The Flash-Triggering Action Potential of the Luminescent Dinoflagellate *Noctiluca*

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ABSTRACT The action potential which elicits luminescence in *Noctiluca* is recorded from the flotation vacuole as a transient all-or-none hyperpolarization in response to either local or general application of inward (bath to vacuole) current. Experiments were performed to determine whether the unorthodox polarities of both the stimulus current and the potential response resulted from uncommon bioelectric mechanisms or from special morphological features of this species. The findings all indicate that the action potential belongs to the familiar class of responses which have their origin in voltage- and time-dependent selective increases in membrane permeability, and that morphological factors account for the observed deviations from normal behavior. Both the stimulus and the response have orthodox polarities provided the vacuole is designated as an "external" extracytoplasmic compartment. Differential recording between vacuole and cytoplasm showed that the action potential occurs across the vacuolar membrane, with the cytoplasmic potential, which at rest is negative with respect to the vacuole, overshooting zero and reversing sign to become transiently electropositive. The rising phase of the action potential therefore depends on active current flow through the vacuolar membrane from the vacuole into the cytoplasm. Propagation of the action potential over the subspherical cell from the locus of stimulation is thought to depend largely on the core conductor properties of the thin perivacuolar shell of cytoplasm which is bounded on its inner surface by the excitable membrane and on its outer surface by inexcitable membranes.

INTRODUCTION

In accordance with predictions based on other stimulus-response systems (Harvey 1952), it was found that the emission of light by luminescent dinoflagellates is mediated by specific bioelectric events interposed between the stimulus and the biochemical production of light (Eckert, 1965 *a*, *b*, 1966, 1967; Eckert and Reynolds, 1967). Mechanical or electrical stimulation of *Noctiluca* elicits an all-or-none action potential which propagates over the cell

from the zone of stimulus application, triggering the luminescent organelles in its path (Eckert, 1965 *b*; Eckert and Reynolds, 1967).

Noctiluca exhibits, in addition to the flash-triggering action potential, a spontaneously recurring potential wave form responsible for regulating contractions of the food-gathering tentacle (Eckert and Sibaoka, 1967; Sibaoka and Eckert, 1967). This tentacle-regulating potential (TRP) is largely independent of the electrical activity which triggers luminescence, and therefore it will receive only passing reference here.

The most striking feature of the flash-triggering action potential of *Noctiluca*, as recorded from the vacuole, is its unorthodox polarity, for it consists of a transient negative-going hyperpolarization (Chang, 1960; Eckert, 1965 *a*, *b*). The polarity of the effective stimulus is equally peculiar, a positive current passed in the inward (seawater to vacuole) direction. The central purpose of this study was to determine the basis for this unusual behavior. Intimately related was the problem of identifying which membrane, the plasmalemma or the vacuole-limiting membrane, carries the action current. Finally, we considered the problem of impulse propagation in this cell of nearly spherical configuration.

Interest in the action potential which initiates luminescence (flash-triggering potential, FTP) in *Noctiluca* is centered not only on general and comparative bioelectric questions, but is especially concerned with the mechanism by which an action potential exerts control over cellular reactions, in this case the luminescence response. The findings presented here will be applied to the coupling problem in subsequent studies.

MATERIALS AND METHODS

The Organism

Gross morphological features of *N. miliaris* and methods of culture are described elsewhere (Eckert, 1966; Eckert and Reynolds, 1967). For present purposes the organism can be reduced in organization to two major cellular compartments: the cytoplasm, and the flotation vacuole which inflates the cytoplasm to form the periplast (thin layer of cytoplasm between vacuole and pellicle), and which represents the major portion of cell volume. In addition to forming the periplast, the cytoplasm is distributed between a perinuclear aggregation near the sulcus and numerous labile radial strands which course through the flotation vacuole between the perinuclear and perivacuolar cytoplasm (see Eckert and Reynolds, 1967, Fig. 1; and Fig. 1 A in this paper). The vacuolar membrane presumably forms a continuum over the surface of cytoplasm facing the flotation vacuole.

Peripheral Morphology of the Cell

The relationship between flotation vacuole, periplast, and pellicle is shown in a diagram (Fig. 1 C) based on electronmicrographs. As a microelectrode is advanced from seawater to vacuole it passes through: (1) plasmalemma, (2) outer and (3) inner

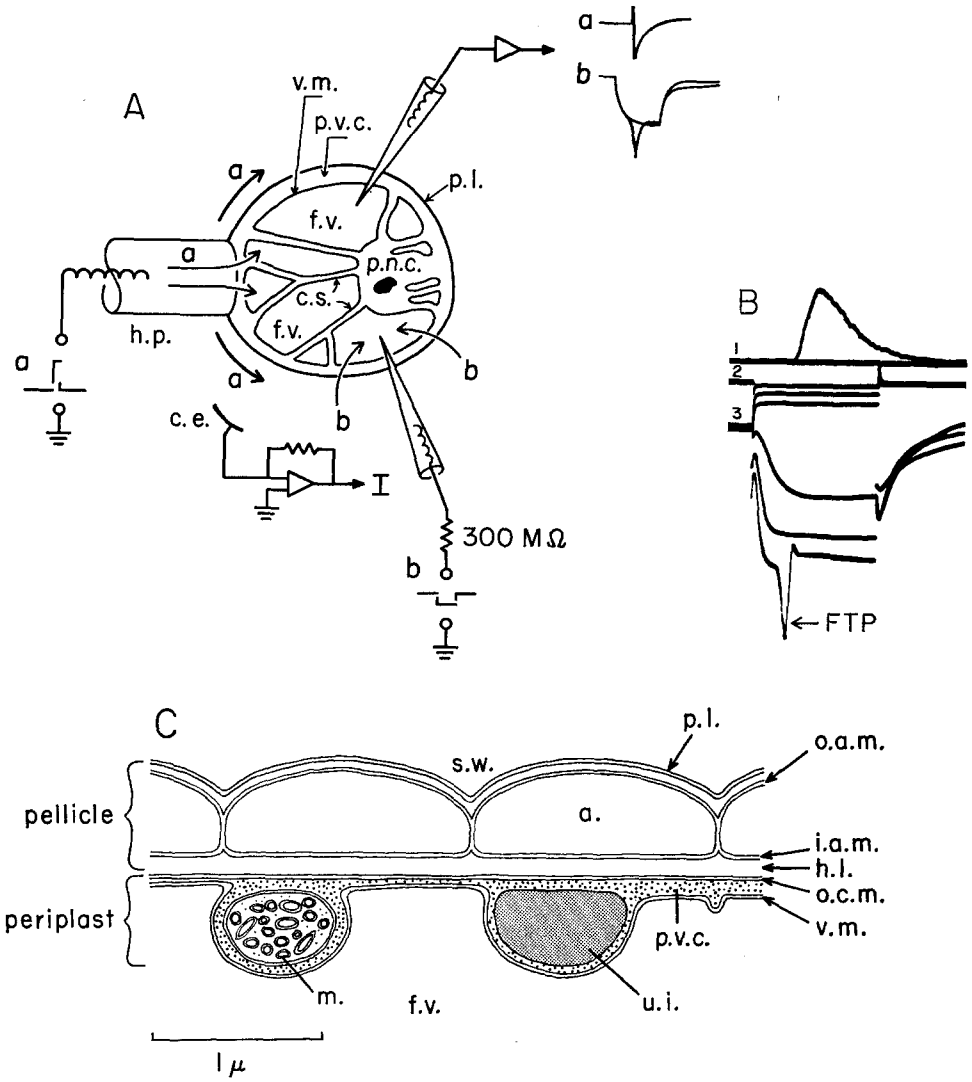

FIGURE 1. A, gross morphology of *Noctiluca* related to the conditions of stimulation. The cell is diagramed out of proportion in order to emphasize the three major divisons of cytoplasm (perinuclear, *p.n.c.*; perivacuolar, *p.v.c.*; and radial strands, *c.s.*); the vacuolar membrane, *v.m.*; the plasmalemma, *p.l.*; and the flotation vacuole, *f.v.* The latter is a single continuous compartment. In stimulus method *a*, a short (0.5–2.0 msec) current pulse was passed locally into the cell from the tip of the holding pipette (*h.p.*). The response to stimulus *a* consisted of a brief, positive displacement of the vacuole, due to the injection of current, followed by the negative-going spike. In method *b*, current was drawn into the vacuole diffusely over the cell surface during a long pulse (15–50 msec) applied with a capillary electrode tip positioned in the vacuole. Sufficient inward current leads, after some charging time, to a negative-going spike somewhat shorter in duration than a spike elicited by method *a*. *c.e.*, calomel electrode; *I*, current display.

