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Green Light (550 nm) Inhibits Electrogenic Cl^- Pump in the *Acetabularia* Membrane by Permeability Increase for the Carrier Ion

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Summary. In *Acetabularia*, the fast, green-light sensitive depolarization (Schilde, C. 1968, *Z. Naturforschung* **23b**:1369) has been reexamined. In the physiological voltage range (−170 to −50 mV), it appears to be a light-induced current source. The dose-effect relationship is linear up to about 1 A m^{-2}; about 10^6 photons (550 nm) cause the transfer of one elementary charge across the membrane. Voltage clamp experiments yield the current-voltage relationship of the fast, light-induced pathway over a voltage range from −240 to +200 mV. This current-voltage-relationship does not intersect with the voltage axis up to membrane potentials of +200 mV; it exhibits maximum current at −190 mV (corresponding to the electromotive force of the electrogenic pump, at which its slope conductance is maximum) and shows smaller current under metabolic inhibition by 2,4-dinitrophenol. External cations, including H^+, have no significant effect on the fast, light-induced depolarization. The lag between the stimulus and the onset of the reaction is smaller than 40 μsec. On the base of actual carrier models for an electrogenic pump, it can be shown by computer simulation that this light-induced pathway reveals the same features as would be expected if the membrane would become permeable for the unoccupied carrier cation in its Cl^- binding state.

Ten years ago, Schilde reported a fast photoelectric effect in *Acetabularia* with maximum sensitivity to green light (540 nm), a lag time less than 40 μsec and a proportional dose-effect relationship up to 2.5 $\times 10^4$ W m^{-2} white light [13]. Such a phenomenon has been novel in plant physiology (it still seems to be unique); only in visual physiology are light-induced depolarizations of comparable speed under investigation. Therefore, Schilde suggested that the mechanism of the fast photoelectric response in *Acetabularia* was similar to the mechanism of early receptor potentials, with a kind of rhodopsin as a receptor [13].

On the other hand, the action spectrum might also remind one of an

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absorption band of some type of cytochrome [4, 8], especially since these pigments are involved in transfer of electrical charge across energy conserving membranes. Cytochrome chains in plant plasmalemma membranes have been proposed repeatedly during the last years, however, without crucial evidence. If the receptor for the fast photoelectric effect in *Acetabularia* turns out to be a cytochrome, this effect would be a direct demonstration of its location and electrical properties in the plasmalemma membrane.

Since the biochemistry of plant membranes is still underdeveloped, we prefer electrophysiological analysis at present. This approach seems promising because there has been, in the last few years, appreciable progress in understanding the electrophysiological properties of the *Acetabularia* membrane [4–8].

These electrical properties can be summarized in an equivalent circuit for the steady-state conditions. This circuit basically consists of a diffusion channel and an electrogenic pump in parallel. The diffusion channel is essentially controlled by potassium ($E_K \approx -90\,\mathrm{mV}$) and its rectifying current-voltage relationship [4–8]. The pump channel consists of an electromotive force, $E_P \approx -190\,\mathrm{mV}$, in series with two nonlinear resistors $P1$ and $P2$, where $P2$ and E_P are shunted by a quasi-capacitor, C_P of some $10\,\mathrm{F\,m^{-2}}$. C_P probably represents the ATP pool of the cells [4, 5]. $P1$ has a current-voltage relationship like a carrier mediated electrogenic pump [3], with saturating current for larger voltage displacements and maximum conductance at its equilibrium (zero current). This element will be focused on in this study. The element $P2$ has a range of negative slope conductance in its current-voltage relationship, which plays an essential role for action potentials; furthermore, $P2$ is suggested to be closely linked to the cell's energy metabolism [4, 5].

The described equivalent circuit has been worked out for steady-state conditions. However, it has been proved to be useful as well for the analysis of dynamic events, such as action potentials [6]. The fast, light-induced depolarizations have now been investigated on grounds of this equivalent circuit.

We do not want to specify this equivalent circuit in detail by complicated formal analogies. The aim is rather to gain some ideas and evidence about the molecular function of the individual elements of the circuit and, in this study, about the element $P1$, which is assumed to represent a carrier mediated electrogenic Cl^- pump.

Some of the results have been presented at the meeting on "Transmembrane Ionic Exchange in Plants" [8].

Materials and Methods

Cells

Young cells of the marine giant green algae *Acetabularia mediterranea* and *Acetabularia crenulata* have been used. No difference in the electrophysiological characteristics could be observed between these two species. The cells have been cultured in Erdschreiber solution as usual [2, 9]. For electrophysiological studies, young cells of 2–4 cm length and 0.2–0.6 mm diameter are favorable, because the relatively soft cell wall near the growing tip region allows rather convenient puncturing. Furthermore, the lack of a cap of the adult specimens justifies the use of linear cable theory for these approximately cylindrical cells.

