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Spontaneous and Induced Changes in the Membrane Potential and Resistance of *Acetabularia mediterranea*

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Summary. The normal resting potential across the *Acetabularia mediterranea* cell membrane is -170 mV and the resistance is about 0.1 $k\Omega \cdot cm^2$. The time courses of potential and resistance changes have been studied in connection with several slow dynamic properties of the membrane. These effects include spontaneous and stimulated depolarizing spikes, spontaneous repolarization after prolonged maintenance of a quasi-stable depolarized condition, and a hyperpolarizing response when current is applied to the cell in this depolarized state. These processes show considerable similarities to each other, which suggests that they might all be explained by changes in permeability to Cl^-. In normal conditions, membrane punch-through occurs with a relatively small hyperpolarizing bias.

For many years, the giant unicellular green alga *Acetabularia* has been used for the study of nucleo-cytoplasmic interactions, but very little attention has been paid to other aspects of its physiology. Schilde (1966, 1968) has examined the effect of light on the membrane potential and finds a resting potential of -160 mV in the light.

The present paper gives some of the results of an investigation of ionic regulation and electrical behavior in *Acetabularia mediterranea.* In normal conditions, the resting potential is about -170 mV, virtually all of which is across the plasmalemma, and the resistance is about 100 $\Omega \cdot cm^2$, also at the plasmalemma (Saddler, 1970*a*, *b*). The large active Cl^- influx (200–700 pmoles $\cdot cm^{-2} \cdot sec^{-1}$) is strongly electrogenic, but the membrane potential is also controlled by the diffusion of K^+. The exact relationship between these two factors and the total potential is unclear, but experimentally they behave quite independently, so that the K^+ diffusion component is apparently unaffected when electrogenic Cl^- influx is abolished,

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and vice versa (Saddler, 1970*b*). Very similar results have been reported for *Acetabularia crenulata* (Gradman, 1970; Gradman & Bentrup, 1970). Electrogenic Cl^- influx has been determined in various equilibrium and dynamic situations (Saddler, 1970*b*); the present paper is concerned with the values of membrane potential and resistance in similar situations.

Materials and Methods

A culture of *Acetabularia mediterranea* was maintained in the laboratory; culture techniques are described in detail elsewhere (Saddler, 1970*a*). Cells were selected for experimental use before they had started to form caps, so that they had a roughly cylindrical shape. They were typically about 3.5 cm long with an average diameter of 0.03 cm. About 24 hours before making any measurements, cells were transferred from the enriched natural sea water medium to an artificial sea water (ASW) of the following composition: Na^+ 467 mN, Mg^{++} 110 mN, Ca^{++} 20 mN, K^+ 9.8 mN, Cl^- 549 mN, SO_4^{--} 55 mN, HCO_3^- 2.3 mN, Br^- 0.8 mN. This was the standard solution for all experiments. Some measurements were also performed with solutions in which the Cl^- concentration was reduced to 21 mN and the deficit made up with SO_4^{--}, so that all the other ions were the same as in ASW. This solution will be called low-Cl^- ASW.

Most of the experimental techniques have been described at length in another paper (Saddler, 1970*a*), and only the most important aspects will be dealt with here. Intracellular potential was measured by standard techniques using KCl-filled glass microelectrodes, Keithley 603 electrometer amplifiers and pen recorders. Reservoirs of bathing solution were connected to the experimental chamber via a six-way tap, which made rapid changes of solution possible. It was not always certain whether the electrode tip was in the cytoplasm or the vacuole of the *Acetabularia* cell, but since the potentials in the two phases are not significantly different this was not a problem.

Membrane resistance was measured by the method of Hogg, Williams and Johnston (1968) using two intracellular electrodes. This method was checked in several cases by using three electrodes and determining the cell space constant. It is necessary to assume that the cell is a uniform circular cylinder, but many cells actually vary quite considerably in diameter along their length. It was estimated that absolute resistance values might be in error by a factor of two either way, but relative changes measured on the same cell are much more accurate.

Results

The response of the membrane potential of an *Acetabularia* cell in ASW at 25 °C to a series of square hyperpolarizing current pulses of increasing amplitude is shown in Fig. 1; Fig. 2 shows the response to depolarizing pulses. The fast response to applied current (a measure of resistance) is so small as to be imperceptible in these traces. Further amplification of the signal shows that the resistance is about 0.1 $k\Omega \cdot cm^2$. The slow potential transients, which under these conditions represent most of the response to applied current, are not a measure of the membrane resistance.

