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"Metabolic" Action Potentials in *Acetabularia*

Dietrich Gradmann

Institut für Biologie I der Universität Tübingen,
Lehrbereich für Allgemeine Botanik und Pflanzenphysiologie, Abteilung Biophysik,
D-7400, Tübingen, Auf der Morgenstelle 1, Germany

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Summary. The transient depolarizations in *Acetabularia* which fulfill the essential criteria of an action potential (all-or-none characteristics, triggering by depolarization, propagation, etc.) are investigated. These action potentials are analyzed by conductance measurements and voltage clamp experiments on the basis of the analog circuit of the membrane (Gradmann, D. 1975. *J. Membrane Biol.* **25**:183). It is concluded that these action potentials do not arise by permeability changes of the passive diffusion channels, but by the active pathway of the electrogenic pump, which consists of a voltage source E_P of about -200 mV in series with two nonlinear conducting elements $P1$ and $P2$, the latter and E_P being shunted by a large quasi capacity C_P of some mF cm^{-2}. The nonlinear current-voltage relationship of the carrier system ($P1$) is not changed during the action potential but has an effect on its time course. However, the elements $P2$ and C_P, which probably reflect metabolic entities, are suggested to control the action potentials.

Action potentials seem to be a universal phenomenon in biomembranes. Besides the classical preparations of nerves and muscles, plants such as *Mimosa* and *Dionaea* (for review *see* [14]) or *Nitella* (reviewed in [10]) and many others, even fungi (*Neurospora* [19]), show action potentials. However, the mechanisms in the particular systems seem to differ, not only in the time base (animal cells in the range of msec, most plants in sec and *Neurospora* as well as *Acetabularia* in min), but also in the ions involved. In contrast to the action potentials in animal cells which normally operate on a Na^+/K^+ basis, plant cells – at least *Nitella* [10] –, usually deficient of much sodium in the external medium, seem to substitute Na^+ by Cl^-. However, *Acetabularia*, having enough sodium outside, has also been reported to exhibit an increased Cl^- efflux during the depolarization of its action potential [6]. In addition, for *Acetabularia*, a large conductance increase during the depolarization of the action potential has been reported [16]. Thus, the phenomenon which has been described repeatedly [2, 6, 16, 18] seemed to fit the conventional mecha-

nism of transient permeability enhancements in particular diffusion pathways.

However, in contrast to the classical preparations, the electrical properties of the *Acetabularia* membrane are, as in *Neurospora*, essentially controlled by an electrogenic pump. The role of this pump within the entire network of the electrical elements in the membrane has been elaborated recently [3]. It could be shown that the measured high Cl^- fluxes [3, 6, 15] have only little significance for the electrical parameters of the plasmalemma. Furthermore, the particular circuiting of nonlinear elements and the existence of a capacity of some mF cm^{-2} in addition to the normal membrane capacity (some μF cm^{-2}) demand a careful interpretation of the electrical measurements, especially of the conductance data. This analysis has been tried in this study with the conclusion that the electrogenic pump is the actual source of the action potentials in *Acetabularia*. Corresponding experiments about the role of ATP, temperature and light in the described phenomenon support the idea of a "metabolic" action potential in *Acetabularia*.

In addition, some attention is given to the (semantic) question, whether the phenomenon observed in *Acetabularia* really fulfills the criteria of an action potential. Originally the term "action potential" was derived from electrical events (in nerves) which cause a biological action (contraction of a muscle). The properties of these action potentials have been investigated in detail. Now, if some of these properties of this event are found in different systems, the corresponding phenomenon is usually also called "action potential", even if any action seems to be missing. In literature there is, however, no agreement about the set of properties which classifies an event as an action potential. The following list of criteria should, however, provide a reasonable definition:

1. Transient change of transmembrane potential difference.
2. All-or-none characteristics.
3. Endogenous energy supply.
4. Propagation.

The phenomenon described here will be shown not only to fulfill these criteria, but also to exhibit many additional properties which are typical for classical action potentials. The main purpose of this paper, however, is to demonstrate the basic mechanism of a new, metabolic type of action potential which obviously occurs in *Acetabularia*.

Materials and Methods

Material

Cells of the giant green marine algae *Acetabularia crenulata* and *Acetabularia mediterranea* have been used. The results of cells of these two different species did not differ significantly. The cells have been cultured in Erdschreiber solution according to [1, 7]. Young cells, without a cap and thus with approximately cylindrical shape, have been used for the experiments. The diameter of these cells varied from 0.2–0.7 mm, the length from 2–6 cm.

General Conditions

The outer medium for the experiments was usually artificial sea-water containing the following compounds in mM: Na^+, 461; K^+, 10; Mg^{++}, 53; Ca^{++}, 10; Cl^-, 529; SO_4^{--}, 28; HCO_3^-, 2; usually buffered with 10 mM Tris/HCl at pH 8.0. The normal illumination was 0.1 mW cm^{-2} diffuse white light, usually referred to darkness *D* compared to bright illumination *L* of 100 mW cm^{-2} white spot light from a quartz iodide lamp. The normal ambient temperature was 23 ± 2 °C. Temperature changes have been brought about by a thermostated perfusion system around the measuring chamber.

Electrical Experiments

A detailed description of the setup and analysis used is given by previous papers [2, 3, 4]. Briefly, standard microelectrode techniques have been applied for recording intracellular potentials and for current injection. Conductance measurements have been performed and analyzed according to linear cable theory [9]. The voltage clamp circuit has been described explicitly [2]. Differential amplifiers with high impedance input ($>10^{11}\,\Omega$) have been used. The signals were recorded by a storage oscilloscope and a pen chart recorder.

Cl^- Fluxes

The exact method for simultaneous recording of voltage and $^{36}Cl^-$ efflux is given in a previous study [6]. Cells preincubated with $^{36}Cl^-$ were mounted in a lucite vessel, which allowed puncture of the top of the cells by microelectrodes under continuous rinsing with nonradioactive medium. The radioactivity released from the cell to the medium could be sampled in 30 sec intervals and determined in a liquid scintillation counter.

ATP Measurements

For the experiments reported here, cells of similar size have been transferred each within less than 1 sec from the particular experimental condition for 5 min extraction in 1 ml boiling water plus one drop 100 mM NaOH solution. 2 ml buffer (100 mM H_3PO_4 plus 20 mM $MgCl_2$) has been added to the samples. After addition of 50 µl firefly extract (10 g l^{-1}, Sigma) the bioluminescence was determined in a liquid scintillation counter. The resulting relative ATP concentrations (in cpm) for different conditions have been related to the absolute ATP level (*D*), the measurement of which is described elsewhere [3].

Results

General Phenomenology

Triggering of action potentials by light-off. It has been reported that photosynthetically active light raises the normal resting potential of *Acetabularia* [17]. Upon light-off, there is an undershoot, depending on the intensity and duration of the preceeding light, before the membrane voltage, V_m, reaches its stable dark level. If the light pulse exceeds a threshold, upon light-off the undershoot becomes very large and does not increase any more with stronger stimuli.

