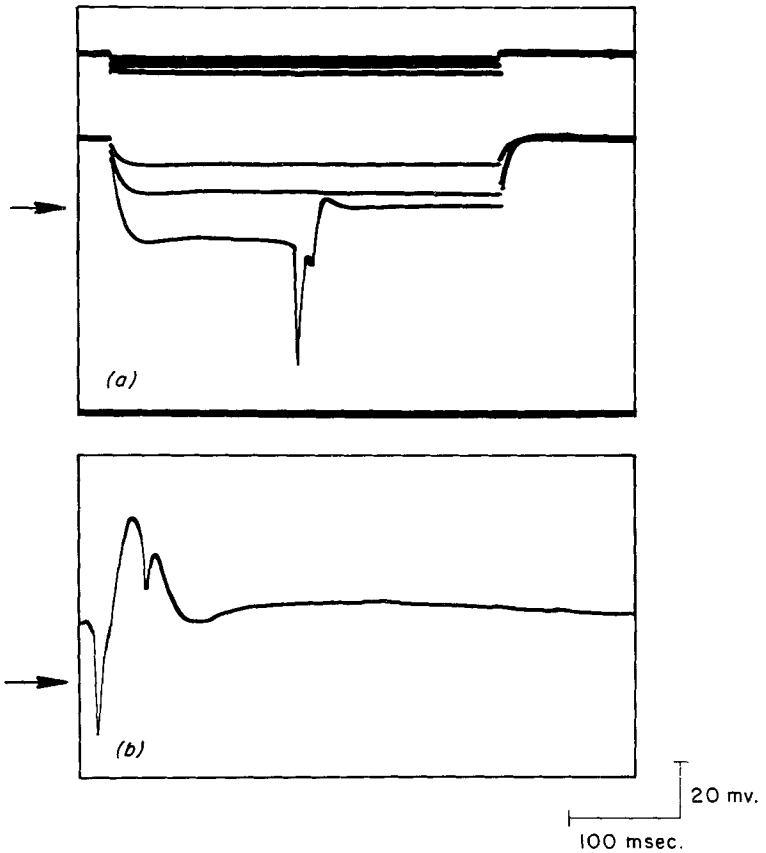

Figure 2A. Intracellularly recorded transient responses to 2,4 and 7 $\times$ 10^{-8} amp. cathodal current pulses. Spike response had an amplitude of -42 mv.

Figure 2B. Record produced by mechanical stimulation immediately after the record shown in A. Response had an amplitude of -41 mv. In these and subsequent records the black arrows to the left indicate the 0 mv. level with positive polarity being up.

and generally showed no resistive or capacitative coupling, it was apparent that both of the electrodes were not within the cell membrane.

It therefore appears that initially during the penetration of a cell the electrodes were in fact intracellular and recording intracellular potentials. However, within 1 to 2 min the tip of the penetrating electrodes was encapsulated by the cell and electrically and physically isolated from the cell interior. In this encapsulated state the electrodes could remain "penetrating" the cell for several hours or could be completely rejected with the animal becoming physically detached.

It does not appear feasible to quantify the intracellular resting potential on the basis of the records obtained because the recorded D.C. potential fluctuated a great deal during the minute or so of intracellular recording and because the recorded potential must certainly have dif-