B, the emission of light occurs as a flash (trace 1) in response to current pulses (trace

sections of the alveolar membrane, (4) homogeneous dense layer, (5) outer cytoplasmic membrane, (6) cytoplasm, and finally the inner cytoplasmic membrane (i.e., vacuolar membrane, tonoplast). The alveolar structure of the pellicle is a common one in naked protozoans. However, the pellicle of *Noctiluca* differs from that of certain other species (Mornin and Francis, 1967) in lacking an additional unit membrane between the alveolar membrane and the homogeneous dense layer. In all, there appear to be five unit membranes interposed between the vacuole and the exterior. The membrane between two adjacent alveoli appears to be a single common unit membrane continuous with the inner and outer surface membranes of both alveoli. It is uncertain whether this is uniformly the case, or whether there are occasional spaces between adjacent alveoli.

Experimental Methods

Experiments were performed at room temperature (20–24°C) on the stage of an inverted compound microscope (Eckert, 1965 *a*). Hydrostatic pressure of 2–8 millimeters water held the specimen (300–600 μ in diameter) in seawater to the end of a pipette which had an internal tip diameter of approximately 70 μ. Specimens were stimulated either by application of 0.5 msec inward constant current pulses through the holding pipette or by application of current with a cathodal polarizing electrode in the vacuole. The relationship between stimulus mode and response is shown in Fig. 1 A. External electrical recordings were taken from the surface of the cell with glass capillaries (15–25 μ in tip diameter) filled with seawater. Internal electrodes were normally positioned with tips in the large hydrostatic vacuole. This was by far the most convenient and most reproducible method of electrode placement. For certain experiments electrode tips were inserted into the perinuclear cytoplasm. Electrode placement in the periplast proved to be virtually impossible because of the thin (<1 μ) perivacuolar cytoplasm. In both internal and external recordings conventional (negative downward) display polarity was employed. Recording and reference leads were either Ag-AgCl or calomel. Standard electrophysiological equipment and techniques were employed as described elsewhere (Eckert and Sibaoka, 1967; Siboaka and Eckert, 1967).

In addition to the common display of voltage against time, potential changes were

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2) only in conjunction with the all-or-none action potential (trace 3). This recording was made with stimulus method *b*.

C, cross-section through pellicle and periplast of *Noctiluca* reconstructed from electron micrographs. The pellicle consists of membrane-limited alveoli (*a*) overlaid by a continuous unit membrane which we will term the plasmalemma (*p.l.*), and supported below by a continuous homogeneous layer (*h.l.*). The inner (*i.a.m.*) and outer (*o.a.m.*) membranes of the alveoli are continuous and appear to form a single unit membrane between adjoining alveoli. The periplast consists of the perivacuolar cytoplasm (*p.v.c.*) sandwiched between the outer cytoplasmic membrane (*o.c.m.*) and the vacuolar membrane (*v.m.*). The cytoplasm contains familiar organelles such as mitochondria (*m.*), Golgi complexes, etc., as well as densely staining unidentified inclusions (*u.i.*). The pellicle is bathed in seawater (*s.w.*), while the vacuolar membrane delimits the flotation vacuole (*f.v.*).

displayed in the plane of V and dV/dt (Cole, Antosiewicz, and Rabinowitz, 1955; Jenerick, 1963). Both registrations were displayed and photographed simultaneously from a single CRO screen. Vacuolar potential was displayed on the horizontal axis (x) with increasing negativity proceeding toward the left. The rate of change of potential (dV/dt), given in v/sec, was displayed on the vertical axis (y) with a negative-going voltage change plotted downward. A straight segment in the phase plane trajectory represents an exponential time function having a rate constant proportional to the slope of the linear component (Jenerick, 1963). The reciprocal of the slope is therefore proportional to the time constant of the exponential potential change. The techniques used in generating phase plane displays were similar to those described previously (Eckert, 1967).

Impedance measurements were made with an AC bridge in the manner described by Sibaoka and Eckert (1967).

RESULTS

Passive Electrical Properties

Before entering into discussion of the active properties of this cell, it is appropriate to consider its passive electrical behavior. This is especially desirable because stimulating currents were passed and recordings were made across several membranes in series (Fig. 1 C).

When an electrode was inserted, it severely dimpled the surface before popping through the pellicle and periplast into the vacuole. With the electrode inserted, the highly motile cytoplasm of the periplast and radial strands gradually crept up the electrode shank to form a minute vacuole around its tip within several minutes. Subsequent recordings resembled those obtained externally with surface electrodes. In order to delay electrical ejection of the electrode and thereby prolong the usefulness of the preparation, electrode tips were inserted as far as 50 μ into the flotation vacuole.

Cells initially showed a relatively low DC vacuolar potential and membrane resistance, presumably due to the severe dimpling and the deep penetration of the electrodes. While this is normally taken as an indication of damage to the cell in nerve and muscle studies, it was unavoidable here. A gradual slow increase with time in both cellular input resistance and vacuolar potential followed electrode insertion, and is ascribed to recovery from damage attending electrode insertion (Eckert and Sibaoka, 1967). This recovery was not correlated with the extension of streaming cytoplasm around the tip of the electrode as suggested by Hisada (1957). Effective resistances between vacuole and cell exterior range between 1 and 10 megohms, while DC potentials recorded in the vacuole range from several millivolts positive (perhaps a tip potential artifact) to approximately -20 mv.

Action potentials elicited after recovery from damage of electrode insertion are accompanied by drops in both vacuolar DC potential and input resistance of the cell. These parameters then increase in value until a steady level is

attained or another action potential results in another drop in resistance and potential. The FTP elicits a small twitch contraction of the cell; hence, it is not certain whether the reduction in resistance and resting potential is a direct aftereffect of the action potential, or the result of mechanical disturbance of the seal around the inserted electrode. Because of the necessity for repetitive stimulation in this study, most of the FTP's recorded were elicited at relatively low DC vacuolar potentials and cell resistances. In undisturbed free

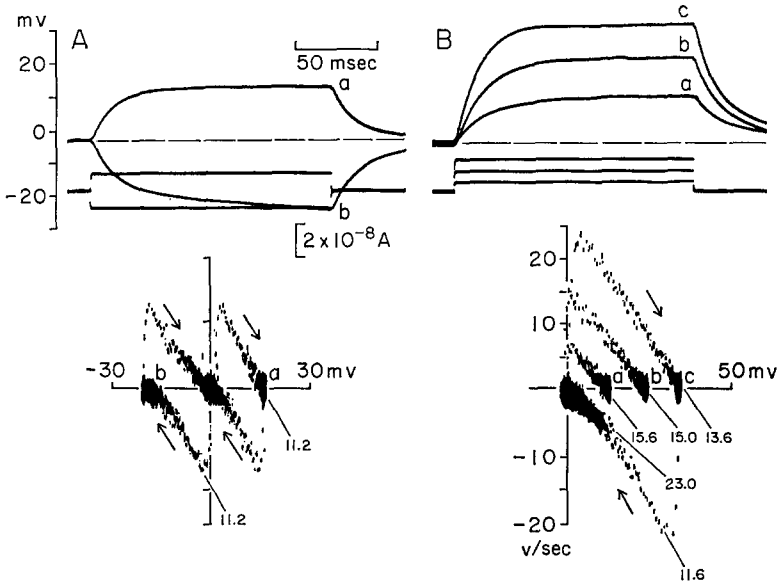

FIGURE 2. Passive changes in vacuolar potential in response to current pulses. Top to bottom: potential, current, and phase plane displays. The numbers at the ends of slope extension lines in the phase plane records are the time constants in milliseconds of the exponentials. A and B from same specimen. Lowercase letters label corresponding points in time for both normal and phase plane plots. Arrows in phase plane records show progression of time.

specimens (which normally exhibit spontaneous tentacle movement) the potential of the vacuole should be relatively high (ca. −20 mv), because studies reported elsewhere (Sibaoka and Eckert, 1967) showed that the tentacle-regulating potential occurs spontaneously in the −20 mv range of vacuolar potential and is completely inhibited at lower vacuolar DC potentials.

It is unlikely that postpenetration recovery is an artifact resulting from KCl leakage from the electrode (Chang, 1960), for Kesseler (1966) has shown vacuolar concentrations of K^+ and Cl^- similar to those of seawater; an appreciable change in the transmembrane $[K^+]$ gradient would require enormous leakage into the volume of the vacuole.