This situation is illustrated by the results of an experiment given by Fig. 1. Light pulses of 1 min duration and increasing intensity have been applied to a cell of *A. crenulata.* Upon 0.1 mW cm^{-2} there is a slow and small increase of the resting potential with a slight undershoot upon light-off. Qualitatively the same result but more pronounced is obtained by a stronger light pulse of 1 mW cm^{-2}. If the intensity of the light is increased again by the factor 10, the on-effect is again faster and larger; however, the off-effect is qualitatively completely different. First, the depolarization slows down normally during the first seconds, but then it speeds up again to a rapid and large depolarization below the equilibrium potential of potassium E_K of about -90 mV [2, 3, 16]. From there on the depolarization is retarded again until it reaches the peak before repolarization. The repolarization starts rather fast but slows down to a shoulder near E_K. In many cases this shoulder can even form a plateau. As soon

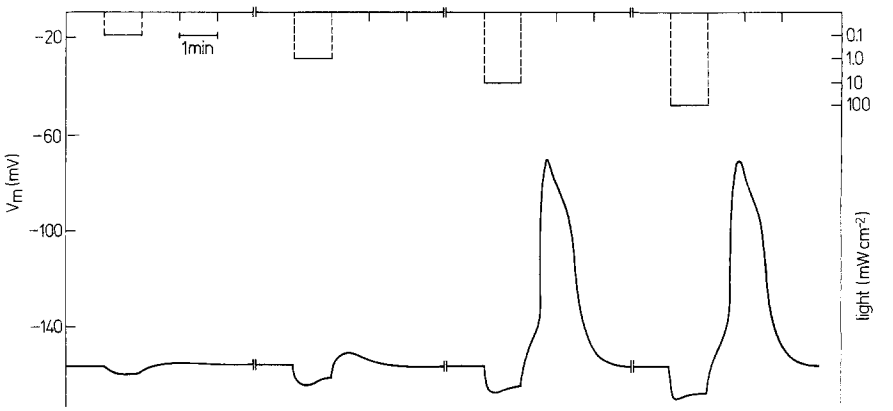

Fig. 1. Example of the response of the membrane voltage V_m of *A. crenulata* upon 1 min pulses of white light of different intensity. The light-off response becomes an action potential after a preceeding pulse of 10 mW cm^{-2} or more. 23 °C

as the voltage becomes more negative than E_K, the repolarization becomes fast again and the voltage then slowly approaches the new resting level. This characteristic time course is not changed, if the light stimulus preceeding the triggering light-off signal is increased again by an order of magnitude. This typical all-or-none behavior fulfills the first two essential criteria of an action potential according to the above definition.

Triggering action potentials by depolarization. Action potentials of the classical preparations can be triggered by depolarization. This is also possible in *Acetabularia.* Fig. 2 shows an example where voltage clamp depolarizations to -110 mV have been applied 4 times, each lasting 10 sec. The first depolarization is sufficient to trigger an action potential; the second one is not because of the refractory period. The last two stimuli suffice again to trigger an action potential, because the time after the preceding action potential is larger now with respect to the refractory period. It could also be shown that two subthreshold depolarization

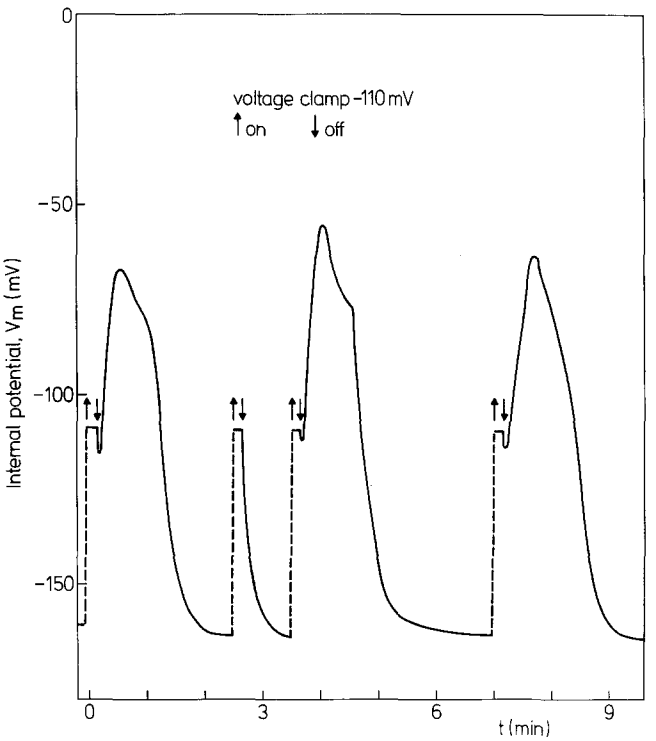

Fig. 2. Triggering action potentials by 10 sec point-clamp depolarization to -110 mV. Second stimulus (2.5 min after successful triggering) subthreshold due to refractory period. 4th stimulus (4 min after successful triggering) sufficient to trigger an action potential. Note small repolarizations upon clamp-off, before V_m merges the depolarizing course of an action potential. *A. crenulata*, 23 °C, 1.5 mW cm^{-2} white light

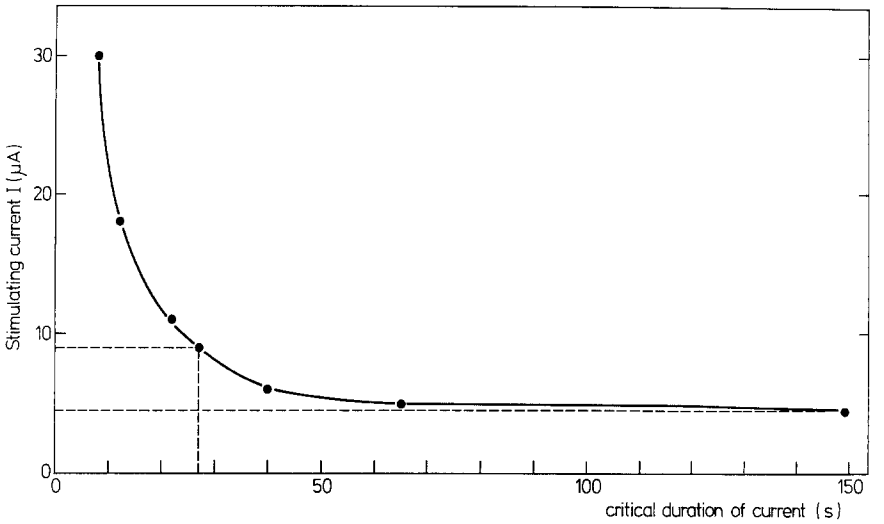

Fig. 3. Hyperbolic strength-duration curve of the critical triggering depolarization by an injected current pulse. Dashed lines mark chronaxie and rheobase. *A. crenulata*, 23 °C, 1.5 mW cm^{-2} white light

stimuli, separated by a short intermission, can add up to a triggering stimulus [2]. These properties, the refractory period and the summation of stimuli, are typical for physiological processes.

The results of a series of experiments, common in conventional electrophysiology, are given by Fig. 3. The intensity of rectangular depolarizing current pulses is plotted against the critical duration of these pulses to elicit an action potential. This strength-duration-curve is roughly hyperbolic. The smaller the current, the larger the necessary duration, with a minimum current of 4.5 μA for "infinite" pulse length (rheobase). The chronaxie of 27 sec could then be determined as well (cf. Fig. 3).