General Conditions

Usually, the temperature was 23 ± 2 °C. Low temperature was obtained by cooling the measuring chamber by a thermostated perfusion system. Normally, the external medium was artificial seawater with 461 mM Na^+, 10 mM K^+, 53 mM Mg^{++}, 10 mM Ca^{++}, 529 mM Cl^-, 28 mM SO_4^{--} and 2 mM HCO_3^-, usually buffered with 10 mM Tris/HCl at pH 8.0 or varied between pH 6 and 9.

Light

In order to eliminate circadian rhythms, the cells were exposed at least one week before the experiments to continuous white light of about 500 lux. For preparation and puncturing, the cells could be exposed to continuous white light of maximum 1 kW m^{-2} from a 250 W quartz-iodide lamp, focused on the object. With this lamp and interference filters, monochromatic light from 408–738 nm wave-length and a constant quantum flux density of 83 $\mu E\ m^{-2}\ s^{-1}$ could be obtained. The appropriate energy flux density for each filter could be continuously adjusted by the heating voltage of the light source. Continuous light has been measured by a calibrated optometer (UDT 40 A). The intensity of white light was modulated by neutral grey reduction filters at constant heating voltage in order to keep the spectral characteristics constant. A mechanical shutter operated within less than 1 msec.

For fast and high intensity effects, we used a commercial electronic flash light for photographic use (Mecablitz). The measurement of the time course and intensity of these light flashes was performed with a fast photodiode operating under short-circuit conditions (50 Ω) with a storage oscilloscope. The photodiode was calibrated with neutral glass reduction filters down to intensities, which were also obtained by continuous light. Peak intensities of 250 kW m^{-2} could be obtained by appropriate focusing.

Electrical Measurements

Detailed descriptions of the electrical setup and of the analysis of the raw data have been given in previous papers [4, 5]. Briefly, standard microelectrode techniques have been applied. For fast voltage recordings and for current injection, the electrode tips have been broken to obtain low impedance (ca. 200 kΩ) electrodes. The input capacitance of the measuring circuit could be kept down to about 10 pF by mounting the differential preamplifiers ($R_i > 10^{12}\ \Omega$) directly behind the microelectrode. To record the data, we used a chart recorder and a storage oscilloscope with a camera.

The electronic device for our voltage clamp experiments has been described explicitly [4]. Since insertion of coaxial current electrodes was not successful in our experiments, we used a method with three microelectrodes inserted [1]. Two voltage recording electrodes (V_0 and V_1) and one current injecting electrode (I_0) were located close to the cell end so that the longitudinal voltage drop inside the cell under voltage clamp could be assumed to be linear. In this case, the voltage difference between the two voltage recording electrodes is proportional to the membrane current. In our experiments, this voltage difference never exceeded 10 mV, though voltage displacements up to 370 mV have been recorded. The scaling factor between $V_0 - V_1$ and the membrane current, i_m, has been taken from the linear case (small voltage displacements) and usual resistance measurements with 3 microelectrodes in the middle of the cell and linear cable analysis [5, 10].

Results

General Observations

As reported repeatedly, the normal resting potential of *Acetabularia* is about -170 mV (inside negative). That is much more negative than the most negative passive equilibrium potential (E_K of about -90 mV). This high resting potential and its sensitivity to changes of the energy metabolism is demonstrated in Fig. 1. Upon the onset of white light, there is a fast and small depolarization at first (marked by encircling), which is the main subject of this study, followed by a much slower and larger hyperpolarization with a temporary maximum. This hyperpolarization has been shown to have the same action spectrum as the photosynthetic pigments in *Acetabularia*, with the usual maxima in the blue and red range [12].

Upon light-off, the voltage response appears to be (in principle) symmetric to the light-on effect: first, there is a small and fast hyperpolarization (also marked by encircling) followed by a transient depolarization below the control value, which is reached some minutes later. If the preceding light pulse has been strong and long enough, this transient depolarization becomes rather large and exhibits all-or-none characteristics. It has been described as a "metabolic" action potential because of its intimate correlation with energy metabolism [6]. The dotted curve gives the voltage response after a subthreshold light stimulus.

The fast responses are more pronounced in the cold, when the slow metabolic reponses are inhibited. Figure 2 shows an example; the resting potential is now around -80 mV and could be explained as a pure diffusion potential. However, voltages more positive than E_K can still