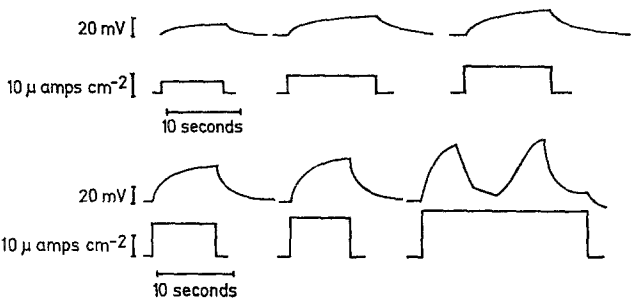

Fig. 1. Recorder traces showing the effect of hyperpolarizing current pulses on the membrane potential

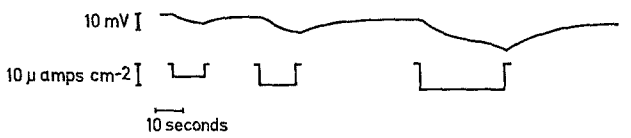

Fig. 2. Recorder traces showing the effect of depolarizing current pulses on the membrane potential

Barry and Hope (1969*a*, *b*) have studied transients of this type in *Chara* and provide a convincing explanation in terms of local changes of concentration adjacent to the membrane which are the consequence of the difference in transport numbers between the membrane and adjacent solutions. They have termed this the "transport number effect". In certain conditions, to be described below, the membrane resistance of *Acetabularia* is very much greater than 0.1 $k\Omega \cdot cm^2$; when a current pulse is applied, the fast resistance component of the response and the slow "transport number" transient are of approximately equal amplitude.

In the final trace of Fig. 1, hyperpolarization is sufficient to induce the rapid decrease in membrane differential (slope) resistance, termed punch-through by Coster (1965). The bias at which this occurs is only -60 mV, representing a total potential of about -230 mV, which is much lower than the punch-through potential of other (fresh water) algae. Coster (1969) has shown that this potential is very sensitive to pH, being lower at low pH; in *Chara* at pH 7.8 (the pH of ASW), it is between -400 and -430 mV (a bias of about -280 mV). In *Nitella*, at unspecified pH, the bias is -300 mV (Williams & Bradley, 1968). While making a comparison between these algae and *Acetabularia*, in which most of the normal membrane current is carried by Cl^-, it is interesting to note that Cl^- conductance rises rapidly with increasing potential bias in *Chara*, and near punch-through Cl^- carries most of the current (Coster & Hope, 1968).

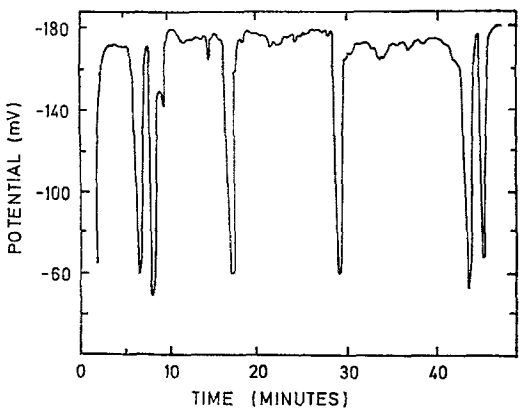

Fig. 3. A typical recorder trace of potential for 50 min following microelectrode insertion

The last trace of Fig. 2 shows the beginning of an increase in the rate of depolarization with no further current increase. A longer pulse than shown here causes large erratic fluctuations of potential going as low as −120 mV, or, in some cases, the initiation of a spike. Spikes can be seen in Fig. 3, which is a recorder trace of potential for the first 50 min after electrode insertion. Two features stand out. In the first place, the resting potential is extremely variable, oscillating apparently at random with an amplitude of about 10 mV. Secondly, there are a number of spikes of depolarization of 1 or 2 min duration. Although the minimum potential of the spikes in Fig. 3 is fairly constant at around −60 mV, repeated observations have shown a range of −30 to −100 mV. Their initiation is often apparently spontaneous, as in Fig. 3; they are often stimulated by a depolarization of 30 mV, but sometimes cannot be induced by one as great as 60 mV; sometimes anode break – the sudden cessation of a large inward current causing hyperpolarization to about −210 mV – is effective, but this often has no effect. Mechanical shock sometimes initiates a spike or a series of spikes and so does a change in composition of the external solution, e.g., reducing the K^+ concentration to half. A localized membrane depolarization (e.g., by partly withdrawing a microelectrode, thus breaking the seal and allowing vacuolar contents to escape) is often not propagated as a spike, showing that the membrane is frequently inexcitable. When it is excitable, the propagation velocity is very low, i.e., less than 1 cm · sec^{-1}.