Spontaneous action potentials. As mentioned previously [2], there are large variations of the stimulus threshold between individual cells. Some cells could never be forced to produce action potentials. In many cases, however, single or repetitive action potentials occurred spontaneously and could produce "bursts" as illustrated by the example given in Fig. 4. This phenomenon clearly shows that it is not the energy applied by the stimulation process which drives the action potential. It must be fueled by endogenous energy sources. Therefore, the third criterion of the above definition of an action potential is met as well.

Propagation. It has been reported that action potentials arise preferentially on the apical end of the *Acetabularia* cell [13]. In fact, if two voltage recording electrodes are impaled some distance apart in the cell,

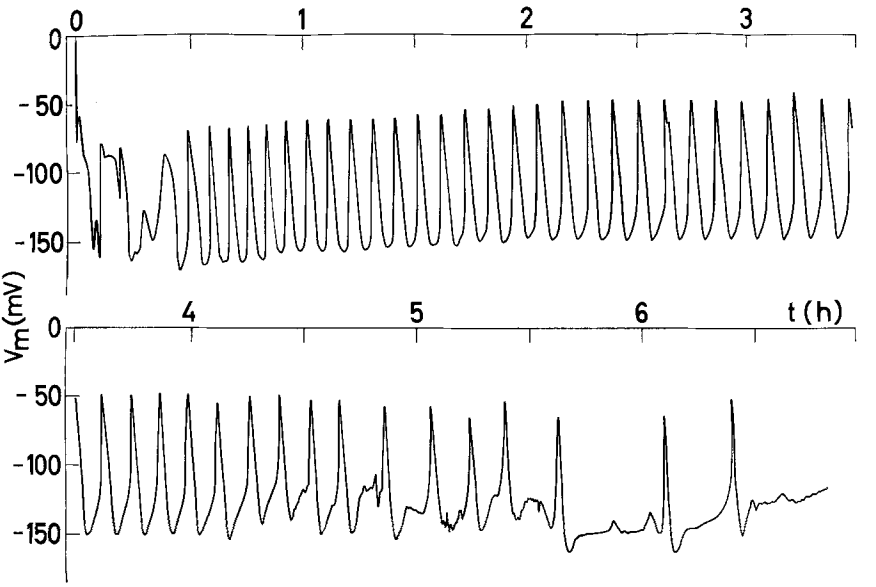

Fig. 4. Series of spontaneous action potentials. Time after puncture. *A. crenulata*, 1.5 mW cm^{-2} white light, 23 °C

Table 1. Velocity of propagation of action potentials (mm sec^{-1}) in *A. mediterranea*[a]

	Propagation (mm sec^{-1}) mean	±SEM
I	0.80	0.46
II	1.36	0.12
III	0.60	0.13

[a] I: propagation from apex to basis, action potential triggered by light-off after 1 min illumination of the entire cell with 100 mW cm^{-2} white light; II: propagation from apex to basis, action potential triggered by pulse of depolarizing current (4 μA) in apex; III: propagation from basis to apex, action potential triggered by pulse of depolarizing current (4 μA) in basis.

the peak of a light-triggered action potential occurs earlier at the apical point than at the basal one (cf. Table 1). This result does not necessarily imply propagation from the apex to the base. The time difference between the two peaks is small compared with the duration of the entire action potential; and this difference could be due to the lower excitability or slower time course of the (nonpropagated) action potential in the basal region of the cell.

Similarly, if the action potential is electrically triggered on the apical end, the peak occurs some seconds later at the base (Table 1), but the cell

is not long enough compared to the electrical length constant of the cable to make sure that the action potential is really propagated, rather than the electrotonic depolarization simply triggering a (later or slower) nonpropagated action potential on the base.

Fig. 5 shows an example where the cell has been depolarized by a 4 μA current of 13 sec duration in the basal part. Here, the peak depolarization after the triggering current pulse occurs 17 sec later at the apical electrode, 8 mm apart from the basal electrode. On the one hand, this could again be due to the smaller depolarizing current density at the apical end (cf. Fig. 3). On the other hand, the higher excitability of the apical part would favor an increased velocity from the base to the apical part over the opposite way. However, the results compiled in Table 1 show a smaller velocity for propagation from the base to the apex. These results suggest that there is an intrinsic gradient which facilitates an earlier

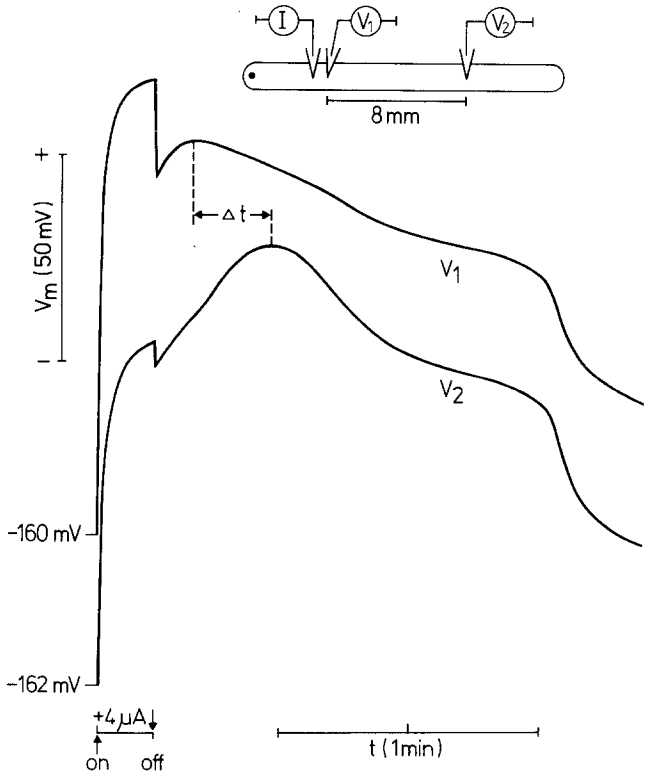

Fig. 5. Propagation of an action potential. Depolarizing current pulse injected in the basal part (with nucleus) of a cell (*see* inset) causes an action potential in the basal part. The peak depolarization in the apical part is larger and appears 18 sec (Δt) later. Numbers on traces V_1 and V_2 mark recorded resting potential in mV. *A. mediterranea*, 23 °C, 0.1 mW cm^{-2} white light

peak on the apex. This gradient enhances propagation of electrically triggered action potentials from the apex to the base of the cell and slows down propagation in the opposite direction. The example in Fig. 5 shows in addition that there is a genuine propagation rather than an electrotonic spreading of the action potential, because the peak depolarization is larger at the apical end though the stimulus has been applied to the base. In conclusion, active propagation of the action potential takes place in *Acetabularia* as well.

Ionic Relations

Since *Acetabularia* cells exposed to "pure" choline chloride solution exhibit normal action potentials, it has been concluded that no cation of the normal external medium is necessarily involved in the action potential [2]. Neither do protons seem to play an essential role for the action potential, since normal action potentials can be observed under various external pH (4–9).