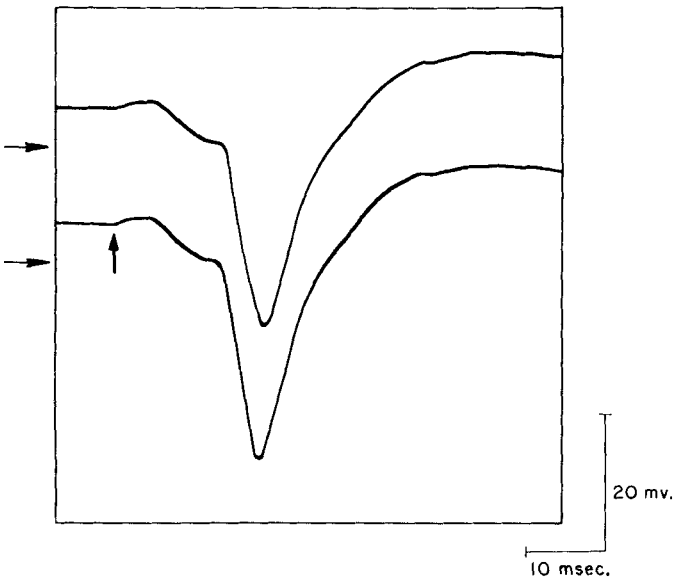

Figure 3. Spike recorded simultaneously from two intracellular microelectrodes. Stimulus was mechanical and began at the vertical black arrow.

fered from that of the intact animal owing to the recent injury involved in the microelectrode penetration. Nevertheless, it was apparent that the recorded potential was anamolous since it generally indicated an intracellular positivity. This positivity cannot be attributed to a tip potential or liquid junction potential change since such shifts were minimized by lowering the microelectrode resistance.

Intracellularly recorded transient responses. When the D.C. potential level and the resistive coupling between the electrodes indicated that they were intracellular, an all-or-none negative-going transient potential was recorded when the animal was stimulated to contract. Figure 2*a* provides an example of such a response produced by electrical stimulation; Figure 2*b* is a record from the same animal during the application of a mechanical stimulus which caused the animal to contract. Mechanically stimulated spikes had an average amplitude of 27 mv (10–60 mv) while electrically stimulated spikes averaged 23 mv (11–54 mv). Spike durations averaged 17 msec (9–27 msec), though secondary spikes and shoulders during the repolarizing phase of some records appeared responsible for this large average measure and upper limit. Large positive-going after potentials (Fig. 2*b*) were frequently but not invariably seen. Maximum rates of potential change were on the order of 10 V/sec and the average threshold stimulus was a 350 msec 5×10^{-8} ($2\text{–}8 \times 10^{-8}$) amp cathodal current pulse. Anodal current pulses were ineffective in producing spikes though a positive-going potential similar to the above mentioned after-potential was sometimes produced.

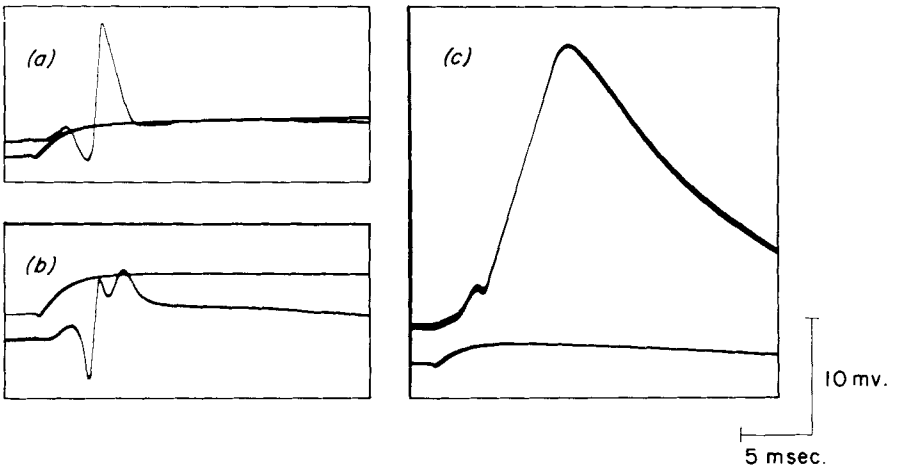

Figure 4. Transient responses recorded from encapsulated electrodes. Exponentially rising curve records the voltage pulse applied to the piezoelectric crystal. Different transient responses arise from different steady potentials as indicated by their position relative to the trace marking the stimulus pulse. The onset of the stimulus pulse trace is positioned at 0 mv. for the voltage electrodes. Transient responses similar to A were recorded in 66% of the cases, similar to B in 6% of the cases and similar to C in 28% of the cases.