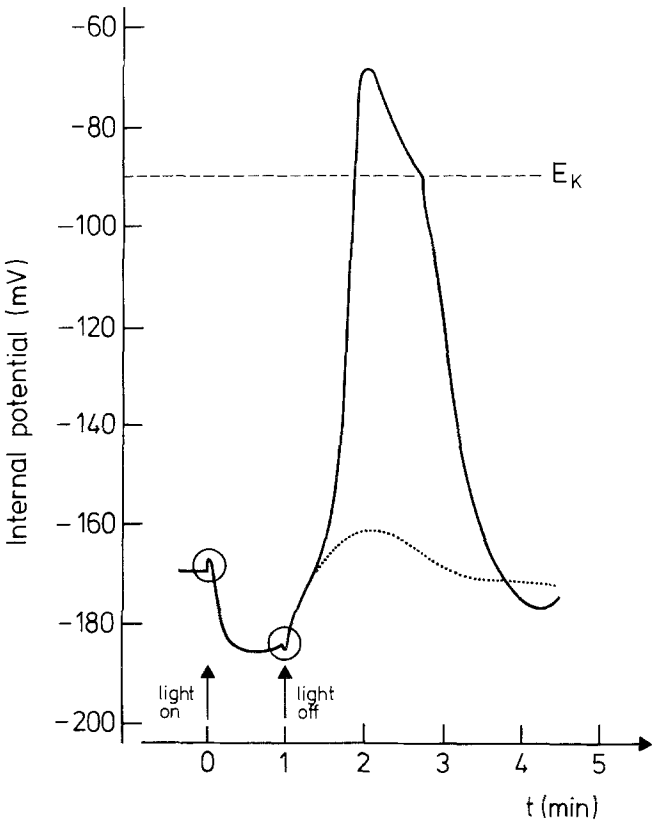

Fig. 1. Basic voltage responses of the *Acetabularia* membrane upon light-on and off. The encircled, fast responses are the main subject of this paper. Dotted line: voltage response after a subthreshold light pulse

include an active component. In Fig. 2, there is only a fast depolarization by a few mV upon the onset of light (the small overshoot is not important here; it can be shown that it is only a relaxation phenomenon of the transient from a high resistance state to a low one, according to the diode characteristics of the potassium diffusion system). Under illumination, the membrane potential stays relatively constant for a while at a more positive level; then, it begins to hyperpolarize due to photosynthesis. When the light is turned off, there is a rapid repolarization at first by about the same amount as the depolarization upon light-on (not very clear in this experiment; more pronounced in [4, 8]). This repolarization is followed by a large, transient hyperpolarization up to voltage ranges, which are again only possible with an electrogenic pump operating. The apparent asymmetry of the voltage response upon light-on and off signal is to a great extent due to the rectifying properties of

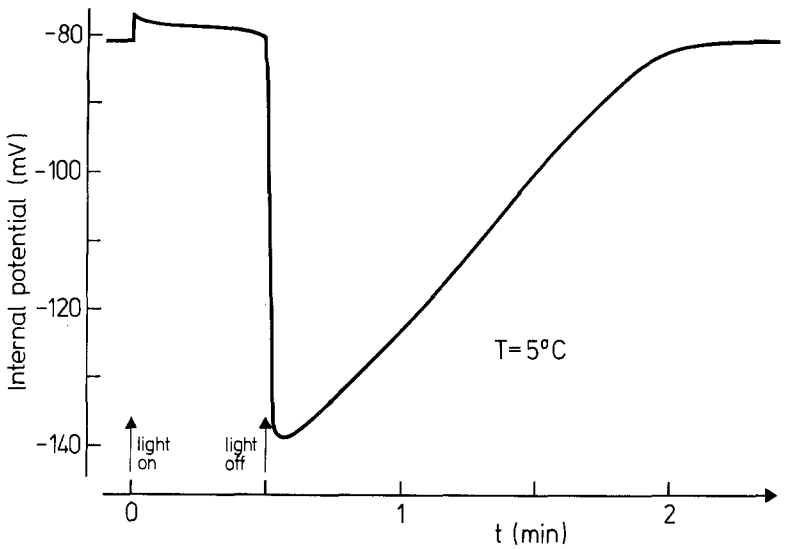

Fig. 2. Characteristic voltage response of the membrane upon light-on and off under inhibition of the energy metabolism by low temperature. Slow hyperpolarization upon light-on and slow depolarization upon light-off are drastically reduced compared to Fig. 1. Therefore, fast depolarization upon light-on and hyperpolarization upon light-off are very distinct

the passive diffusion system; under voltage-clamp conditions, the corresponding clamp currents upon light-on and off do not differ that much [7].

Action Spectra

In order to get more information about these fast effects upon light-on and off, we must investigate their sensitivity to the wavelength of the stimulating light. In many experiments, the end of the depolarization and the beginning of the negativation upon light-on cannot be separated as clearly as in Fig. 2 (*see* Fig. 1). Therefore, the absolute amount of depolarization does not seem to be a good measure for the depolarizing effect; it is not the result of one effect but of two with different signs, amplitudes and time courses. The maximum depolarization rate, however, appears to be a better measure because it can be determined while the hyperpolarizing reaction is still negligible (a similar reasoning has been applied for the slow, photosynthesis-induced hyperpolarization under normal temperatures; in this case, the rates also gave the clearer relationship rather than the absolute differences [4]). Furthermore, we shall see later that the maximum depolarization rate has the physical meaning of a membrane current in these experiments.