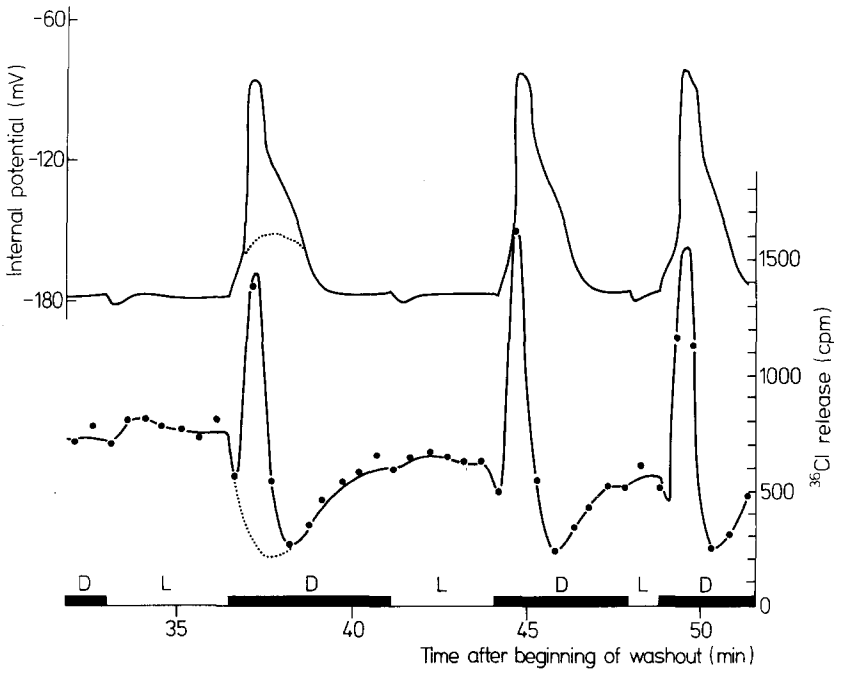

Fig. 6. Internal potential, V_m (———) and $^{36}Cl^-$ release (●——●). Example from an *A. mediterranea* cell in a light/dark regime. Coincidence of action potentials and enhanced Cl^- efflux. Replotted from [6]

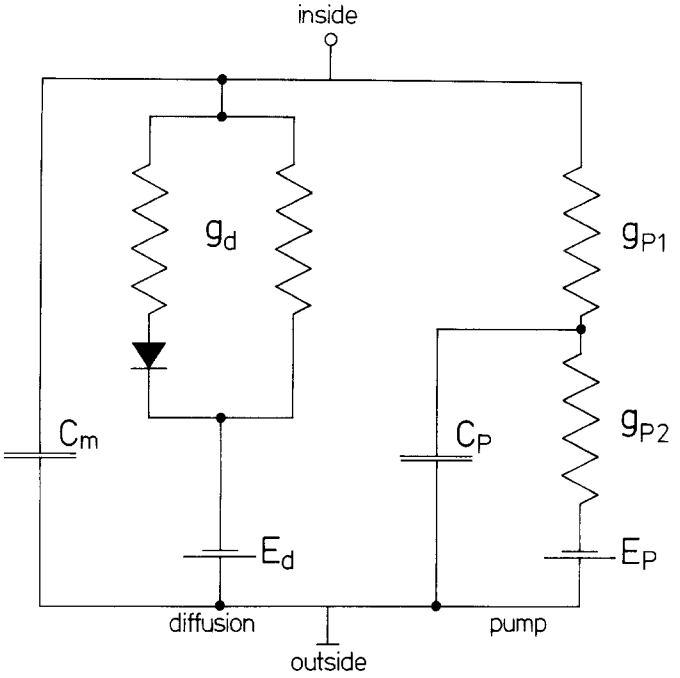

Fig. 7. Simplified analog circuit of the *Acetabularia* membrane in the steady state, according to [3]. C_m: membrane capacity *ca.* 5 μF cm^{-2}; g_d: conductance of diffusion pathways, approaching *ca.* 30 μS cm^{-2} for voltages more negative than E_K (≈ -90 mV) and *ca.* 1 mS cm^{-2} for voltages more positive; E_d: electromotive force of the diffusion pathways. *ca.* -80 mV, close to E_K; g_{P1}: conductance of the carrier system of the electrogenic pump, maximum 6 (4) mS cm^{-2} in $L(D)$ at about -200 mV, approximately symmetrically decreasing to saturation current of 60 (30) μA cm^{-2} in $L(D)$ at 100 mV displacement of V_m; g_{P2}: conductance equivalent of energy metabolism, linear 600 (300) μS cm^{-2} in $L(D)$ from about -300 to -150 mV membrane voltage, then decreasing to negative values down to -500 μS cm^{-2} near -100 mV membrane voltage; C_P: quasi capacitance, *ca.* 3 mF cm^{-2}, probably reflecting ATP pool; E_P: electromotive force of the electrogenic pump, *ca.* -200 mV

Simultaneous recordings of the membrane voltage and the ion fluxes during action potentials, however, have shown that there is a large Cl^- efflux associated with an action potential (illustration in Fig. 6, replotted from [6]) as well as a large K^+ efflux [12]. From these results it might be presumed that the depolarizing phase of the action potential is due to an increased Cl^- permeability causing a passive net Cl^- efflux ($E_{Cl} \approx 0$ mV) and the repolarization reflects an analogous K^+ efflux. However, an interpretation different from this *prima facie* explanation will be presented in the discussion.

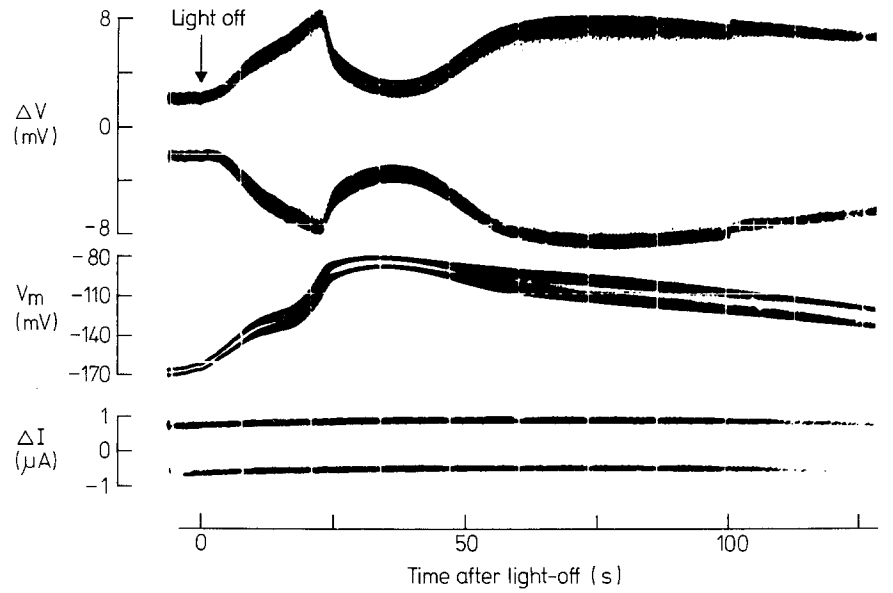

Fig. 8. Example of original recording for conductance (g_m^0) measurement during the first 120 sec of an action potential triggered by light-off. Lower double trace: injected current pulses, ΔI (50 msec $\pm$ 0.8 μA) merged to a continuous double-trace. Middle double-trace: time course of the membrane voltage V_m with voltage response ΔV upon ΔI superimposed. Upper double-trace: time course of ΔV isolated from V_m by a high pass filter and magnified. *A. mediterranea*, 23 °C