During the period when such responses were recorded, both electrodes exhibited the same potential level and changes (Fig. 3) as would be expected of intracellular electrodes.

About half of the animals produced repetitive spikes to a single sustained electrical stimulus or to a mechanical stimulus. Only a small secondary spike is evident in Figures 2*a* and 2*b*, but occasionally the secondary spike was of equal amplitude with the first. Since animals require more than 15 sec to reextend after a contraction, it is apparent that they could have produced only one contraction of their entire body during the time span of a single record ($\frac{1}{2}$ sec). Therefore, the second spike could not have been an artifact resultant from mechanical disturbance of the electrode produced by a large contraction of the animal's body. Since the first spike had the same amplitude and form, it seems likely that it also is not a motion artifact.

Transient responses recorded from encapsulated electrodes. Two or more minutes after the beginning of a microelectrode penetration, tests for electrical coupling indicated the electrodes had been encapsulated. At this time diphasic transient responses were recorded whenever an electrically or mechanically stimulated animal contracted. The form of these potentials, as recorded by a single electrode, was fairly consistent from contraction to contraction but varied markedly from animal to animal. Certain potential patterns were characteristically observed and are reproduced as Figures 4*a*, *b*, and *c*. With but a

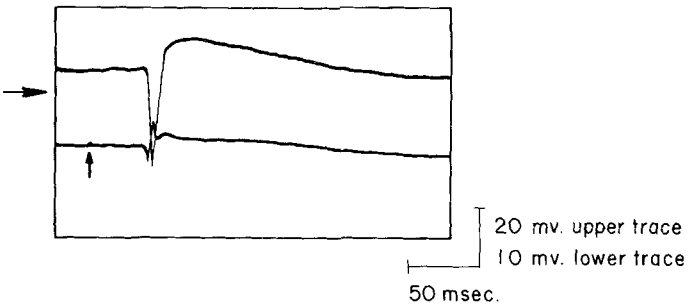

Figure 5. Simultaneous recording from an intracellular electrode (upper trace) and an encapsulated electrode (lower trace). No prepotential is evident and the latency of the response is long. Mechanical stimulus onset occurred at the vertical black arrow.

few exceptions potential patterns recorded simultaneously from two electrodes "penetrating" the same animal were different. Hence, a particular potential pattern appears characteristic of the locus of the electrode tip and not of the animal as a whole.

The amplitude of such diphasic spikes ranged continuously from 0 to 30 mv. Characteristically the responses were largest during initial contractions and decreased in amplitude with time. Simultaneously the resistance in series with the electrode decreased and the amount of current required to electrically stimulate the animal increased. These observations suggest the animal was becoming disengaged from the electrodes during a recording session. Recording sessions often ended because the animal freed itself from the electrodes.

Transient responses recorded from encapsulated electrodes will hereafter be referred to as diphasic to differentiate them from negative-going potentials recorded from intracellular electrodes.

The temporal correlation of the negative-going and the diphasic response. The fact that transient responses recorded from intracellular and encapsulated electrodes were both observed when extended animals contracted suggests that the two events are correlated. By inserting two microelectrodes and waiting several minutes before mechanically stimulating the penetrated animal, it was possible to record the two types of transient response simultaneously. It appears probable that one electrode had become encapsulated during the waiting period while the other electrode remained shorted to the cytoplasm.

A record of such a simultaneous recording is presented as Figure 5. The temporal correlation of the two potentials is obvious in this and six similar records. It is also apparent that the onset of the negative-going response is the same in both records; this appears as the consistent point of temporal coincidence. Therefore, the negative-going and diphasic potentials occur simultaneously so that recording the diphasic potential is sufficient evidence that an intracellular negative-going response occurred.

The time course of contractions and their correlation to transient responses. To determine the time course of contractions pictures were taken by briefly illuminating the test chamber containing several *Stentor* at variable intervals after administering a suprathreshold shock. Figure 6 is a plot of the length of the contracting animals as a percentage of their prestimulus length against the interval between the electrical stimulus and the film exposure. From the figure it is apparent that *Stentor* contract in 7 to 8 msec following a 2 to 3 msec latent period after the shock onset. Slight irregularities in the curve are probably attributable to alterations in the animals' length or orientation after the prestimulus control picture was taken.