The presence of several biological membranes between the vacuole and the external reference suggested the possibility of overlapping *RC* time constants

of potential change in response to a square step of current applied across the membrane series. To examine this possibility, it was necessary to use a specimen with relatively low resting potential in order to minimize the activity associated with the tentacle-regulating potential (Sibaoka and Eckert, 1967). Responses to constant current pulses were recorded in both normal and phase

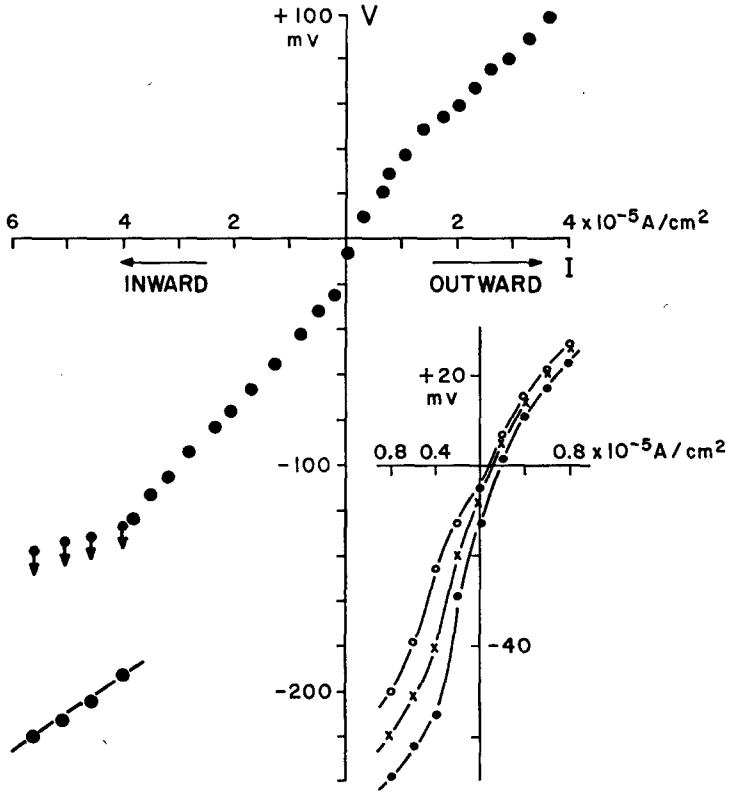

FIGURE 3. Current-voltage curve generated galvanostatically with both current and recording electrodes in the flotation vacuole. Small dots indicate the potential levels from which action potentials arose. Arrows point to the corresponding peak values of the action potentials. The inset shows the current-voltage relationship in the range of small currents in another specimen, 300 sec (open circles), 500 sec (crosses), and 600 sec (solid circles) after inserting electrodes. The ordinate gives the vacuolar potential relative to the potential of the bath.

plane registrations (Fig. 2). The positive-going potential changes showed only one distinct time constant, as seen in the linearity of the corresponding segments of the phase plane trajectories. Time constants of far longer durations would not, admittedly, have been resolved, but shorter series time constants would have been detected as jumps in the potential trace at make and break of the current pulse. In contrast to the apparently single time constant of the rising

phase of the *RC* response, two components were resolved during recovery from positive-going polarizations (Fig. 2 B, sweeps *a–c*) and during the development of the negative-going polarization (Fig. 2 A, sweep *b*). The longer time constant in both cases represents the beginning of activity associated with the tentacle-regulating (TR) potential. Fig. 6 A–F illustrates the slow TR component which occurs frequently in response to negative-going potential changes. The reason for differing time constants of rise in the three responses of Fig. 2 B is unclear; perhaps the resistance of the cell was increasing during recovery from electrode insertion.

Current-voltage curves were obtained by plotting the plateau potential

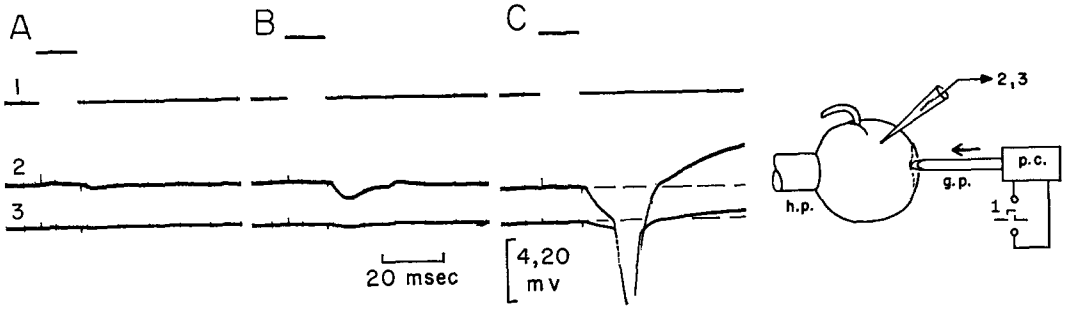

FIGURE 4. Action potential evoked by mechanical stimulation. Diagram shows method of tapping cell with fine glass probe (*g.p.*) driven by piezoelectric crystal (*p.c.*). Trace 1 displays the voltage pulse applied to the crystal. The displacement of the probe against the specimen increased from A to C. The sensitivity of trace 2 is five times that of trace 3, so as to show development of the graded subthreshold response with increasing mechanical stimulation. *h.p.*, holding pipette.

deflections as a function of applied current (Fig. 3). The galvanostatic I-V curve is relatively linear except for a reduction in slope near resting potential. The relative linearity of the current-voltage plot may reflect the ohmic properties of the inactive membranes which are in series with the active membrane. The I-V plot resembles that made by Chang (1960) for a non-luminescent form of *Noctiluca*.

While we cannot calculate the specific resistance of the active membrane by itself, it is possible to determine the summed resistances of the membranes in series (assuming them to be smooth concentric spheres having the same diameter as the specimen). The resting resistance of the specimen plotted in Fig. 3 was about 10 kilohm·cm².[1] If the five membranes between vacuole and bath were to have equal resistances and were of the same surface area, the value would be about 2 kilohm·cm² for each membrane. This may be compared with resistances of 700 ohm·cm² for crab nerves (Katz, 1948), 100

[1] This figure is more typical than the value of 10^5 ohm·cm² given earlier (Eckert and Sibaoka, 1967).

kilohm·cm² for *Nitella* (Curtis and Cole, 1937), and 30 kilohm·cm² for *Chara* (Oda, 1961). Our present measurements of the aggregate capacitance of the series membranes of *Noctiluca*, averaging 1.5 μf/cm², agree with Chang's (1960) figure for the nonluminescent variety of *Noctiluca*.

The flotation vacuole, because of its configuration and electrolyte content (Kesseler, 1966), was suspected of being relatively isopotential. This was tested by inserting two recording electrodes with their tips at widely separated positions within the vacuole to record the action potential elicited by stimulus method *a* (Fig. 1 A). The signals recorded with the two electrodes were synchronous and identical in all parameters even though the action potential was propagated over the periplast. Since the action potential passed the points of electrode insertion at different times, we conclude that the vacuole is isopotential. This is fundamental to the interpretation of certain experiments described below.

Effective Forms of Stimulation

Mechanical energy is the normal stimulus for the luminescence flash in dinoflagellates. A mechanical stimulus which elicits a flash also evokes the action potential (Fig. 4). The flash and the action potential are both all-or-none and share the same mechanical threshold (Eckert, 1965 *b*). Since the action potential is also followed by the flash in the absence of mechanical stimulation (Fig. 1 B), the action potential is seen as an intermediate process coupling the luminescence response to the mechanical stimulus. The problem of mechanoreceptive transduction will not be considered here except to note that the mechanical stimulus elicits a graded subthreshold wave, which in turn appears to give rise to the action potential (Fig. 4). This resembles the behavior of other mechanoreceptive cells (Katz, 1950; Gray and Sato, 1953; Eyzaguirre and Kuffler, 1955).

For routine convenience in these experiments the flash-triggering action potential (FTP) was elicited by elecrical stimulation. The two principal methods are shown in Fig. 1 A. In both the local (*a*) and the general (*b*) methods of electrical stimulation it was found that the flow of positive current from seawater across the periplast into the flotation vacuole, rather than the reverse, is the effective electrical stimulus. Moreover, it cannot be the potential of the vacuole per se which leads to electrogenesis, for local application of inward current (stimulus method *a*) causes a positive displacement of the vacuolar potential, whereas method *b* requires a negative displacement of the vacuole to draw current inward across the periplast (Fig. 1 B).