Electrical Parameters

In order to obtain relevant information about the electrical events during the action potential, it is necessary to know the electrical properties of the normal membrane in the steady state. According to [3], a simplified analog circuit of the membrane in the steady state is given by Fig. 7. One outstanding feature of this system is the existence of two clearly distinct time constants. The small one, τ^0, in the range of msec is due to the membrane capacity C_m (5 μF cm^{-2}) and the high conductance element $P1$ (some mS cm^{-2}) in the pathway of the electrogenic pump, shunted by the conductance g_d of the diffusion pathways, which is small (28 μS cm^{-2}) for voltages more negative than E_K and becomes large (500 μS cm^{-2}) for more positive membrane voltages.

$$\tau^0 = C_m/(g_d + g_{P1}). \tag{1}$$

The large time constant τ^∞ in the range of 10 sec is due to the large capacity C_P (3 mF cm^{-2}) in the pump pathway, which has been analogized with the cell's ATP pool (under various conditions, the energy of the ATP

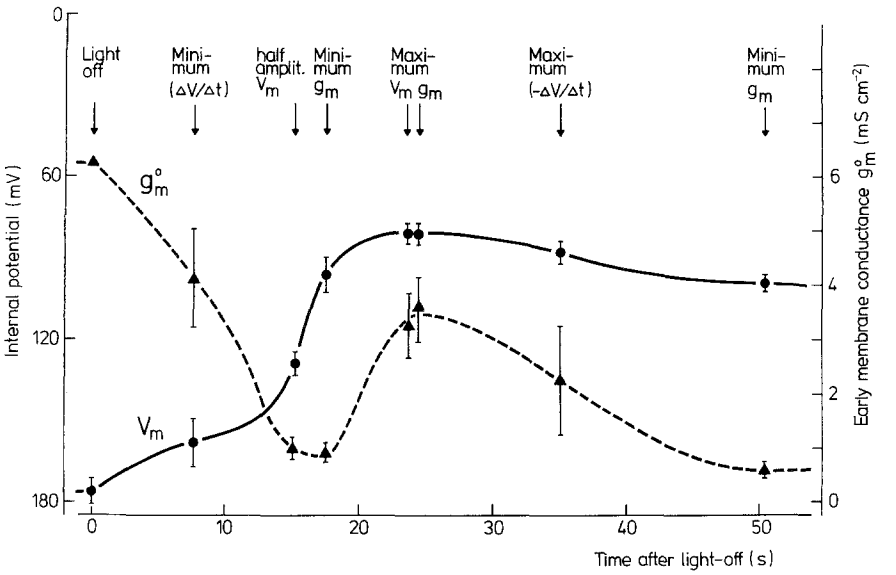

Fig. 9. Average time course of the membrane voltage V_m and the early membrane conductance g_m^0 during the first 50 sec of an action potential triggered by light-off. 5 experiments as in Fig. 8. Measuring points ±SE in states marked by arrows; conductance ($\sim \Delta V^{-2}$ in Fig. 8) values, related on 6.25 mS cm^{-2} at the resting potential in *L* [3], may be too high in these experiments. Note: g_m^0 generally decreases drastically, but for V_m more positive than E_K (here probably near −100 mV), g_m^0 rises. *A. mediterranea*, 23 °C

pool (1 M ATP ≈ 10 kcal) turns out to be about the electrical energy equivalent stored in C_P), and the conductance g_{P2} of the second element *P*2 of the pump channel (normally about 500 μS cm^{-2}) in series with *P*1, shunted by the conductance g_d of the diffusion pathway with g_{P1} in series

$$\tau^\infty = C_P/(g_{P2} + g_d \times g_{P1}/(g_d + g_{P1})). \quad (2)$$

The conductance of *P*1 is not constant but decreases from its maximum value near $V_m = -200$ mV for hyper- and depolarizations. So, the slope conductance of *P*1 is only a few % at a membrane potential of about −100 mV. *P*2 is also nonlinear: its slope conductance decreases with depolarization of the membrane potential to zero near −130 mV and reaches with further depolarization even negative values, down to about −500 μS cm^{-2} or more (*see also* legend to Fig. 7).

Conductance. If small and short ($\tau^0 \ll 50$ msec $\ll \tau^\infty$) current pulses ΔI are injected into a cell during an action potential, fast voltage responses ΔV are superimposed on the voltage course of the action potential. ΔV can be separated by a high-pass filter (ac input of oscilloscope). An original recording of V_m, ΔV and ΔI during a light-triggered action

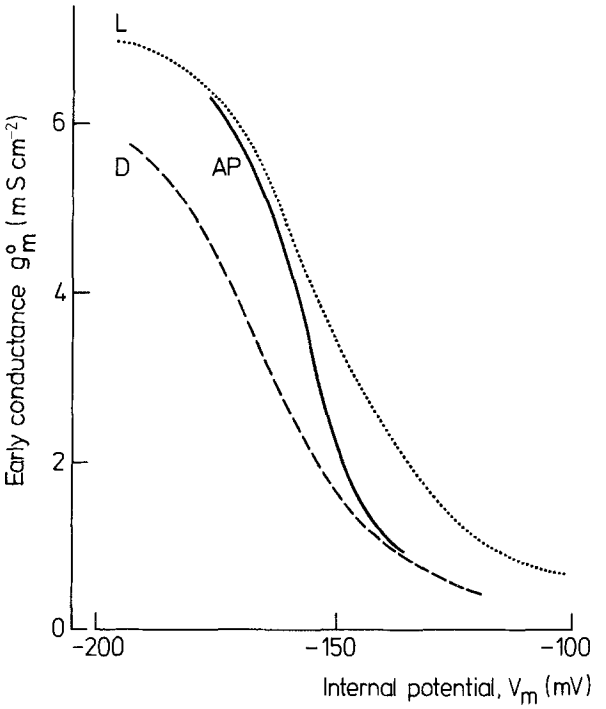

Fig. 10. Early conductance g_m^0 plotted versus the actual membrane voltage V_m during depolarizing phase of an action potential triggered by light-off (solid line, data from experiments to Fig. 9) fits in the transition of the steady state functions $g_m^0(V_m)$ from *L* to *D* conditions (*L*: ..., *D*: ---, replotted from [3])

potential is given by Fig. 8. Analysis by linear cable theory [9] then gives the time course of the "early" conductance, $(g_d + g_{P1})$, during an action potential. Fig. 9 shows the average results during the first 50 sec of 5 action potentials, such as the example of Fig. 8. The time course of the measured conductance can be interpreted quantitatively on the basis of the steady state current-voltage relationships of the diffusion system and of the element *P*1. The large conductance decrease during the depolarizing phase is mostly due to the current-voltage relationship of *P*1, which approaches saturation current at a membrane voltage of about -100 mV. In addition, the conductance of *P*1 becomes smaller in the dark. Therefore, the early conductance, g_m^0, decreases even faster during the depolarization of a light-triggered action potential than just following its voltage dependence. This situation is illustrated by Fig. 10. Here, the early conductance, g_m^0 (dotted curve: *L*, dashed curve: *D*) in the steady state is plotted as a function of the membrane voltage, V_m; these data are adopted from [3]. The solid curve gives the average early conductance during a light-