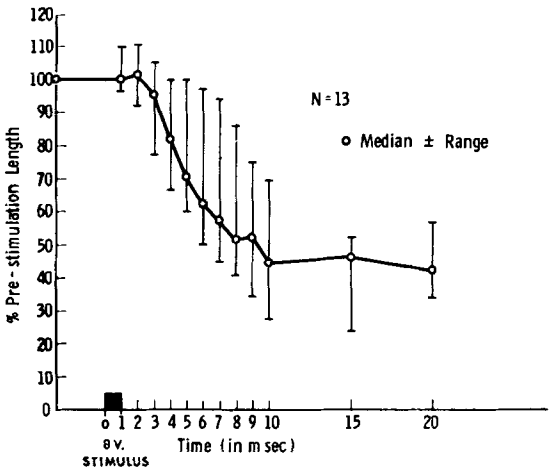

Figure 6. Percentage of prestimulus extended length against time after stimulus. Duration and time of stimulus is indicated by the black box on the abscissa.

These data were confirmed by use of the same test chamber and the photomultiplier. By this method a median contraction latency of 2.9 msec (range 2.0–5.5 msec) was determined. Contraction time courses, as determined from photomultiplier records, were generally briefer than indicated by the photographic data because the animal's anterior was withdrawn from the area focused on the photomultiplier before the contraction was complete, i.e., only part of the contraction was recorded.

Finally, the photomultiplier and baffles were arranged so that only a small spot of light containing the image of a single *Stentor's* tail was focused on the photomultiplier surface (see Fig. 1*b*). This animal previously had been penetrated by a microelectrode, and transient responses were recorded. The top trace of Figure 7 monitored the photomultiplier output and hence the animal's contractions, while the lower trace recorded the microelectrode potential. The onset of the contraction occurred 2.5 msec after the onset of the diphasic transient response. In all cases positioning the masks and stimulating needle required several

Figure 7. Simultaneously recorded transient response from an encapsulated microelectrode and voltage output of the photomultiplier tube. Photomultiplier output is uncalibrated.

minutes, hence only diphasic transient responses from encapsulated electrodes were obtained.

25 records from 7 animals showed the contraction onset occurred 1.8 msec (range: 0.8–2.6 msec) after the onset of the transient response. These data leave no question as to the correlation of contractions and transient responses. Further, the initial segment of the diphasic response cannot be attributed to artifact due to the contraction since it precedes the contraction.

Specific membrane resistance and capacitance. Membrane resistances were studied by means of rectangular cathodal current pulses of varying amplitudes. Slope resistances were determined from the resulting E-I graphs. For the calculation of specific membrane resistance the animal's surface area was estimated from the measured diameters of the contracted animal assuming it was spherical in shape. For six well-studied animals the pre-response specific membrane resistance was 2900 Ω cm^2 (1600–3600 Ω cm^2).

Membrane capacitances were determined by the time constant method. The six animals mentioned above had an average specific membrane capacitance of 8.8 μf/cm^2 (4.0–14.4 μf cm^2). This value may be too large by a value of 2 or so since the surface of the contracted animal was convoluted and its surface area larger than that used in the above calculation. Possibly overbalancing this surface area factor was the series capacitance present in the form of the pellicle. Were the pellicle to have a capacitance equal to that of the external plasma membrane the calculated specific membrane capacitance would be only half of that which actually characterizes the plasma membrane.

Prepotentials. Prepotentials were observed in records produced by mechanical stimuli. During intracellular records (Fig. 2*b*, 3) this response was generally negative-going and had the appearance of a receptor potential. Prepotentials recorded from encapsulated electrodes were positive-going (Fig. 4).