Fig. 5 shows some response variants to the general application of current across the periplast. Recording A shows the most normal case, in which the cell also exhibits TR (tentacle-regulating) activity. The TR spike (Sibaoka and Eckert, 1967), shown to better advantage in Fig. 6 A–E, is seen on the

smallest negative-going response. The FTP in this cell originated at a vacuolar potential of about −135 mv. This level varied among specimens from about −100 mv to −200 mv. The true potential threshold across the active mem-

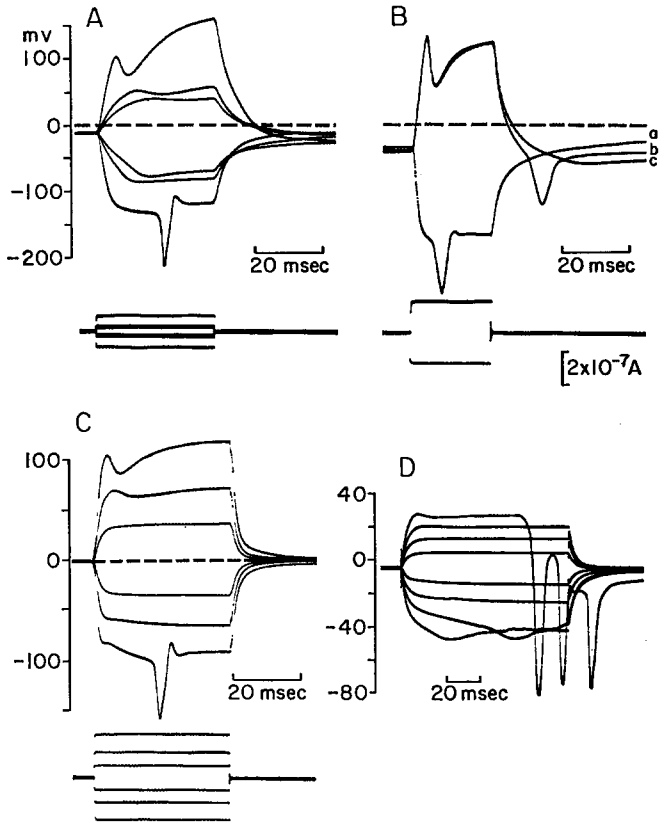

FIGURE 5. The flash-triggering action potential (FTP) arising in different ways in response to inward and outward polarizing current pulses. A, most typical case; cell shows active behavior related to tentacle-regulating potential (TRP) as detailed in Fig. 6. B, similar to A, but with FTP during repolarization in trace *b*. C, similar to A, but with little or no TRP activity present. D, train of three FTP's initiated by outward current. This occurred occasionally, but in a poorly reproducible manner. Current pulses in D were of 1, 2, 3, 4 × 10^{-8} A. Recordings A to D were made on four different specimens.

brane is obscured, of course, by the *IR* drop across the unknown series resistance of the inactive membranes.

A break response is shown in Fig. 5 B, trace *b*. It was slower in time course than the action potential elicited during hyperpolarization of the vacuole (trace *a*). This may have been due to asynchrony resulting from local threshold differences and conduction of the action potential (compare with Fig.

13). The cell represented by Fig. 5 C showed only residual TR activity during the inward current pulse, but its action potential is similar in appearance to that of Fig. 5 A.

Anomalous firing of the FTP during outward current (Fig. 5 D) occurred occasionally and inconsistently when the vacuolar potential was low. We have

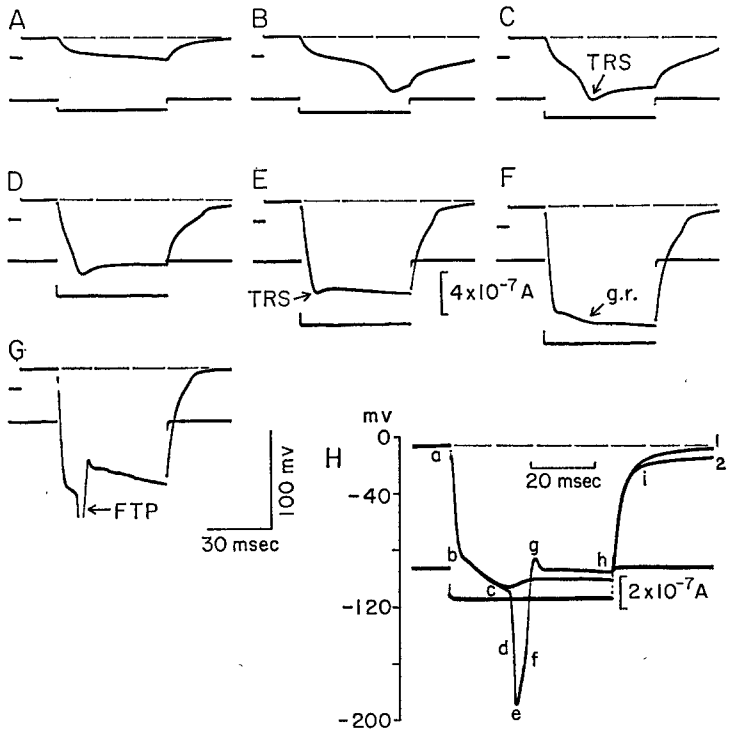

FIGURE 6. Development of the flash-triggering potential (*FTP*) with increasing current strength. A, largely passive response. B–E, tentacle-regulating spike (*TRS*) superimposed on passive response with progressive reduction in latency. F, graded subthreshold response (*g.r.*) which gave rise to flash-triggering action potential in G. The step at the beginning of each potential trace is a 50 mv calibration pulse. H, two superimposed sweeps each showing a response to current pulses of the same intensity. In sweep 1 the subthreshold response subsided, while in sweep 2 it developed into an action potential.

no satisfactory explanation for this. These action potentials always have a slower time course than synchronous FTP's elicited by inward current, and hence probably arise locally and propagate over the cell.

At sufficient outward current levels, the positive-going response showed a prominent negative-going notch beginning several milliseconds after current onset (Fig. 5 A–C). This component has not been explored except to note that it does not lead to a flash, and therefore does not appear to be a variant of the FTP.

The development of the FTP is shown in more detail in Fig. 6. A–G were from a cell showing TR (tentacle-regulating) activity (Eckert and Sibaoka, 1967), while H was from a cell with greatly reduced TR potentials. At sufficient current strength (E–G) the TR spike is confined to the rising phase of the potential response. In F the graded subthreshold wave which develops into the FTP is seen after the TR spike. In recordings G and H the subthreshold response developed into the FTP. The slow, graded wave is seen in response to near-threshold current pulses, and is reminiscent of local responses recorded near threshold in other electrically excitable cells.

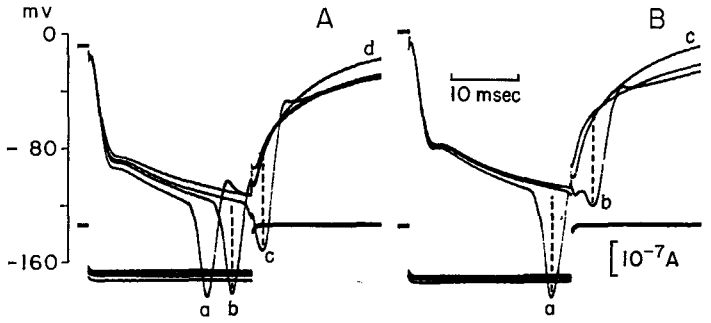

FIGURE 7. The flash-triggering potential (FTP) during and after pulses of applied current. A, two action potentials completed during the current pulse (*a*, *b*) and one initiated just prior to termination of the stimulus current (*c*). B shows an almost completely poststimulus action potential (*b*). Traces A*d* and B*c* are without action potentials. When measured from the curve of passive decay, the poststimulus FTP's are similar in amplitude to those which completed their course during the applied current pulse. Amplitudes of the spikes measured from the base line of passive decay are indicated by the vertical dashed lines. This was done with the assumption that the series resistance of the inactive membranes remained largely unchanged during the action potential.

Fig. 6 H illustrates the wave shape of a characteristic synchronously initiated FTP. The FTP arises from a graded subthreshold response (*c*), reaches a peak (*e*), and descends to an undershoot (*g*) after passing through a shoulder (*f*). The potential drop (*h–i*), which occurred when the stimulus current was terminated, approached a more negative level following a spike (trace 2) than did recovery from a subthreshold hyperpolarization (trace 1). This observation suggests that the conductance pattern immediately following the FTP has an equilibrium potential negative with respect to the pre-stimulus vacuolar potential.

Orthodox Nature of the Flash-Triggering Action Potential

Recordings of the FTP such as those of Figs. 5 and 6 superficially resemble the "hyperpolarizing response" of lobster muscle (Reuben et al., 1961), in which

spikelike voltage transients can occur during constant current hyperpolarization. Frog skin and toad bladder show a related behavior (Finkelstein, 1964; Lindemann and Thorns, 1967). The energy for these voltage transients is supplied by the applied current. The hyperpolarizing spike results as an ohmic potential from a transient increase in membrane resistance at a threshold hyperpolarization. If the applied current is terminated during the hyperpolarizing response the latter is abruptly terminated.

Fig. 7 shows the termination of stimulus current before, during, and after the FTP. The persistence of the action potential without attenuation after termination of the stimulus current indicates that its energy derives from a source other than the applied current. The interpretation of this experiment obtains support from recordings shown in Figs. 5 B, D, 12, and 13, in which the FTP runs its course following the application of current.