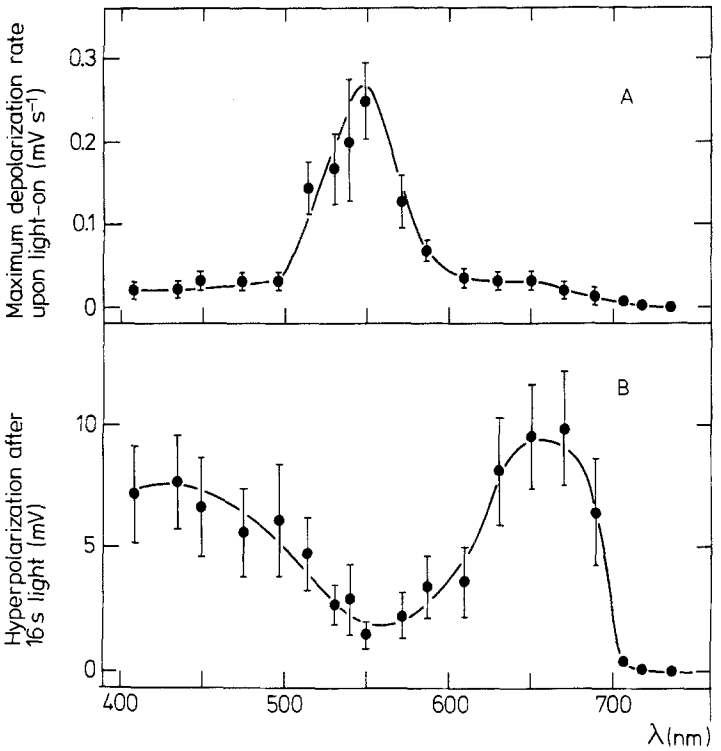

Fig. 3. Sensitivity of the fast depolarization (*A*) upon light-on and the hyperpolarization upon light-off (*B*) upon monochromatic light of different wave-length and constant photon flux density (83 μE m^{-2} s^{-1}). Choice of the coordinates is discussed in the text. Data replotted from [8]

The spectral sensitivity of the light-induced depolarization rate is given in Fig. 3*A*. There is a clear maximum near 550 nm. This spectrum is in good agreement with the absolute depolarization under normal temperatures, which has been reported to be most sensitive to 540 nm [13]. In order to decide whether this depolarization is only an unspecific heat effect of any irradiation minus the light which is absorbed by photosynthetic pigments (this could also result in a maximum around 550 nm), it is important to notice that light with wave length more than 715 nm is completely ineffective. Therefore, a simple heat effect can be excluded as the cause of the fast depolarization.

It is now of interest to see whether the hyperpolarization upon light-off is analogous to the depolarization upon light-on; in this case we would expect that the maximum hyperpolarization after irradiation would occur around 550 nm. The opposite is the case. The transient hyperpolarization upon light-off is minimum after green light and maxi-

mum after red and blue light, just like the slow light-induced hyperpolarization under normal conditions [12]. Since the time course of the transient hyperpolarization upon light-off is rather complex (details are given and discussed in previous papers [4, 8]), we took the absolute amount of the voltage peak for a convenient and qualitatively sufficient measure (Fig. 3*B*).

Time Course

In order to decide whether the light acts directly on the membrane or indirectly *via* cytoplasmic receptors, it is important to look for a possible time lag between the onset of the stimulus and the beginning of the reaction. If the receptor were about 1 μm away from the membrane, where the effect is measured, free diffusion would cause a delay of about 1 msec or more. Essential smaller delays point to the receptor being located inside the membrane itself.

The time course of the intensity of our standard maximum light flash is given in Fig. 4. The maximum intensity is reached after about 200 μsec. The intensity decreases then with a time constant of about 1 msec. The voltage response is, of course, much slower because of the low-pass characteristics of the membrane ($R_m \times C_m > 1$ msec). Therefore, the absolute depolarization, which occurs much later than the peak intensity, is not a good measure for the quantitative relationship between light and the electrical effect.

The depolarization rate seems to be a better parameter. This can be seen in Fig. 5, where some original data are presented in an expanded time scale (here, the light-coordinate is nonlinear due to the characteristics of the photodiode). The voltage response shows increasing depolarization rate as long as the light-intensity rises. Such a behavior is typical for a low-pass of first order, when the first derivative is maximum at the time of maximum input signal. These two events coincide in our experiments within the accuracy of our setup (ca. 40 μsec), which also limits the determination of a possible lag between the onset of light and the beginning of the reaction. Therefore, the finding that if there was any time lag, it must be smaller than 40 μsec is sufficient to postulate the receptor to be located in the membrane or very close (<200 nm) to it.

Figure 5 shows the superimposed tracings of five subsequent experiments plus one control tracing. The tracings of the five experiments coincide rather well. This fact can be taken for the good reproducibility, at least for measurements on the same cell.