Fig. 4 shows the changes in membrane potential and chord conductivity during a typical spike, measured by the response to current pulses of constant amplitude. It has been emphasized that conductivity values are only approximate, but, relative to each other, they are accurate. For obvious

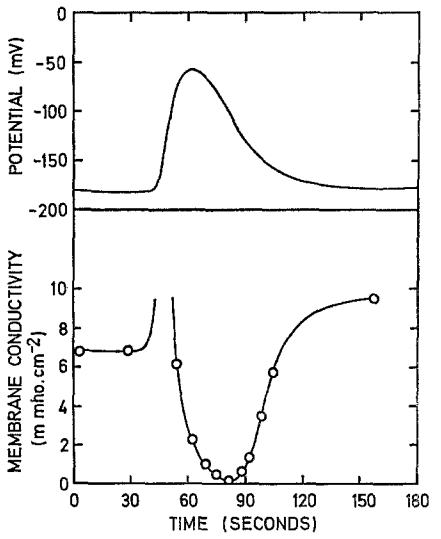

Fig. 4

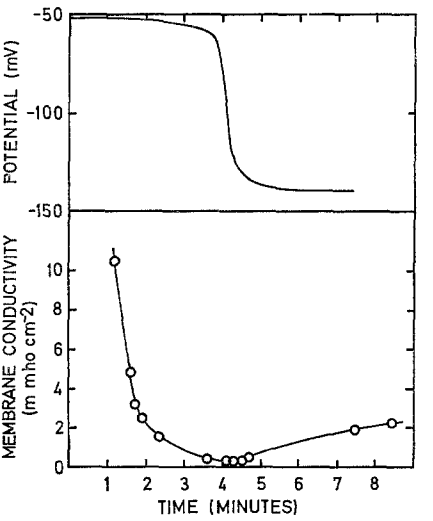

Fig. 5

Fig. 4. The time course of potential and chord conductivity changes during a depolarizing spike in ASW at 25 °C. Potential record is a continuous trace; conductivity was measured by brief current pulses at the points shown

Fig. 5. Same as Fig. 4, but showing time course of potential and chord conductivity changes during spontaneous repolarization in low-Cl^- ASW at 25 °C

reasons the current pulses were kept very short during the spike, so that there was no time for transients to appear. The discontinuity in the conductivity curve between 40 and 50 sec corresponds to a phase during the spike when conductivity is so high (resistance so low) that even very large applied currents produce no perceptible potential change at the highest amplification used; this coincides with the depolarizing phase of the potential trace. Conductivity starts to decrease again just before the maximum level of depolarization is reached, and minimum conductivity is attained when repolarization is about half completed.

The phenomenon of repolarization has been described previously (Saddler, 1970*b*). When a cell is placed in low-Cl^- ASW, potential falls rapidly and resistance remains very low (less than 0.1 $k\Omega \cdot cm^2$). After some time at steady low values, resistance and potential start to increase, slowly at first, then more quickly until a new stable level is reached. This course of events is shown in Fig. 5. As the conductivity decreases, repolarization occurs at first very slowly, but eventually builds up to about the same rate as is observed during a spike, with minimum conductivity coinciding with the half-way stage in repolarization. The process is very much like the second

(repolarization) phase of a slowed-down spike. Such behavior is observed in CCCP and in darkness, as well as in low Cl^-. The speed of repolarization and the final stable level depend on the conditions. In CCCP and in very low Cl^- concentrations (5.5 mN), repolarization may take up to 1 hr and the stable repolarized potential is only about -130 mV; the conductivity may also remain rather low (as in Fig. 5). In darkness, repolarization occurs almost immediately and attains the normal -170 mV of a cell in the light; it is difficult to say whether this should be described as repolarization or spike behavior, indicating the essential similarity of the two phenomena. If a cell is exposed to intermittent periods of darkness of, say, 5 min followed by 10 min of light, it will show extensive depolarization and repolarization of about 90 mV amplitude for the first two or three exposures to darkness. Thereafter one observes only smaller transients of about 15 mV amplitude and 3 min duration.

Gradman (1970) has carried out a very thorough study of the electrical properties of *Acetabularia crenulata*. The resting potential is about -170 mV under normal conditions and about -70 mV at low temperature; in many other respects, it is very like *A. mediterranea*. The slow response to applied current, membrane resistance at low temperature, small potential transients under intermittent illumination at 25 °C, and the occurrence, amplitude and time course of spikes all seem to resemble those described in this paper. By exposing cells of *A. crenulata* to alternating periods of light and dark, each of 4 min duration, Schilde (1966) was able to induce regular oscillations in the membrane potential with an amplitude of about 40 mV (20 mV either side of the resting potential). The amplitude and period of these oscillations suggest that they correspond to a series of small transients, of the type described above and by Gradman (1970), run together by changing the light regime just as each transient was beginning to return to the resting level.