In conventional action potentials the potential energy of electrochemical gradients is released in a controlled manner by specific voltage- and time-dependent changes in membrane conductance (Hodgkin, 1958). Synchronous and asynchronous FTP's both show such decreases in impedance during activity (Fig. 8). Maximum conductance (assuming nearly constant capacitance) is recorded during the rising portion of the synchronous FTP. Not surprisingly, the relative timing of the locally recorded conductance change during the asynchronous (propagated) FTP was dependent on the relative locations of the stimulating pipette and the impedance-recording pipette. Thus, with pipettes h and s at opposite sides of the cell, the conduction time was reflected by the appearance of the locally recorded impedance change late in the potential wave form recorded from the vacuole.

The results shown in Figs. 7 and 8 suggest strongly that the FTP is an orthodox action potential arising from specific changes in membrane conductance which control the release of the potential energy of electrochemical gradients.

Assuming that a single active membrane is responsible for the emf of the peak potential, we have represented the passive and active electrical components by the equivalent circuit of Fig. 9 A. A constant current, I, passing from a to b, will cause a steady-state passive potential drop of

$$E_{a-b} = I\left(R_1 + \frac{1}{\frac{1}{R_2} + \frac{1}{R_3} + \frac{1}{R_4}}\right)$$

in which R_1 and R_4 represent the series passive resistances, and R_2 and R_3 the variable resistances which control the active ionic currents.

If, during activity, one or both of the variable conductances, R_2 and R_3, decrease in resistance, the passive potential drop (E_{a-b}) would decrease accord-

ing to the equation. This was the basis of the experiment shown in Fig. 9 B. During activity (peak of action potential), the *IR* drop attributable to imposed current was 0.75 (tan 36°) of that during rest. If one or both of the variable

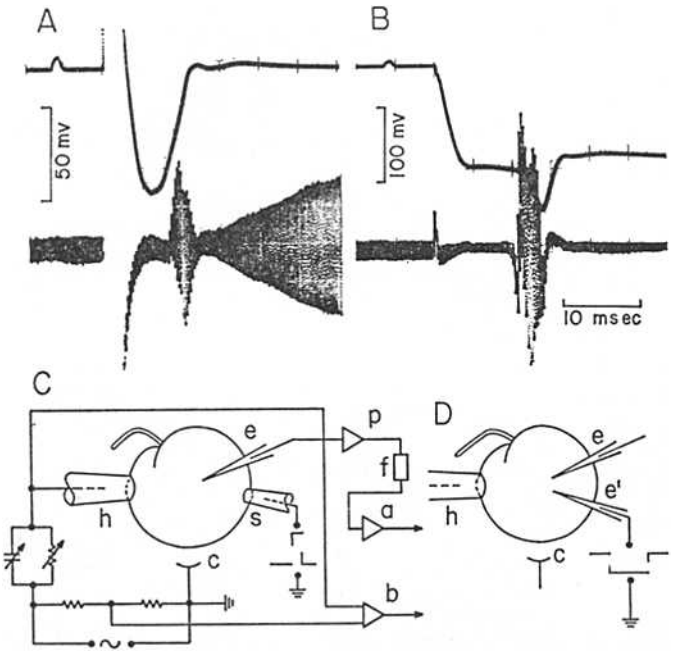

FIGURE 8. Impedance drops associated with asynchronous (A) and synchronous (B) FTP's. *Upper trace*, vacuolar potential. *Lower trace*, bridge balance. Increments in the trace width are proportional to the recorded impedance change. Local surface recorded potential changes at the holding pipette are superimposed on this trace. Schematics of the experimental setup for eliciting asynchronous (C) and synchronous (D) action potentials are shown at the bottom. *h*, holding pipette with tip diameter of about 100 μ. *c*, calomel electrode in seawater. *e*, recording microelectrode. *s*, surface pipette used for applying an inward current locally to initiate asynchronous FTP. *e'*, polarizing microelectrode used for applying an inward current to elicit the synchronous action potential. *p*, neutralized capacity unity-gain electrometer. *f*, high-cut filter. *a*, DC amplifier. *b*, differential amplifier. A 4000 Hz sine wave was applied to the bridge. The slow impedance drop in the latter half of record A is probably due to increased leakage around the holding pipette produced by the small contraction of the specimen which always follows the action potential.

resistances R_2 and R_3 had approached zero at the peak of the action potential, the passive *IR* drop would have been entirely across the series passive resistance R_1, and it would follow that R_1 contained 75% of the total resting resistance. Since the active membrane must, however, have had a finite conductance during activity, the passive series membranes must have contributed somewhat less than three-quarters of the total resting resistance.

Which Membrane Generates the Action Potential?

Recordings made with electrodes in the vacuole or on the outer surface of the cell cannot distinguish activity of the vacuolar membrane from activity of the plasmalemma or another of the five known peripheral membranes. Record-

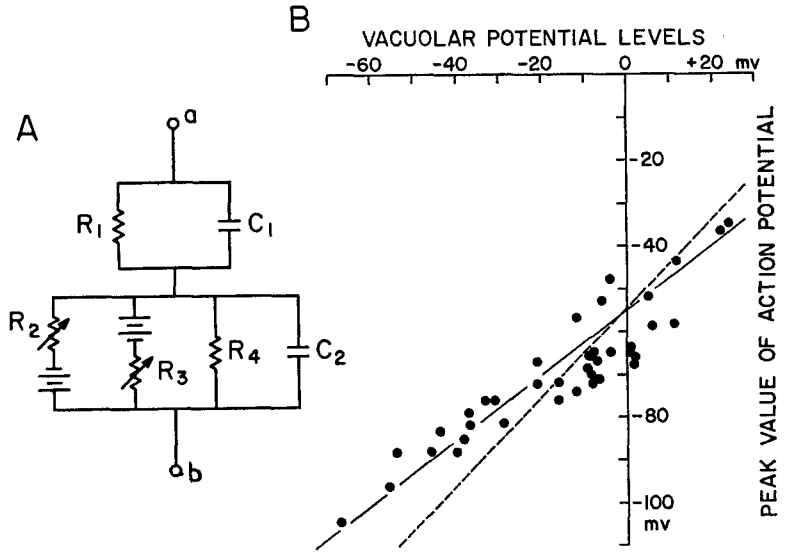

FIGURE 9. A, simplified equivalent circuit of the series active and inactive membranes between the exterior (*a*) and the vacuole (*b*) of *Noctiluca*. R_1 and C_1 represent the lumped resistances and capacitances of the inactive membranes (pellicle and outer cytoplasmic membrane, Fig. 1 C). R_2 and R_3 are ionic conductances in the vacuolar membrane responsible for the rising and falling phases of the action potential; R_4 is the nonvarying resistance and C_2 the capacitance of the vacuolar membrane. B, relationship between the DC level of the vacuole and the peak potential attained by the FTP. The vacuolar potential was altered experimentally with polarizing current delivered through a second electrode inserted in the vacuole. The FTP's were initiated locally by stimulus method *a* (Fig. 1 A). The solid line gives the actual slope (36°) of the relationship, while the dashed line is the 45° slope expected if the potential drop due to applied current were the same during activity as during rest.

ings therefore had to be made from the cytoplasm. It is quite difficult to insert an electrode effectively into the perinuclear cytoplasm with a second electrode in the vacuole, and so only a limited number of unusable recordings were obtained from both the cytoplasm and the vacuole. In every case the perinuclear cytoplasm showed a resting potential after recovery from electrode insertion approaching −50 mv with respect to the external bath.

Fig. 10 shows the biphasic shape of the FTP recorded between the perinuclear cytoplasm and the bath. Both synchronous (A) and asynchronous (A′) responses were elicited in specimen A. The initial slow negative wave

which is most prominent in A′ is interpreted as consisting at least in part of an electrotonic potential originating from local circuit current which entered the perinuclear cytoplasm as the propagated action potential approached the side of the cell containing the perinuclear mass.

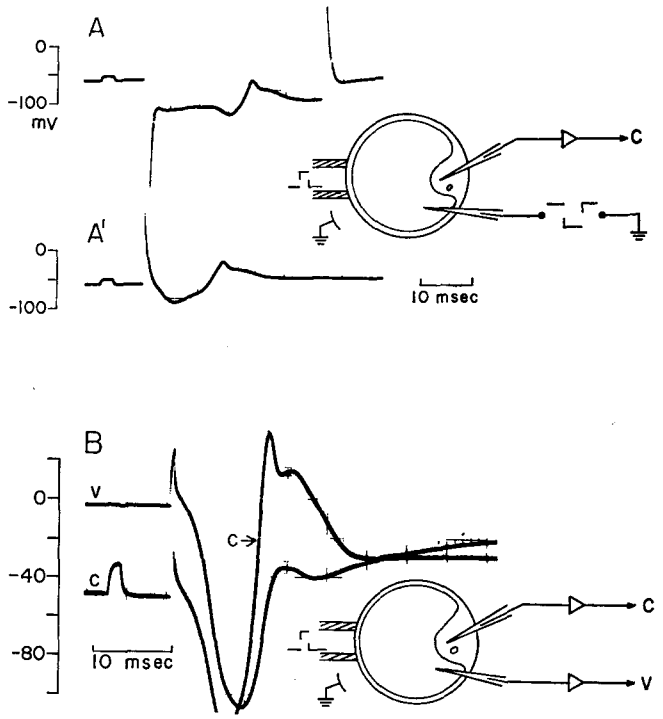

FIGURE 10. Recordings from the perinuclear cytoplasm. A, general (*b*, Fig. 1A) and A′, local (*a*, Fig. 1A) 0.5 msec stimulus current and the resulting action potentials from the cytoplasm of one specimen. B, simultaneous recording of potential changes during the asynchronous action potential from the vacuole (trace *v*) and from the perinuclear cytoplasm (trace *c*). A propagated action potential was evoked with an 0.5 msec pulse applied through the holding pipette. Pulse at beginning of trace *c* was to monitor electrode resistance. Resting potential level in the perinuclear cytoplasm was −48 mv.