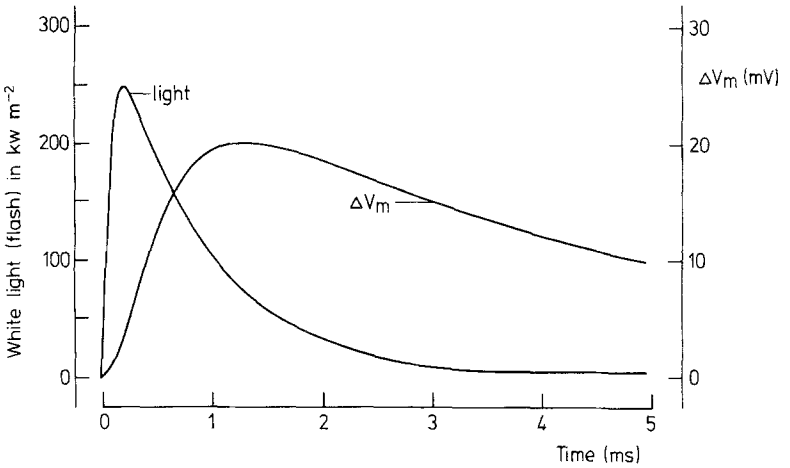

Fig. 4. Time course of standard maximum light pulse and example of the fast voltage response

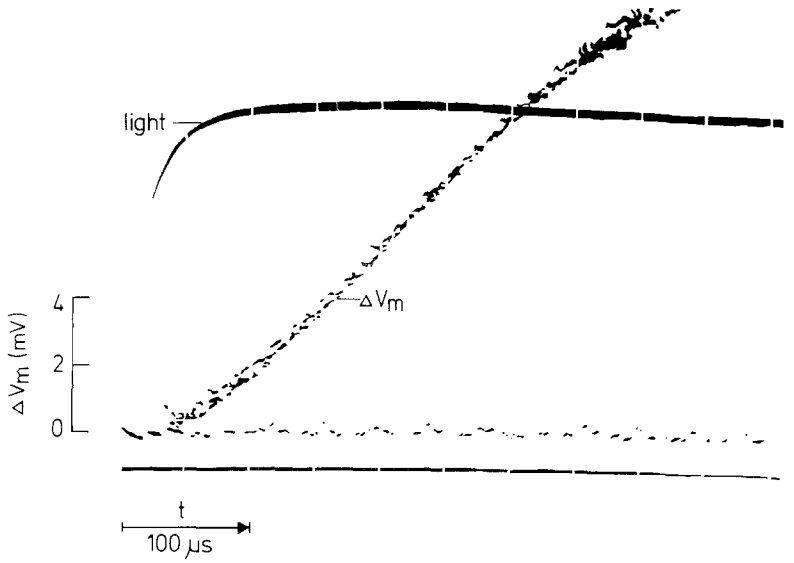

Fig. 5. Oscilloscope tracings of the light-intensity of the standard maximum light pulse (nonlinear scale, because of the characteristics of the electronic light sensor) and the initial voltage response. One control and five subsequent experiments (30-sec intervals)

Dose-Effect Relationship

In Fig. 6 we have an example of a series of experiments to determine the dose effect relationship. For final analysis, we used the maximum depolarization rate rather than the absolute peak depolarization. Fi-

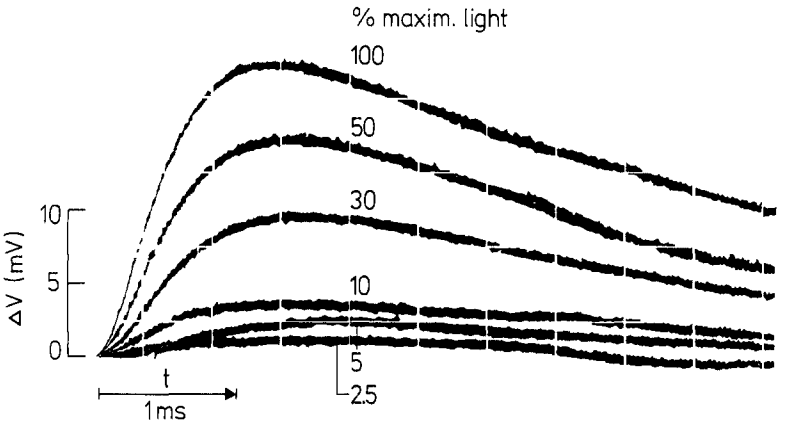

Fig. 6. Example of a series of original oscilloscope tracings for determination of the dose-effect relationship between the intensity of the light (in % of the standard maximum light pulse, not shown here) and the response of the membrane voltage

gure 7 gives a dose-effect curve over a rather wide range. The light intensities of 10^4 to 10^6 W m^{-2} were obtained by our standard maximum light flashes and neutral grey reduction filters, the intensities between 10^1 and 10^3 W m^{-2} were obtained similarly but with continuous light, because the small depolarization rates could be measured better over a longer time than just about 50 µsec (actually they were determined in the cold, when the RC-time of the membrane could be >100 msec). Since the two light sources did not have the same emission spectrum, the intensity scale had to be adjusted.