If a moderate current pulse (about 10 amp $\cdot$ cm^{-2}) is applied when the resistance is increasing during repolarization, there is a rapid dynamic increase in resistance, causing a faster than normal repolarization. When the current is stopped, the potential sometimes falls again, but it frequently remains at a high level. This behavior can be observed in low-Cl^- solutions, in the presence of CCCP, and also in a number of conditions when the cell has been subjected to shock of some kind. For example, it is often found that, when an initial electrode insertion is made, a quasi-stable potential of about -80 mV is maintained for perhaps 15 min, then sudden repolarization to -170 mV occurs; this stable hyperpolarization can be brought about sooner by application of a current pulse.

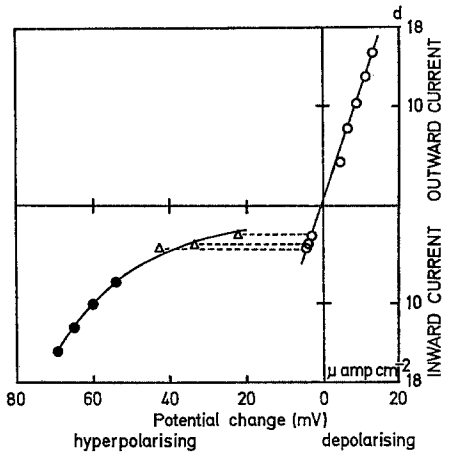

Fig. 6. Current-voltage curve for a cell at 5 °C, showing hyperpolarizing pulse

At low temperature (5 °C), repolarization takes a very long time, and during the first 1 or 2 hr the potential is normally very steady at a level near −80 mV, with none of the fluctuations seen at 25 °C (as in Fig. 3) and no spikes (which confirms that these fluctuations are real effects and cannot be regarded as artifacts produced by instrument error). This steady potential corresponds to the part of the total membrane potential that is controlled by K^+ diffusion. The resistance under these conditions is about 1.0 kΩ · cm².

Fig. 6 shows the current-voltage relation of a cell when the resting potential is steady near −80 mV. For depolarizing pulses, the resistance is about 1.0 kΩ · cm², decreasing slightly with large currents. The application of an inward hyperpolarizing current causes an immediate and rapid dynamic increase of resistance to about 8 kΩ · cm². This can be seen more clearly in Fig. 7 which shows the effect on the potential of hyperpolarizing current, and from which some of the data of Fig. 6 are taken. At −110 mV (30 mV hyperpolarization), the differential resistance is about 8 kΩ · cm²; it decreases to 2.2 kΩ · cm² at −140 mV. Fig. 7 also shows a spike which occurred during the continuous passage of inward current. It is indistinguishable from a normal spike. Furthermore, the sequence of events during this hyperpolarization process with applied current differs very little from the sequence that occurs during repolarization without applied current.

The repolarization phenomenon when current is applied at low temperature in *A. mediterranea* is identical with the hyperpolarizing response which Kishimoto (1966*a*) has studied in *Nitella* cells initially depolarized by high external salt concentrations. Hyperpolarizing response has been investigated in a number of nerve and muscle preparations; most of these

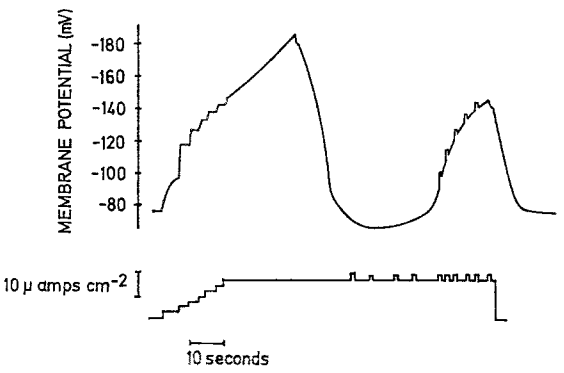

Fig. 7. Recorder traces of potential and applied current during hyperpolarizing response in a cell at 5 °C, showing the occurence of a depolarizing spike

require initial depolarization in high salt concentrations, but lobster muscle fibers do not need this treatment (Reuben, Werman & Grundfest, 1961). While undergoing hyperpolarizing response, this tissue also shows oscillations in the potential which, apart from being an order of magnitude faster, are very similar to the spikes observed in *A. mediterranea.* It seems that *A. crenulata* at low temperature does not show a hyperpolarizing response like that in *A. mediterranea* (Gradman, 1970), but a large and rapid hyperpolarization in the dark is induced by a period of intermittent illumination when the temperature is below 10 °C.