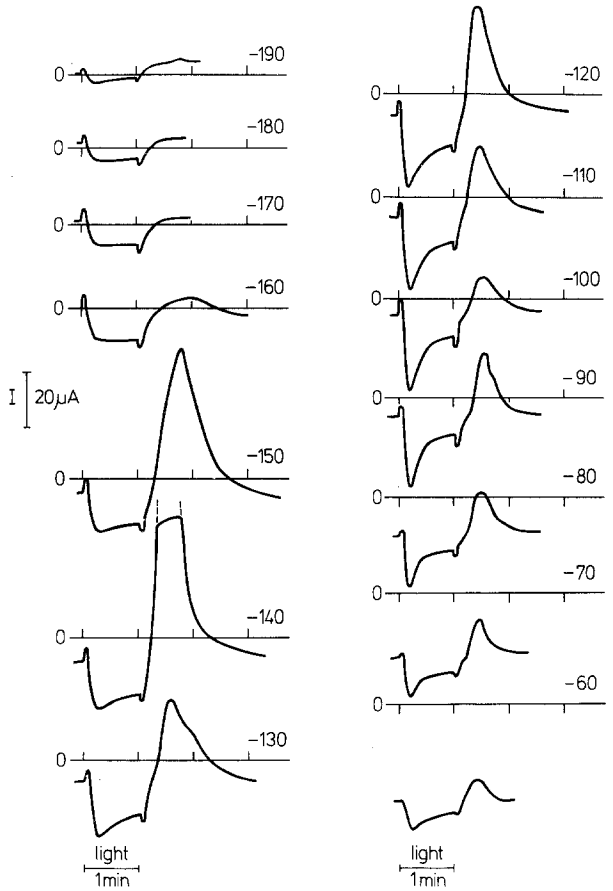

Fig. 11. Series of clamp currents upon pulses of 1 min 100 mW cm^{-2} white light. Numbers on abscissae mark level of point clamp in mV. *A. crenulata*, 23 °C

triggered action potential (same experiments as Fig. 9) as a function of the simultaneously recorded membrane voltage V_m. This curve simply represents the direct transition from the dotted (L) to the dashed (D) curve.

However, it is known that the conductance of the passive diffusion system increases drastically (g_K), if the membrane voltage becomes more positive than E_K (cf. dashed $I-V$ curve in Fig. 12) [3, 12]. Therefore, it is expected that in this voltage range g_d amounts to a considerable portion of g_m^0 ($=g_d+g_{P1}$), especially because g_{P1} becomes very small for larger depolarizations. In fact, Figs. 8 and 9 show that for membrane voltages more positive than E_K, g_m^0 rises again by about 2 mS/cm^{-2}, which is high but in agreement with the statistical limits of the steady state conductance

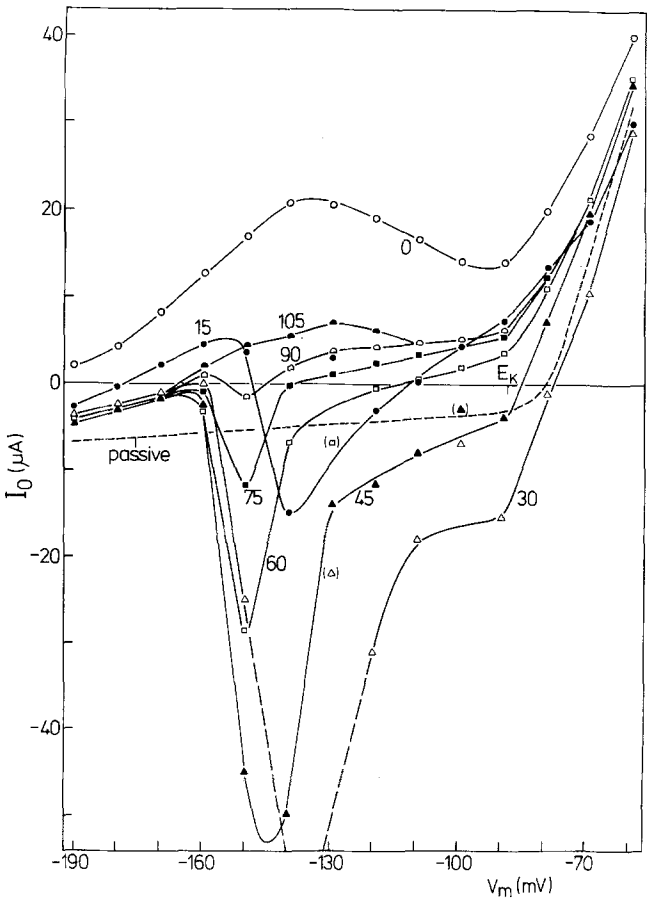

Fig. 12. Input current-voltage curves at different times (marked in sec on curves with different symbols) after light-off. Values taken from experiment to Fig. 11. Points in brackets (ignored for tracing the curves) may be due to temporary disturbations of the cell. Dashed line: current-voltage curve of the passive channels according to [2, 3, 4]

increase in the passive pathways for voltages more positive than E_K [3]. This potassium conductance decreases again, when the membrane voltage repolarizes towards E_K. For further repolarization, g_m^0 decreases again due to $g_d(V_m)$ and finally rises again slowly (Fig. 8) due to the voltage dependence of g_{P1}.

Current-voltage relationship. In order to obtain the probable changes in the non linear current-voltage relationship of the remaining element $P2$ during an action potential, voltage clamp experiments have been carried out. The voltage response upon current pulses would not give the necessary information, because $P2$ is the source of negative conductance in the membrane [3]. Unfortunately, the data available do not permit a quantitative analysis of the electrical parameters related to the membrane

area. However, the performed point clamp experiments give relevant qualitative results, especially since it could be shown that the main electrical properties (including negative conductance) of the *Acetabularia* membrane do appear in point clamp experiments as well [2, 3, 4].