In Fig. 10 B recordings were made simultaneously from both cytoplasm and vacuole, while the cell was stimulated locally to evoke the propagated action potential. Synchronous excitation would have been desirable, but the introduction of a third electrode proved technically not feasible, and current passing bridges behave badly in the range of currents required to stimulate. The recording shows a cytoplasmic resting potential of almost −50 mv even though the vacuolar potential is only several millivolts. Stimulation was followed by a diphasic wave in the cytoplasm, which had a negative component somewhat lower in amplitude than the FTP recorded from the vacuole. The positive-going wave had a characteristic notch on the falling phase.

More easily interpreted recordings were obtained by electronically subtracting the potential of the vacuole (recorded with respect to the bath) from the potential of the cytoplasm (also recorded with respect to the bath) as shown in Fig. 11.

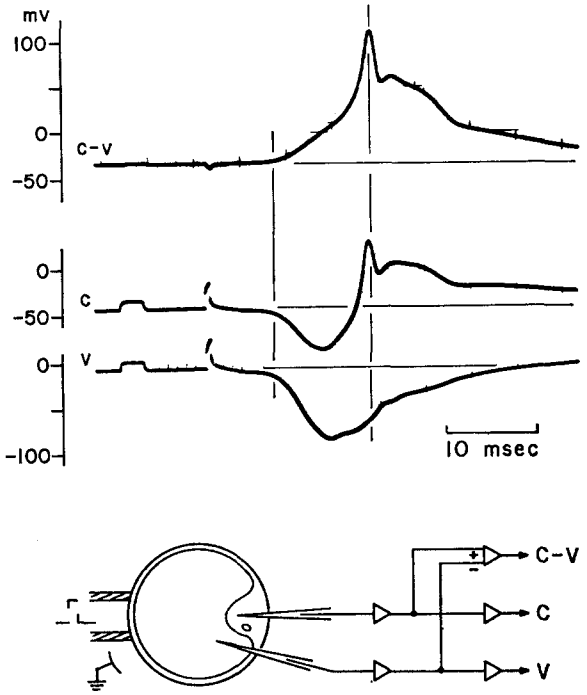

FIGURE 11. Differential potential recording across the vacuolar membrane (trace $c - v$), obtained by electronically subtracting the signal recorded from the vacuole (trace v) from the signal recorded in the perinuclear cytoplasm (trace c). The FTP was propagated from the region of stimulus under the holding pipette. Note that the spike component of the differential record came late in the wave form recorded from the vacuole. This is to be expected because the action potential is propagated from the opposite end of the cell before reaching the perinuclear mass. The slow wave from which the spike emerges in the differential record ($c - v$) is interpreted as due in part to a passive depolarization from electrotonic current with the approach of the propagated action potential, and in part from the asynchrony of recording explained above and in the text.

The differential recording was somewhat distorted in time because the potential recorded from the vacuole arose from all asynchronously active portions of the vacuolar membrane, while the potential recorded from the cytoplasm arose only from activity in areas of membrane within electrotonic reach of the perinuclear cytoplasm. In spite of this, the result demonstrates the reversal of cytoplasmic polarity with respect to the vacuole, and unequi-

vocally points to the vacuolar membrane as the site of activity. In this example the potential of the cytoplasm (referred to the vacuole) reversed from a resting value of −35 to a peak of +110 mv.

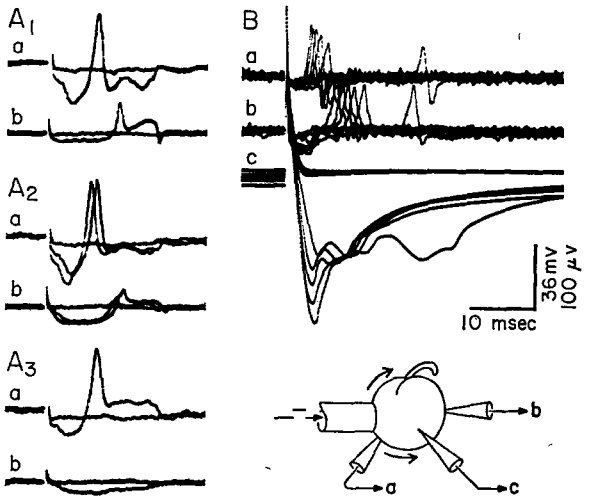

FIGURE 12. Surface recordings of the propagated FTP. Trace *a* in each frame is from the surface electrode nearest the locus of stimulation, while trace *b* was recorded from a point more distant. A_1 to A_3, chronological sequence of electrogenic failure under the *b* electrode. Note the increase from A_1 to A_2 in the negative-going passive current wave in trace *b*. With spike failure under electrode *b*, the response under *a* shows a progressively smaller passive current deflection. B, repetitive stimulation at 10 per sec. There was a progressive increase in latency from stimulus to the electrode *a* response and in conduction time from electrode *a* to *b*. This was accompanied by a slowing of the asynchronous action potential recorded from the vacuole. There was also a double discharge in the final sweep, with the reversed order of the externally recorded spikes suggesting that the second action potential in the final sweep originated at a region other than the action potentials which were directly elicited under the holding pipette.

Propagation of the Action Potential

The propagation of the flash-triggering action potential has already been demonstrated (Eckert, 1965 *b*), but some additional observations are appropriate here.

Large portions of the cell occasionally fail to luminesce in response to electrical stimulation of another portion of the cell (Eckert and Reynolds, 1967). It was postulated that this results from regional failure of invasion by the FTP (Eckert, 1967). Fig. 12 A shows an example of such regional failure. The reduction in the passive current deflection under electrode *a* suggests that the failure in electrogenesis seen under electrode *b* involved a large area of vacuolar membrane. The reason for regional failure of conduction is not known.

Repetitive local stimulation at short intervals (Fig. 12 B) results in a slowing of propagation manifested both by an increase in the latency of externally recorded FTP's (correlated with distance between recording and stimulating electrodes), and a concomitant slowing of the potential wave shape recorded from the vacuole. It appears, therefore, that the slowing of the flash during

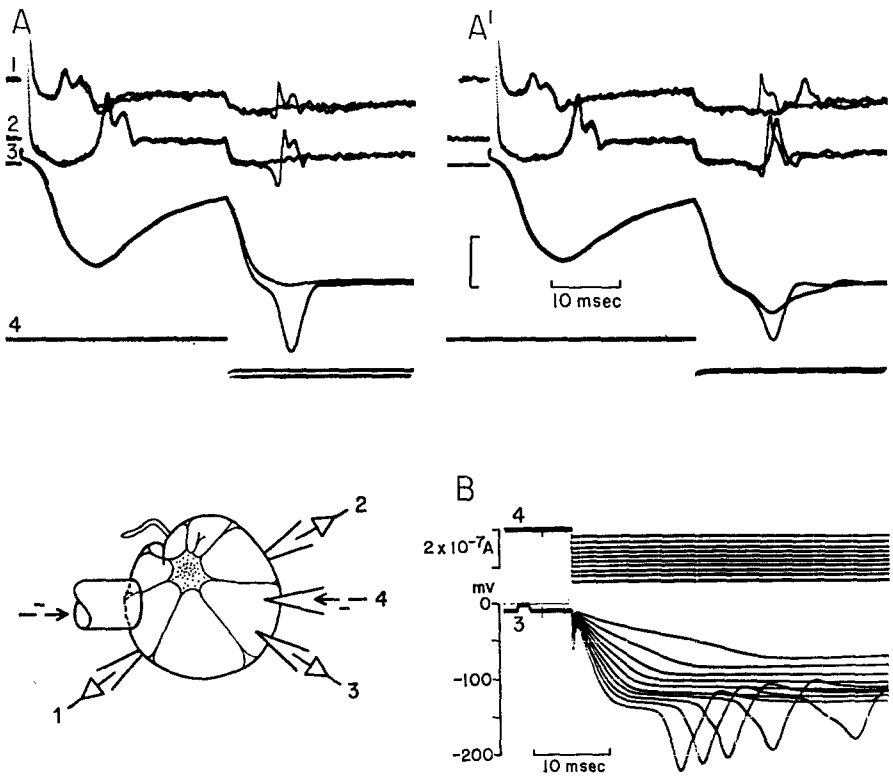

FIGURE 13. Synchrony, asynchrony, and time course of the action potential as recorded from the vacuole. Traces are labeled according to diagram. A, first half of each of two sweeps shows propagated action potential evoked by local current stimulus (method *a*, Fig. 1 A), and second half of each sweep shows synchronous FTP in response to general current stimulation (method *b*, Fig. 1 A). In one sweep the general current stimulus was subthreshold; in the other it resulted in an FTP seen in the external recordings (upper two traces) to be synchronous. A', same as A except that in the sweep with the less intense general current stimulus an asynchronous FTP resulted in response to that stimulus as seen both from the staggered signals in the external recordings and from the concomitant slowing of the wave form of the internally recorded FTP. It is unclear why the areas and amplitudes of the FTP differ with the method of stimulation. B, change in time course of the FTP with increasing stimulus current. Stimulus was general inward current (method *b*, Fig. 1 A). There was a gradual shortening of the latency and an increase in the degree of synchrony of the action potential with increasing stimulus current intensity.