The striking result is that the dose-effect curve is proportional up to 10^6 W m^{-2} white light or (better) to 20 V s^{-1} in this experiment. In other experiments (over a smaller range) depolarization rates up to 50 V s^{-1} could be observed. In these cases, the results were fitted slightly better with an exponential function saturating around 200 V s^{-1}, rather than a linear fit.

For the real relationship between light and our electrical effect, white light is not a well defined parameter. Therefore, we calculated how many photons correspond to the transition of one elementary charge across the membrane under illumination with the most efficient monochromatic light (550 nm). We can take the data fron Fig. 3*A*. At a photon flux density of 83 µE m^{-2} s^{-1}, we obtained from the maximum depolarization rate and from the membrane capacitance a membrane current, $i_m = dV/dt_{\max} \times C_m$. This simple proportionality between $dV/dt_{\max}$ and i_m with the membrane capacitance, C_m, as the proportionality factor, de-

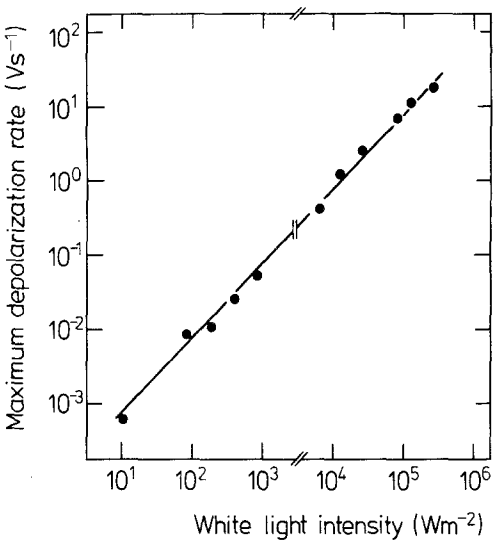

Fig. 7. Dose-effect relationship between intensity of white light and maximum initial depolarization rate. The diagram is composed of two series of experiments: intensities $< 10^3$ W m^{-2} by continuous white light, modulated by neutral grey reduction filters and a shutter; intensities $> 10^3$ W m^{-2}, peaks of light flashes modulated by neutral grey reduction filters. Intensity scales are adjusted for different emission spectrum of the two light sources

rives from the time course of the voltage drop across a simple membrane model consisting of a resistor and a capacitor in parallel, upon constant current pulses; in this case, the initial voltage change only depends on the capacitance and not on the resistance. So we calculate a green-light-induced membrane current of 1.25×10^{-5} A m^{-2} per 83 μE m^{-2} s^{-1} with a known C_m of ca. 5×10^{-2} F m^{-2} [5].

Photometric measurements on cell segments with depleted chloroplasts (by gentle centrifugation the plasmalemma stays electrically intact) did not reveal an extinction at a resolution of 10^{-3} at 550 nm. So, not more than 10^{-3} of the irradiated photons (550 nm) can be absorbed by the *Acetabularia* membrane. With this number and the calculated current, we can figure an efficiency of maximum 10^3 absorbed photons per charge transported.

External Medium

In order to know which ions are involved, we changed the external medium. Among the cations of seawater, none turned out to be essential for the light-induced fast depolarization because, even in "pure" choline-

chloride solution with the osmolarity 1.1 osmol of the seawater, the effect did not differ significantly from the effect under control conditions. It should be mentioned here again, that in such a choline chloride environment, *Acetabularia* cells can basically show the same electrophysiological properties, including high resting potential (even a little more negative due to the lack of external K^+) and action potentials, as under normal conditions [4].

In many systems the external pH has turned out to be very important for the electrical membrane properties, especially when electrogenic pumps are involved. In *Acetabularia*, such an influence of the external pH on the membrane properties could not be found (that is one reason why it has been concluded that Cl^- is transported electrogenically into the *Acetabularia* cell). To make sure that the fast, light-induced depolarization is also pH insensitive, we carried out experiments like that presented in Fig. 2, varying the external pH between 6 and 9. No effect of the pH could be observed, either on the resting potential or on the depolarization upon light-on or on the hyperpolarization upon light-off. Only an insignificant maximum at pH 8 (2 mV) could be observed for the light-induced depolarization.

Voltage Dependence

It is known from previous investigations that the membrane properties of *Acetabularia* are very voltage sensitive. Therefore, we applied our standard maximum light pulse under different membrane voltages. In a first series of experiments this has been achieved by triggering action potentials. Figure 8*B* gives an example of an action potential during the time course of which light flashes have been applied. The resulting fast voltage responses are given in Fig. 8*A*. The different states of the membrane potential are marked in Fig. 8*A* and *B* by numbers.