Action currents of a cell have been recorded, when the voltage was clamped at different levels. The results for electrically triggered excitations have been reported previously [2]. Here, the data in Fig. 11 and 12 represent excitations triggered by light-off. In this experiment, stimuli (1 min × 100 mW cm^{-2} white light) as well as time between stimuli (3.5 min) have been kept constant, since the amplitude of light triggered action currents strongly depends on the dose of the preceding light pulse and on the refractory period. From the continuous recordings of the light-induced clamp currents at different clamped voltage levels (Fig. 11) the current-voltage relationships of the system could be constructed for different times after the triggering light-off stimulus (Fig. 12). In these results, the current-voltage relationships for 0 and 105 sec after light-off are equivalent to the steady state $I-V$ curves of the membrane in L and D, presented previously [3]. Resting potential and conductance are enhanced under illumination, and a region of negative slope conductance occurs between *ca.* −130 and −90 mV under space clamp as well as under point clamp conditions. This finding is expected, since at zero conductance ($g_m=0$) of the membrane (near −130 and −90 mV) the length constant ($\lambda=(g_i/g_m)^{0.5}$, g_i is the conductivity of the cell interior) becomes infinite and each point of the membrane has the same voltage, also in point clamp experiments, since $V_x=V_0 \exp(-x/\lambda)$, where x is the distance from the current injecting electrode.

The transition between the steady state current-voltage curves from light to dark conditions does not, however, consist of simply intermediate curves. There is a dramatic break-down of current to large negative values, far below the current-voltage curve of the passive system, which has been investigated separately [3] and which will be shown in the discussion (on the basis of the results in Figs. 9 and 10) to remain constant during an excitation. With proceeding time, the negative peak currents are slightly shifted to more negative membrane potentials, corresponding to the results of electrically triggered excitations [2].

Metabolic Relations

Temperature. So far, these results suggest that the electrogenic pathway is essentially involved in the action potentials of *Acetabularia*. Therefore,

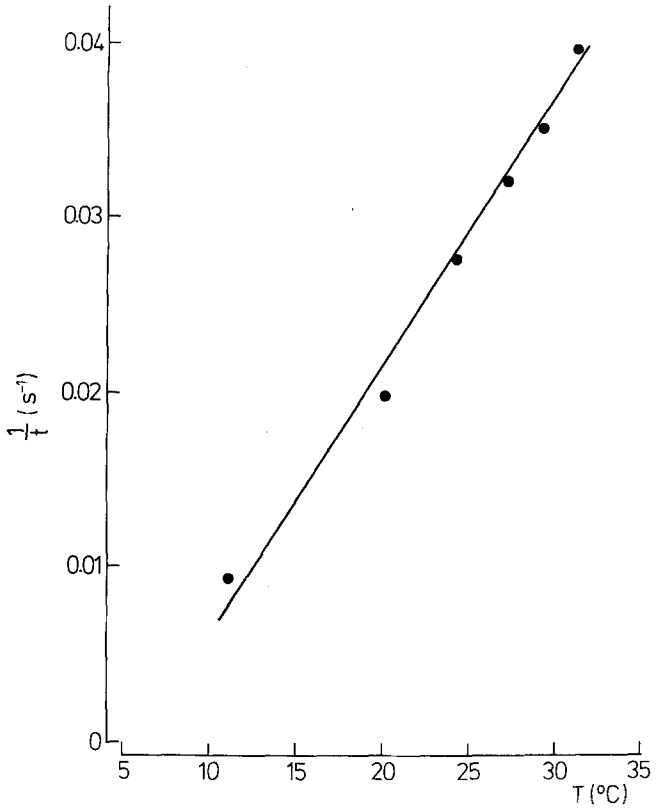

Fig. 13. Example for temperature sensitivity of the action potential. Ordinate: reciprocal of duration of the action potential in sec^{-1} (time between depolarizing and repolarizing passing half the amplitude). One cell of *A. crenulata*, replotted according to [2]

it is expected that these action potentials strongly depend on the energy metabolism, which in turn depends on the temperature. It has been reported previously that the velocity (i.e. 1/time between the depolarizing and repolarizing passage of half the amplitude of the action potential) strongly depends on temperature [2]. The results are replotted in Fig. 13. The clear, linear relationship between temperature and velocity indicates that metabolic events are involved in the action potentials. However, this evidence alone does not suffice to prove the pure metabolic nature of the action potential in *Acetabularia*, since the time course of conventional action potentials can also be slowed down by low temperatures [8].

ATP. A close relationship between the ATP pool and the steady state electrical properties of the *Acetabularia* membrane has been demonstrated [3]. In order to obtain direct information about the correlation between action potentials and energy metabolism, one culture of cells has been used to determine the average time course of the action potentials

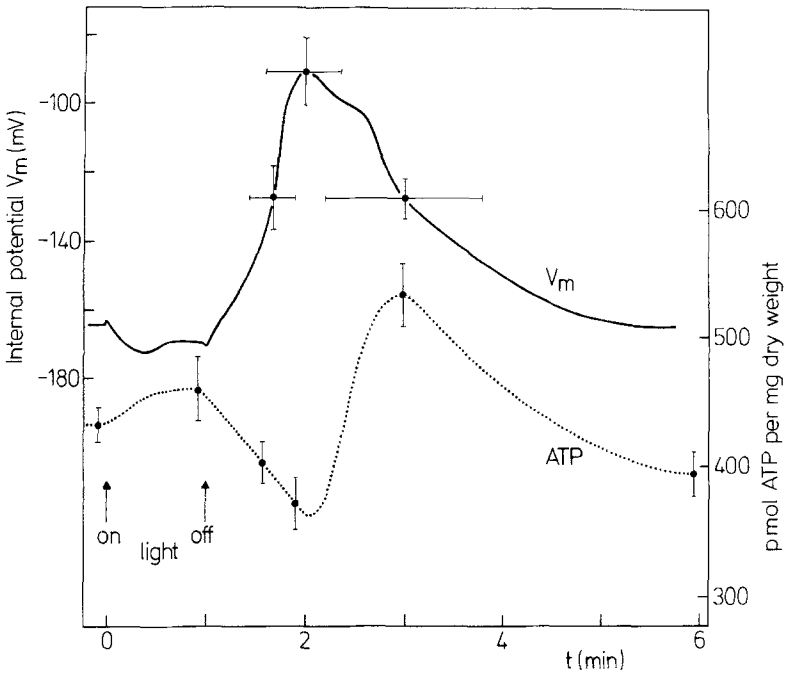

Fig. 14. Time course of average action potential and ATP level of one culture of *A. mediterranea* during a light/dark regime triggering action potentials in 60% of the cells. Bars mark ±SE

of this particular population. Fig. 14 shows this average time course. The resting potentials in *D* and *L* are mean values. The points which mark the average time course of the action potentials (half the depolarization, peak, half the repolarization) of this population are given as means ±SEM, both in voltage and time. The remainder of the time course of the voltage, also found in these experiments, is reconstructed "by eye," according to the well known behavior of the voltage under these conditions.

For these main phases of the action potential, plus for *L* and *D* conditions before light-off (and *D*, 5 min after), the ATP concentration has been determined and plotted in Fig. 14 as well. The slight increase of ATP after 1 min in the light is not significant. However, the ATP level decreases during the time of depolarization of the action potential and increases during the repolarizing phase significantly above its control level in the dark, 5 min after the light-off stimulus.

Light. Since photosynthetically active light raises the resting potential of *Acetabularia* [16], it seems to be possible that the light has also an effect on the action potential *via* the energy metabolism. In fact, it has

been shown that the action currents under voltage clamp conditions strongly increase with the dose of the preceding light pulse [2]. Like the free-running voltage during a light pulse, the action currents upon light-off increase continuously (no all-or-none characteristics) with the intensity of the light pulse and pass a maximum when the duration of the light pulse is increased.