fatiguing stimulation (Eckert, 1967, Fig. 8 A) is at least partially due to slowing of propagation as the action potential sweeps over the cell.

The difference in duration of the synchronous and asynchronous FTP was illustrated earlier (Eckert, 1965 *b*, Fig. 2). The longer duration of the asynchronous FTP recorded from the isopotential vacuole is assumed to be due to the distribution of propagated activity in space and time. Unless it is assumed that the potential threshold for initiation of the FTP is completely uniform

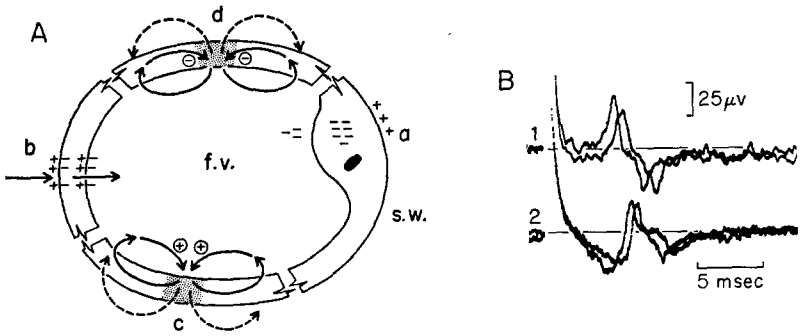

FIGURE 14. A, active current flow across the vacuolar membrane. Segment *a* illustrates the relative potentials of seawater (*s.w.*), cytoplasm, and vacuole (*f.v.*). Segment *b* shows inward stimulating current polarizing the plasmalemma and depolarizing the vacuolar membrane. Sections *c* and *d* show the alternative possibilities of cationic and anionic current flow from the vacuole across the vacuolar membrane into the cytoplasm. The dashed arrows indicate positive leakage current through the seawater volume conductor. B, triphasic passive-active-passive shape of the externally recorded propagated action potential. Trace 1 was recorded near the stimulated region, while trace 2 was recorded at the far end of the cell. The initial negative deflection following the stimulus artifact is not part of the artifact (Eckert, 1965 *b*, Fig. 2).

throughout the cell, the strength of inward current distributed over the cell surface (stimulus method *b*, Fig. 1 A) should be adjustable to initiate the FTP at the region of lowest threshold analogous to the spike-initiating zone in neurons. Direct activation of regions of progressively higher threshold should lead to a progressive decrease in asynchrony in the initiation of the FTP. This prediction was confirmed by such experiments as those shown in Fig. 13; increasing the current strength (within limits) in some specimens increased the degree of synchrony of activation, and concomitantly compressed the time course of the FTP recorded from the isopotential vacuole.

Local circuit current, flowing from the vacuole into the cytoplasm and then completing its circuit back into the vacuole through inactive areas of vacuolar membrane, must divide according to Kirchhoff's laws between the cytoplasm and the seawater bath. Some of it must flow longitudinally in the thin periplast, while the remainder must pass centrifugally across the pellicle, into the

volume conductor of the bath (Fig. 14 A, *c* and *d*). The triphasic potential changes recorded extracellularly with surface electrodes (Fig. 14 B) must result from the leakage current which flows across the inactive surface membranes into the seawater volume conductor.

This interpretation was examined, as shown in Fig. 15, by providing a low-resistance return between seawater and vacuole. The effects of such shunting were to increase the amplitude of the spike component recorded externally while decreasing the passive (negative-going) component. Both effects are consistent with the introduction of a low-resistance shunt for return current flow from the bath into the vacuole.

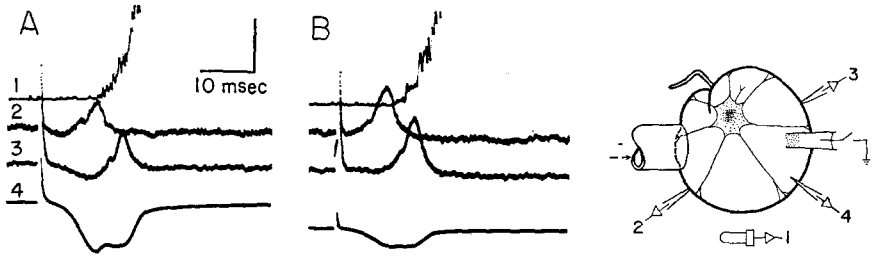

FIGURE 15. Partial short circuit of the flotation vacuole to the bath. A 30 kilohm KCl-agar-filled pipette was inserted with its tip in the vacuole. Traces are labeled according to the diagram. A, recording with switch from vacuole to ground open. B, recording with switch closed. The effective resistance between vacuole and bath with the switch open was about 100 kilohms (primarily leakage around the inserted pipettes), and that with the switch closed was 30 kilohms. The voltage calibration bar indicates 0.2 mv for exnal recordings and 40 mv for the internal recording.

Another approach to testing the conclusion that the vacuolar membrane rather than the plasmalemma is the site of electrogenesis would have been to increase the external resistance by bathing the preparation in isotonic sucrose. Unfortunately, *Noctiluca* deteriorates rapidly when the external sodium concentration is below 40 mM (Chang, 1960), so this experiment was not feasible.

DISCUSSION

Noctiluca is the only cell, to our knowledge, in which an electrogenically active internal membrane system has been demonstrated. In the large excitable cells of the algae *Nitella* and *Chara*, which also have extensive vacuolar compartments, the tonoplast is electrically inexcitable (Findlay, 1959), and the action current arises across the plasmalemma.

Noctiluca is admirably suited for in vivo studies of bioluminescence (Eckert and Reynolds, 1967; Eckert, 1967), but the occurrence of the electrically active membrane within the cell, plus the occurrence of inexcitable membranes closely in series with the active membrane, complicates electrical studies. To add further complexity, the cell generates not one, but two all-or-

none potential wave forms, each controlling a separate and distinct effector response, luminescence and tentacle contractions (Eckert and Sibaoka, 1967). On the basis of our present findings a number of conclusions are nevertheless possible as summarized below.

By ignoring for the moment the fine radial strands of cytoplasm, we can imagine the excitable vacuolar membrane to be spheroid in configuration, and separated by a thin layer of cytoplasm from a slightly larger spheroidal membrane, the outer cytoplasmic membrane. If one then considers a limited area of the surface, the periplast resembles a sheet with the innermost of its two surface membranes excitable, and the outermost inexcitable, with the inner and outer membranes each in contact with separate extracytoplasmic compartments.

The cytoplasm, at least the perinuclear component, is electronegative with respect to both the vacuole and the bath. An imposed current from bath to vacuole will cause a potential drop across each of the series membranes which is the product of the current strength and the resistance of that membrane. As a consequence, the passage of inward current from bath to vacuole (through pellicle, cytoplasm, and vacuolar membrane) increases cytoplasmic positivity with respect to the vacuolar potential. Since the cytoplasmic resting potential (as measured in the perinuclear cytoplasm) is negative with respect to both the vacuole and the bath, the result of inward current is to depolarize the vacuolar membrane. This is true both of the periplast under the holding pipette during the local application of current (Fig. 1 A, method *a*) and of the entire periplast during the general application of current (method *b*). A stimulus current passed in the effective (inward) direction is therefore seen to depolarize the vacuolar membrane while hyperpolarizing the outer cytoplasmic membrane (Fig. 14 A, *b*). Since depolarization rather than hyperpolarization classically results in regenerative electrogenesis, it was not surprising to find the action potential localized across the vacuolar membrane.