Discussion

The results described in this paper must be interpreted in the light of two important facts that have previously been established (Saddler, 1970*b*). Firstly, the membrane potential can be described as the sum of two independent components – K^+-dominated diffusion and electrogenic Cl^- influx. Secondly, the fluxes are such that an *Acetabularia* cell which has been in light or dark or any other conditions under which it survives for some hours must, after about 3 hr, be in a state of flux equilibrium with respect to all the major ions, including K^+. Furthermore, the steady-state membrane potential of -170 mV lies outside the range of the Nernst potentials of all the permeating ions. No diffusion theory can provide a complete explanation of these results. The K^+-rectification mechanism advanced by Gradman and Bentrup (1970) and Gradman (1970) in explanation of their results depends on a net current of K^+ ions into or out of the *Acetabularia* cell; it will be clear that, although this may explain some of the results

obtained in dynamic situations, it cannot account comprehensively for all the steady-state observations.

The measurements described in this paper are not sufficient to suggest a complete explanation of the ionic regulation mechanism in *Acetabularia*, but some distinctive membrane properties have been demonstrated. Despite their superficial diversity, the three principal phenomena – spikes, repolarization, and hyperpolarizing response at low temperature – show very considerable similarities. The time course of potential and resistance changes during a typical spike is generally slower than but otherwise resembles the changes observed during a Characean action potential (Findlay & Hope, 1964; Kishimoto, 1966*b*). It has been established that this action potential is caused by changes in membrane permeability to Cl^- (Gaffey & Mullins, 1958; Haapanen & Skoglund, 1967). Reuben *et al.* (1961) have suggested that the oscillatory characteristic of the lobster muscle hyperpolarizing response may be caused by changes in permeability to Na^+ and Cl^-, and they have also established that the primary hyperpolarizing response is the result of a voltage-dependent decrease in permeability to K^+ (hyperpolarizing K^+ inactivation).

Repolarization and spike initiation in *Acetabularia* can be most simply explained by changes in permeability, modified to some extent by various environmental factors (light, CCCP, low temperature, etc.). It has previously been shown that Cl^- fluxes are an order of magnitude greater than fluxes of K^+ and Na^+, and that in dynamic situations, when net fluxes of Cl^- occur, these net fluxes may be balanced by H^+ fluxes but certainly cannot be balanced by K^+ and Na^+ net fluxes (Saddler, 1970*b*). Changes in permeability to K^+ and Na^+ alone could not, therefore, account for the observed effects, whereas Cl^- permeability could. In other words, Cl^- (and probably H^+) permeability changes must be the major factors, and changes in K^+ and Na^+ permeability, if they occur, can only be of secondary importance.

It might be thought that an explanation in terms of permeability changes would be sufficient to account for all the effects of inhibitors on the membrane potential, and that there is no need to postulate the existence of electrogenic Cl^- influx. This is not so, for although depolarization from -170 mV merely requires that the treatment initiate a large increase in permeability which could short-circuit either an electrogenic flux or a diffusion potential, the subsequent hyperpolarization recovery to and maintenance of the -170 mV potential could not occur without an electrogenic mechanism. The striking way in which normal 25 °C membrane properties can be induced by applying inward current (outward through

the membrane) to a cell at 5 °C shows that this is electrically equivalent to the activity of the electrogenic Cl^- influx pump (producing inward negative current through the membrane). In both cases, the voltage drop of the back current through the membrane resistance generates a potential which depends on the applied current (or electrogenic flux) and the membrane resistance. The same potential may be generated by a large current through a small resistance or a small current through a large resistance.

To conclude, the simplest explanation for the observations described in this paper is provided by the presence of electrogenic Cl^- influx at the plasmalemma, and the occurrence of Cl^- permeability changes. A simple quantitative model based on the Goldman equation with electrogenic Cl^- influx and passive diffusion in parallel across the membrane can account for the potential changes in certain dynamic conditions when the membrane resistance is high (e.g., at low temperatures). It must be emphasized once more, however, that the normal resting state of *Acetabularia*, with resistance apparently very low and all major ions in flux equilibrium, cannot be satisfactorily explained. There is little point in elaborating crude quantitative models for the potential until this central problem is elucidated. This will depend on more sensitive flux determinations undertaken in conjunction with appropriate electrical measurements.

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