Discussion

Similarities to Conventional Action Potentials

The phenomenon described clearly fulfills all criteria of the above definition of an action potential: it is a transient depolarization with all-or-none characteristics (Figs. 1 and 2), endogenous energy supply (Fig. 4) and propagation (Fig. 5). In addition, it displays some typical properties of conventional action potentials, such as summation of subthreshold stimuli [2], refractory period (Fig. 2) and an approximately hyperbolic strength-duration-curve for a sufficient stimulus (Fig. 3). It can be triggered by electrical stimuli (Figs. 2 and 5), but also by light-off signals (Figs. 1, 6, 8) and temperature changes [2]; chemical stimuli have not yet been tested systematically. Single and repetitive action potentials can occur spontaneously (Fig. 4). All these features are typical for the action potentials of classical preparations, such as nerves and muscles in the animal kingdom or plants like *Nitella*, *Mimosa*, *Dionaea*, and many others.

The question is, however, whether all action potentials of biomembranes obey the general mechanism of temporary permeability increase. As for the depolarizing phase, in principle, two mechanisms are possible: (1) opening of a pathway with an electromotive force more positive than the resting potential (i.e., the conventional type); or (2) closing of a pathway with an electromotive force more negative than the resting potential. This type has already been suggested to occur in *Acetabularia* (though on weaker experimental grounds) [2]. Since in *Acetabularia* a large efflux of Cl^- (Fig. 6) and potassium [12] is associated with the action potential, it has been concluded that the depolarization is due to a net efflux of Cl^- across the plasmalemma and the repolarization due to a K^+ efflux, just as it has been suggested for the action potential in *Nitella* [11].

Differences from Conventional Action Potentials

It could be shown, however, that the large Cl^- fluxes in *Acetabularia* do not reflect a corresponding transport of electrical charge across the plasmalemma, since the measured conductance of the membrane is – even in the steady state – much smaller than a membrane conductance calculated from the Cl^- fluxes, if these were charge carrying [3]. Furthermore, the large decrease of the early conductance $g_m^0 = g_d + g_{P1}$ (cf. Figs. 8, 9, 10) during the depolarization of the action potential strongly suggests that among the possible conductance changes causing depolarization there is only a (voltage- and light-dependent) decrease of the conductance of the element $P1$ of the active pathway (cf. Fig. 7) and no conductance increase in the passive pathways (only a fraction of the following temporary conductance increase might be due to the increased Cl^- efflux). This result definitively excludes the depolarization being due to the conductance increase of a pathway with an electromotive force more positive than the normal resting potential. Therefore, it is concluded that the depolarization of the action-potential of the *Acetabularia* membrane arises in the pump pathway.

Changes in the Active Pathway

The conductance change (g_m^0) of a light-triggered action potential can completely be understood as intermediate states during the transition from the steady state conductance-voltage relationship in L and D (Fig. 10). Therefore, it is suggested that no active changes take place in the element $P1$. However, its conductance decrease (according to its steady state current-voltage relationship [3]) during depolarization will also shift the membrane potential closer to the electromotive force of the passive pathway (cf. Fig. 7).

So far, the element $P2$ is left as being the main source of the action potential in *Acetabularia*. It has been shown that the region of negative conductance in the current-voltage relationship of this element $P2$ is responsible for the N-shape of the overall steady state current-voltage relationship of the membrane [3]. Fig. 12 clearly demonstrates a dramatic increase of negative conductance in the current-voltage relationship during the time course of an action potential. This fact indicates that the element $P2$ is the actual source of the action potential in *Acetabularia*. This element has been demonstrated to reflect the coupling between the electrogenic pump ($P1$) and the energy metabolism, or (parts of) the

energy metabolism itself [3]. Therefore, it is concluded that the apparent action potential actually reflects a metabolic event. The correlation between energy metabolism and the action potential has already been pointed out in *Results* (Figs. 13 and 14).

Changes of the Element P2 and of the ATP Level

The normal steady state activity (outward current) of the pump channel causes the current-voltage relationship of the normal intact system to lie above the current-voltage relationship of the passive system (only for voltages more positive than the electromotive force of the electrogenic pump, *ca.* -200 mV [3, 4]. During both electrically triggered action potentials [2], and light triggered action potential (probably initiated by a transient energy deficit upon light-off), the current-voltage relationship of the total system comes to lie below the $I-V$ curve of the isolated passive system, indicated by the dashed line in Fig. 12, which does not change its characteristics. In this state, the pump current is supposed to change its sign (inward) compared to normal (outward). If the pump normally operates on ATP hydrolysis, this inversion of the pump current during an action potential should synthesize ATP, provided the system is reversible. In fact, the results in Fig. 14 show that upon action potential triggering light-off signals, the ATP level first decreases (probably due to temporary energy deficit) and then rises during the repolarizing phase, when the pump current flows inwards (normally outwards), above the control level, indicating increased ATP synthesis.

On the other hand, an increased ATP level can also reflect reduced ATP consumption of the entire cell. On a quantitative basis, it is not possible yet to decide which process really takes place, especially since the electrogenic pump is normally fueled by only about 5% of the total ATP turnover [3]. Furthermore, fluctuations of the ATP level upon metabolic downshifts have been found in other systems too, without correlation to action potentials [5]. Therefore, the time course of ATP during a light-triggered action potential (Fig. 14) does not necessarily imply a direct causal relationship.

Operation of the "Metabolic" Action Potential

In this concluding paragraph the mechanism is described by which the action potential in *Acetabularia* is proposed to operate, according to the preceding results and conclusions. As for the ions involved, the electro-

genic pump operates in the steady state by Cl^- import [2, 15]. Its blockade or inversion during the action potential results, therefore, in a formal Cl^- export, which has not been identical with the measured Cl^- efflux (Fig. 6). If, during an initial depolarization, the peak current of the N-shaped current-voltage relationship drops below zero current (Fig. 12, at -160 mV, 30 sec after light-off), the voltage jumps "discontinuously" (actually damped by C_P) to the only possible value (-78 mV in Fig. 12). For voltages more positive than E_K (≈ -90 mV), there is a large potassium conductance, and potassium will leave the cell down its electrochemical gradient, shifting the membrane voltage towards E_K. If the cell is in low energy state (by low temperatures or metabolic inhibition), this plateau can be prolonged. If the pump does operate, (i.e., imports Cl^- again), the repolarization takes place relatively quickly first, slowing down later. This time course can also be reconstructed from the $I-V$ curves in Fig. 12 (obtained by 14 successive excitations), if one picks out the voltages at zero current at different times.

These action potentials in *Acetabularia* are two orders of magnitude slower than most action potentials in plants and even 4 orders of magnitude slower than usual action potentials in animal cells. This slow time course is due to the existence of the large capacity C_P, which has been analogized with the ATP pool [3]. Comparable slow action potentials have been found in the fungus *Neurospora* [19] where, however, no capacity in the range of mF cm^{-2} could be demonstrated. On the other hand, these action potentials in *Neurospora* are the only example besides the action potentials of *Acetabularia*, which can be interpreted as arising in the pathway of the electrogenic pump.

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