When the vacuole is considered as a compartment external to the cytoplasmic compartment, the vacuolar membrane is seen to respond to stimulus current of the orthodox direction; an imposed current flowing from bath to vacuole is an outward (cytoplasm to vacuole) depolarizing current for the vacuolar membrane. In this context the action potential is also seen to be of conventional polarity, showing a cytoplasmic reversal of sign from electronegative to positive with respect to the reference potential of the vacuole (Fig. 11).

With localized application of inward current (Fig. 1, A, method *a*), direct excitation is restricted to the stimulated region, and is followed by conduction of the action potential over the remainder of the cell surface at about 60 μ per msec (Fig. 12; Eckert, 1965 *b*). A spherical excitable membrane of uniform threshold enclosing a nearly isopotential space, and immersed in a

volume conductor, would not be expected to propagate an action potential at so slow a rate. However, the presence of resistive inactive membranes limiting the cytoplasm to a thin spherical sheet undoubtedly lends a high core resistance to the cytoplasm, and the limited space constant which must result permits local circuit current flow and propagation of the action potential. Hence it is proposed that electrotonus and impulse propagation in *Noctiluca* differ from the classical example of the cylindrical axon primarily in that they occur along two spatial dimensions rather than along only one.

The progress of the action potential over the spherical perivacuolar cytoplasm has not been mapped in detail, but it is assumed that propagation is away from the point of stimulus toward the opposite side of the cell in the most direct manner as the meridians connecting the north and south poles of a globe. This view is supported by experiments such as those of Figs. 12 and 15. In addition to the clear correlation between latency and distance, a relationship was routinely noted between the position of the surface recording electrode relative to the point of stimulation and the sequence of passive current components in the externally recorded potential wave form. In the region opposite the stimulus pipette, about where the advancing front of excitation would be expected to converge and cancel, the source-current (negative-going) potential component was most prominent prior to the spike, and much reduced after the spike. The reverse is true at loci near the point of stimulus (Fig. 14 B).

The biphasic shape of the "synchronous" FTP recorded from the perinuclear cytoplasm (Fig. 10) is thought to result from a residual asynchrony of excitation between perinuclear and perivacuolar regions of the vacuolar membrane in spite of the general application of current (method *b* in Fig. 1 A). In the perinuclear region the area ratio of pellicle to vacuolar membrane is lower (owing to the convoluted surface of the perinuclear cytoplasm and the elaboration of radial strands) than for the periplast. Stimulus current density through the vacuolar membrane of the perinuclear cytoplasm should therefore be lower (assuming uniform current flow throughout the pellicle) than it is through the vacuolar membrane of the periplast. Thus, it is reasonable that the vacuolar membrane of the periplast should be excited first, and the action potential should subsequently invade the perinuclear region. The initial negative deflection of the FTP recorded from the perinuclear cytoplasm (Fig. 10) is interpreted in this light as an electrotonic potential resulting from local circuit current.

We have assumed that the vacuolar membrane alone exhibits electrogenesis, but limited excitability of one or all of the other four membranes has not been ruled out. The experiment of Fig. 11 suggests a crude proportionality between the number of inactive membranes and their contribution to the total resting resistance. The passive properties of the vacuolar membrane

may therefore be similar to those of the other membranes. This is supported by the simple *RC* behavior exhibited by the five membranes in series (Fig. 2).

Another assumption made here is that the radial cytoplasmic strands which course through the vacuole contribute only secondarily to the electrical behavior of *Noctiluca*. It is not unlikely that they are invaded during excitation, but it is unlikely that they are essential to the propagation of the action potential; healthy specimens in which the strands are much reduced or absent do not show qualitative differences in electrical behavior, and microsources (Eckert and Reynolds, 1967) continue to flash in response to local (Fig.1 A, method *a*) stimulation applied to distant regions of the cell.

The experiments of Figs. 5, 7, and 8 provide no details regarding the electrochemical nature of the action potential generated across the vacuolar membrane, but they do provide strong evidence that it is self-sustaining, independent of applied current, and accompanied by a conductance increase. These are characteristics to be expected of a common Hodgkin-Huxley-Katz action potential.

The following observations give further support to the orthodox nature of the FTP: It can be initiated in an all-or-none manner by current pulses far shorter than its own duration (Eckert, 1965 *a*), it can be initiated by nonelectrical stimuli (Fig. 4), and it is nondecrementally self-propagating (Eckert, 1965 *b*; and Figs. 12, 13, and 15). On the other hand, there is no evidence which suggests that this action potential is based on principles other than those described by the inonic hypothesis (Hodgkin, 1958). Those elecrical features which initially appear unusual are merely consequences of atypical morphology.

Further effort is now required to determine which ions carry the current of the FTP, and to elucidate the relationship of the action current to the initiation of the luminescence reaction.

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REFERENCES

CHANG, J. 1960. Electrophysiological studies of a nonluminescent form of the dinoflagellate *Noctiluca miliaris*. *J. Cellular Comp. Physiol.* **56**:33.

COLE, K. S., H. A. ANTOSIEWICZ, and P. RABINOWITZ. 1955. Automatic computation of nerve excitation. *J. Soc. Ind. Appl. Math.* **3**:153.

CURTIS, H. J., and K. S. COLE. 1937. Transverse electric impedance of *Nitella*. *J. Gen. Physiol.* **21**:189.

ECKERT, R. 1965 *a*. Bioelectric control of bioluminescence in the dinoflagellate *Noctiluca*. I. Specific nature of triggering events. *Science*. **147**:1140.

ECKERT, R. 1965 *b*. Bioelectric control of bioluminescence in the dinoflagellate *Noctiluca*. II. Asynchronous flash initiation by a propagated triggering potential. *Science*. **147**:1142.

ECKERT, R. 1966. Excitation and luminescence in *Noctiluca miliaris*. *In* Bioluminescence in Progress. F. H. Johnson and Y. Haneda, editors. Princeton University Press, Princeton, N. J. 269.

ECKERT, R. 1967. The wave form of luminescence emitted by *Noctiluca*. *J. Gen. Physiol.* **50**:2211.

ECKERT, R., and G. T. REYNOLDS. 1967. The subcellular origin of bioluminescence in *Noctiluca miliaris*. *J. Gen. Physiol.* **50**:1429.

ECKERT, R., and T. SIBAOKA. 1967. Bioelectric regulation of tentacle movement in a dinoflagellate. *J. Exptl. Biol.* **47**:433.

EYZAGUIRRE, C., and S. W. KUFFLER. 1955. Processes of excitation in the dendrites and in the soma of single isolated sensory nerve cells of the lobster and crayfish. *J. Gen. Physiol.* **39**:87.

FINDLAY, G. P. 1959. Studies of action potentials in the vacuole and cytoplasm of *Nitella*. *Australian J. Biol. Sci.* **12**:412.

FINKELSTEIN, A. 1964. Electrical excitability of isolated frog skin and toad bladder. *J. Gen. Physiol.* **47**:545.

GRAY, J. A. B., and M. SATO. 1953. Properties of the receptor potential in pacinian corpuscles. *J. Physiol. (London).* **122**:610.

HARVEY, E. N. 1952. Bioluminescence. Academic Press, Inc., New York.

HISADA, M. 1957. Membrane resting and action potentials from a protozoan, *Noctiluca scintillans*. *J. Cellular Comp. Physiol.* **50**:57.

HODGKIN, A. L. 1958. Ionic movements and electrical activity in giant nerve fibres. *Proc. Roy. Soc. (London), Ser. B.* **148**:1.

JENERICK, H. 1963. Phase plane trajectories of the muscle spike potential. *Biophys. J.* **3**:363.

KATZ, B. 1948. The electrical properties of the muscle fibre membrane. *Proc. Roy. Soc. (London), Ser. B.* **135**:506.

KATZ, B. 1950. Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J. Physiol. (London).* **111**:261.

KESSELER, H. 1966. Beitrag zur Kenntnis der chemischen und physikalischen Eigenschaften des Zellsaftes von *Noctiluca miliaris*. *Veröffentl. Inst. Meeresforsch. Bremerhaven.* **2**:357.

LINDEMANN, B., and U. THORNS. 1967. Fast potential spike of frog skin generated at the outer surface of the epithelium. *Science*. **158**:1473.

MORNIN, L., and D. FRANCIS. 1967. The fine structure of *Nematodinium armatum*, a naked dinoflagellate. *J. Microscopie*. **6**:759.

ODA, K. 1961. The electrical constants in *Chara braunii*. *Sci. Rept. Tohoku Univ., Fourth Ser.* **27**:187.

REUBEN, J. P., R. WERMAN, and H. GRUNDFEST. 1961. The ionic mechanisms of hyperpolarizing responses in lobster muscle fibers. *J. Gen. Physiol.* **45**:243.

SIBAOKA, T., and R. ECKERT. 1967. An electrophysiological study of tentacle regulating potentials in *Noctiluca*. *J. Exptl. Biol.* **47**:447.