Two observations are significant: the amplitude is rather voltage sensitive, but not the maximum depolarization rate. The dependence of the voltage amplitude on the actual membrane voltage is very similar to the voltage response upon injected constant current pulses. For voltages more positive than E_K, the diffusion properties yield a high conductance state of the membrane due to the high internal potassium concentration and the high potassium permeability; for voltages more negative than E_K, the passive diffusion processes yield a low conductance for the membrane; however, the electrogenic pump pathway becomes now more

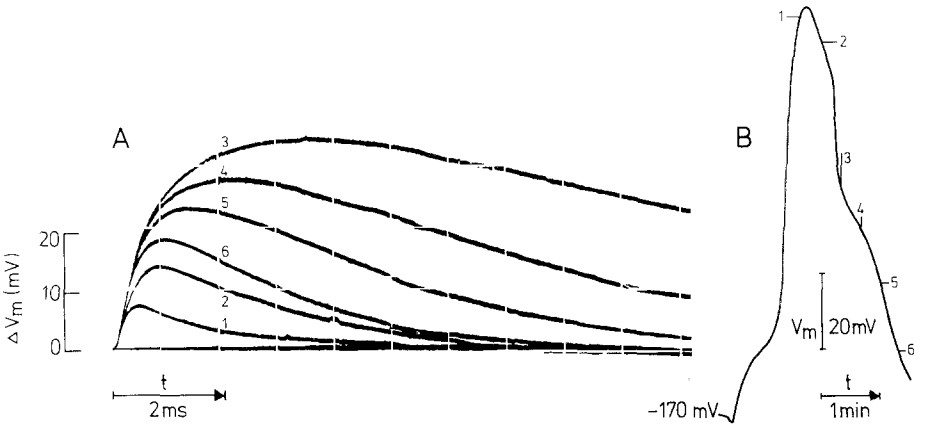

Fig. 8. Example of a series of original oscilloscope tracings for determination of the voltage sensitivity of the fast, light-induced depolarization. (*A*): voltage responses in different states of an action potential, marked in *B*

conducting up to its electromotive force near -190 mV, from whence the pump conductance decreases again. So, we have a conductance minimum between -190 and -90 mV [5]. The apparent amount of the fast, light-induced depolarization just follows this voltage dependence of the membrane conductance.

The apparent constancy of the depolarization rate strongly confirms the idea of light acting *via* a current source. As we have mentioned before, this initial voltage change does not depend on the membrane resistance but only on the membrane capacitance, which is constant, and the membrane current.

More careful readings of the maximum depolarization rates, however, give steeper increases for more negative membrane potentials. Better data should be expected from voltage clamp experiments, by which the investigated voltage range can be made much wider than given by the natural limits of an action potential in *Acetabularia*. Figure 9 shows some original tracings of a voltage clamp experiment. It is important to notice that the voltage clamp circuit is not ideal. Under clamp conditions, there are still small initial voltage changes produced by the light flashes. Therefore, the initial rising rate of the apparent clamp current does not represent the time course of the electrical membrane properties under ideal voltage clamp conditions. At the time of maximum current, however, the membrane voltage seems to be really close to the desired level. By this time the intensity of the light pulse is already decreasing. So, these peak values of $V_0 - V_1$, which should represent the fast, light-induced