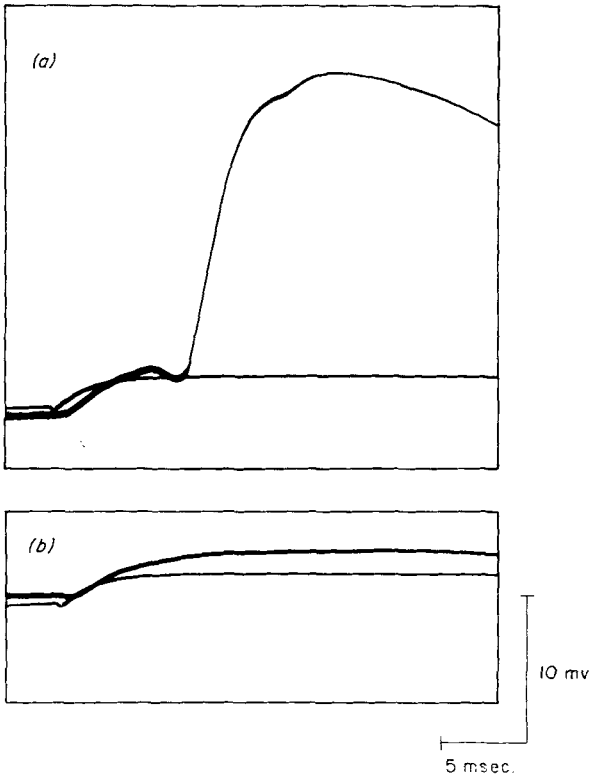

Figure 8A. Diphasic response produced by mechanical stimulation.
Figure 8B. Response to the next stimulus to which the animal did not contract.

Prepotentials could be dissociated from spike potentials. Electrical stimulation produced spike responses but not prepotentials in records from intracellular (Fig. 2*a*) or encapsulated electrodes. Transient responses produced by mechanical stimuli, and occurring with a long latency (>10 msec) were also not preceded by prepotentials (Fig. 5) or they were too small to see in the record. Conversely, in the absence of spikes, graded responses similar to prepotentials were still visible in the records (Fig. 8). Therefore, these prepotentials represent a process which is separable from that producing the spike responses.

A systematic study of prepotential amplitude was not made, but it was obvious that larger stimuli produced larger prepotentials. From the several properties of prepotentials observed it appears that they represent an event associated with mechanical stimulus reception.

DISCUSSION

Intracellular D.C. potentials. The rapidity of the encapsulation process and the instability of the intracellularly recorded D.C. potential prevented the quantitative characterization of the resting potential of

Stentor. It was clear, however, that this potential frequently appeared to be of positive polarity. Other experimenters (Bingley, 1966; Riddle, 1962) have reported positive intracellular resting potentials from fresh-water protozoa only under pathological conditions. Most reported values of transmembrane potentials in fresh-water protozoa have been in the range of −20 to −90 mv (Bingley, 1964a, 1966; Naitoh, 1966; Riddle, 1962; Yamaguchi, 1960; Kinosita, 1954). However, only Bingley (1966) employed both current and voltage electrodes, and objective criteria to determine if his electrodes were actually intracellular.

Changes of the tip potential and liquid junction potential associated with movement of the electrode tip from the dilute culture medium to the cell cytoplasm have previously been cited as sources of error in measuring membrane resting potentials in fresh-water protozoa (Yamaguchi, 1960; Bingley, 1964b). Care was taken in these studies to employ electrodes of low resistance which previously had been shown to produce minimal D.C. potential shifts when transferred into more concentrated media. However, large steady potentials associated with the suspension effect (Tasaki and Singer, 1968) may be expected to have complicated the recording of the membrane resting potential in *Stentor.* Also, large (20 mv) injury potentials have been observed as a result of microelectrode penetration into *Amoeba* (Batueva, 1965), and this factor may have contributed to some of the anomalous potentials recorded.

Transient responses. The principle finding of these studies was of a negative-going spike potential observed when recording from intracellular electrodes. This potential was of reproducible form and amplitude within a 1–2 min period after electrode penetration; i.e., it was all-or-none.

The negative-going responses observed during maintained cathodal stimulation could be attributed to a transient increase in membrane resistance produced by the continuing electrical stimulus as is observed in a variety of preparations (e.g., Bennett and Grundfest, 1967; Reuben, Werman, and Grundfest, 1961). However, since spikes of similar wave form and amplitude were produced by mechanical stimuli, it seems probable that spike production is characteristic of the animals' normal membrane behavior and not solely resultant from the method of stimulation employed.

While movements of the microelectrode tip can be expected to produce potential changes, very little or none of the recorded spike appears to be attributable to movement of the microelectrode produced by the animal's contraction. Certainly the reproducibility of the spike, despite alterations in the animal's orientation or failure to reextend, would not be expected if the contraction per se produced the potential. It was also clear that much of the diphasic potential recorded from encapsulated electrodes preceded the contraction and hence could not be produced by it.

A similar negative-going response of somewhat shorter time course and larger amplitude has been recorded from the sap vacuole of *Noctiluca*

(Hisada, 1957; Chang, 1960; Eckert, 1965a,b; Eckert and Sibaoka, 1968). The hyperpolarizing nature of the *Noctiluca* action potential appears attributable to the fact that it is generated across the membrane separating the sap vacuole from the cytoplasm (Eckert and Sibaoka, 1968). No similar large internal vacuole exists in *Stentor* hence the physiological basis for the negative-going response noted in *Stentor* must differ from the basis of the response noted in *Noctiluca*.

Contractile behavior. Photographic and photomultiplier data confirmed that the contraction is all-or-none as it appears during direct visual observation. 15 to 20 msec after a suprathreshold shock all animals studied had completed contracting; none required more time than this. Likewise, at these times all animals were 60% or less of their prestimulation length; that is, no animal showed partial or incomplete contractions. Contractions in *Stentor* occur with somewhat less rapidity than those observed in *Carchesium* (Sugi, 1960) and *Spirostumum* (Jones, Jahn and Tonesca, 1966), but otherwise appear to be of very similar nature.

Specific membrane resistance and capacitance. *Stentor* was determined to have a specific membrane resistance of 2900 Ω cm^2 and an unusually large specific membrane capacitance of 8.8 μf/cm^2. Specific membrane capacitances have been determined for two other protozoans; *Amoeba* (Batueva, 1965) and *Noctiluca* (Chang, 1960), both of which are reported to have values near 1 μf/cm^2. The values obtained for *Amoeba* and *Noctiluca* are therefore close to those obtained for most membranes while the high value observed in *Stentor* is somewhat anomalous. Fatt and Katz (1951, 1953) obtained values of 8 μf/cm^2 for frog skeletal muscle fibers and 40 μf/cm^2 for crustacean muscle fibers using the time constant method for determination. Subsequently, Fatt and Falk (1964) produced evidence to suggest that these high values were due to capacitance and resistance thought to be associated with the sarcoplasmic reticulum. In their electron micrographic studies, Randall and Jackson (1958) observed systems of tubules and vacuoles in *Stentor* but did not describe these sufficiently to suggest if these tubules could contribute to the apparent specific membrane capacitance in a fashion analogous to that of the sarcoplasmic reticulum.

Prepotentials. A graded potential preceded both negative-going and diphasic spike potentials if they were resultant from mechanical stimulation. The fact that these prepotentials were of positive polarity when recorded from encapsulated electrodes and generally of negative polarity when recorded from intracellular electrodes indicates they did not result from a direct effect of the stimulating probe on the recording electrode. Since these prepotentials could be dissociated from the spike, they appear to represent processes separate from that generating the spike, i.e., the processes associated with the reception of mechanical stimuli.

In summary, the evidence at hand warrants the formulation of a

simple model for *Stentor's* contractile behavior: mechanical stimulus → prepotential → spike → contraction. This is but a temporal sequence not a causal one, though the arrows between mechanical stimulus and prepotential and between spike and contraction appear to indicate causation as well as temporal order.

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