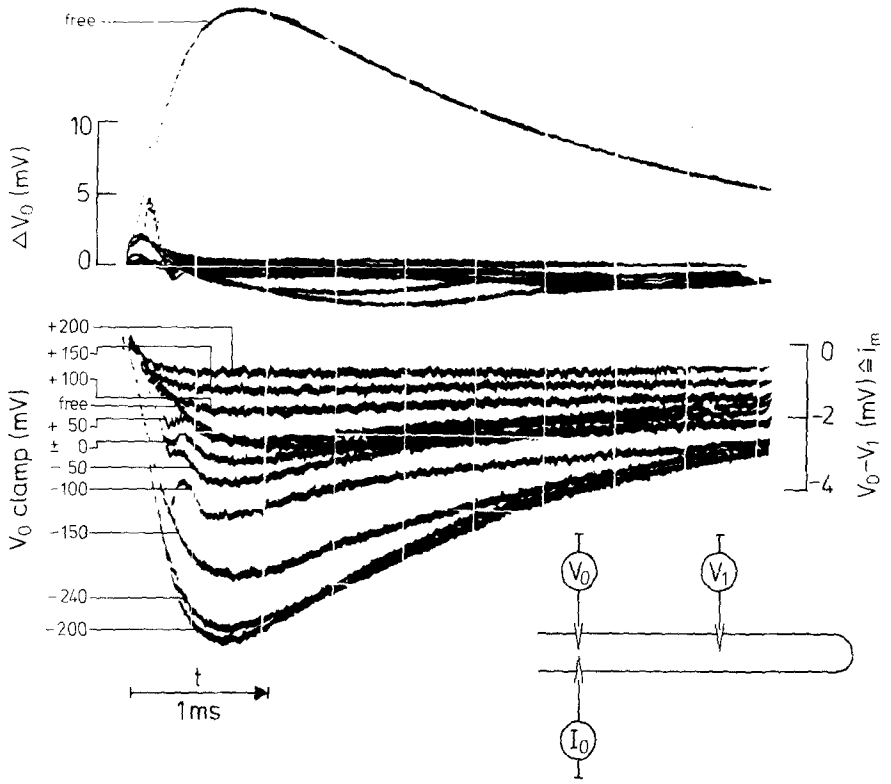

Fig. 9. Voltage clamp experiment, electrode arrangement in inset, $V_0 = V_{clamp}$. *Upper series:* voltage change of V_0 under clamp conditions and free running upon a standard maximum light pulse. *Lower series:* time course of change of $V_0 - V_1 \sim i_m$ upon light pulse under different clamp levels of V_0, marked on individual curves in mV; *Free:* $V_0 - V_1$ change upon light-pulse without clamp

membrane currents, are only relative (smaller) values with respect to the peak light intensity. Nevertheless, $V_0 - V_1$ can be used as a relative measure for the fast, light-induced membrane current.

Under free running voltage, the difference $V_0 - V_1$ is not constant upon the onset of light (Fig. 9, lower part). Obviously, the cells become slightly more sensitive towards the appex. It can be shown that these differences between the places 0 and 1 would not influence the correct reading of that membrane voltage, where the clamp current would change its sign.

In analogy to the voltage clamp experiments with the squid axon, where the clamp current during an excitation does reverse its sign at the equilibrium potential of Na^+, we expected to find such a voltage in our experiments, which would give some information about the ions in-

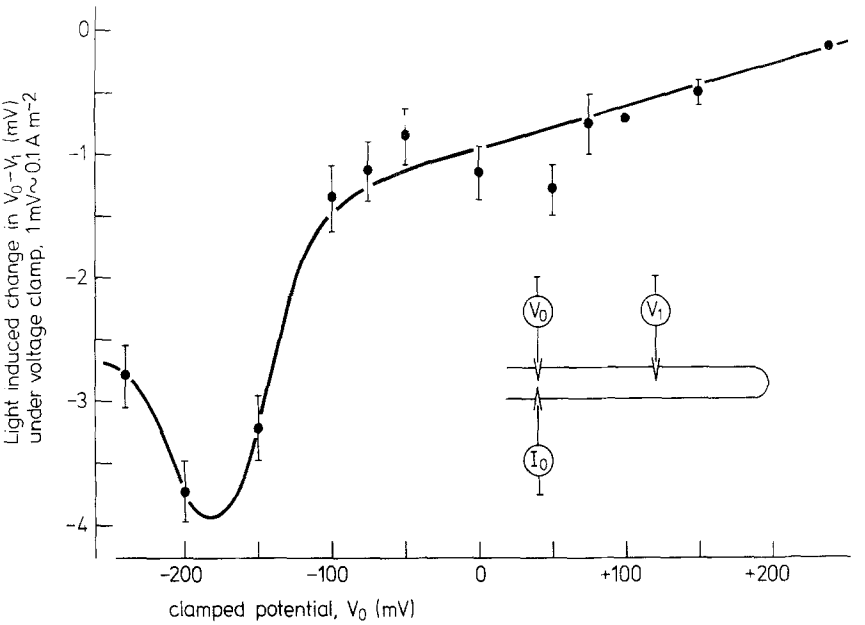

Fig. 10. Current-voltage relationship of the fast, light-induced pathway, obtained from voltage clamp experiments with the electrode arrangement given by the inset; $V_0 = V_{clamp}$; average data ±SEM from 9 experiments (different voltage ranges); peak currents, about 1 msec after peak intensity of light

volved. For instance, with respect to the experiments with different external medium, Cl^- could still be a candidate. In this case the clamp current should reverse at about zero potential, since the equilibrium potential of Cl^- in *Acetabularia* is about 0. In our experiments (Figs. 9 and 10) there is, however, still a clear light-induced inward current at ground potential. Up to +200 mV, we observed inward currents; more positive clamp levels could not be achieved for technical reasons.

In Fig. 10 the data of several experiments are summarized. In these experiments the investigated voltage range has not been always the same; only for a few experiments was the entire voltage range investigated. Therefore, Fig. 10 has somewhat restricted statistical significance. One finding, however, is absolutely reproducible: At a membrane voltage of around −190 mV, the light-induced currents are maximum; for more negative voltages, the light-induced currents decrease again. This finding will be relevant for the discussion, because it is also at −190 mV that the electrogenic pump has its electromotive force and its maximum slope conductance.

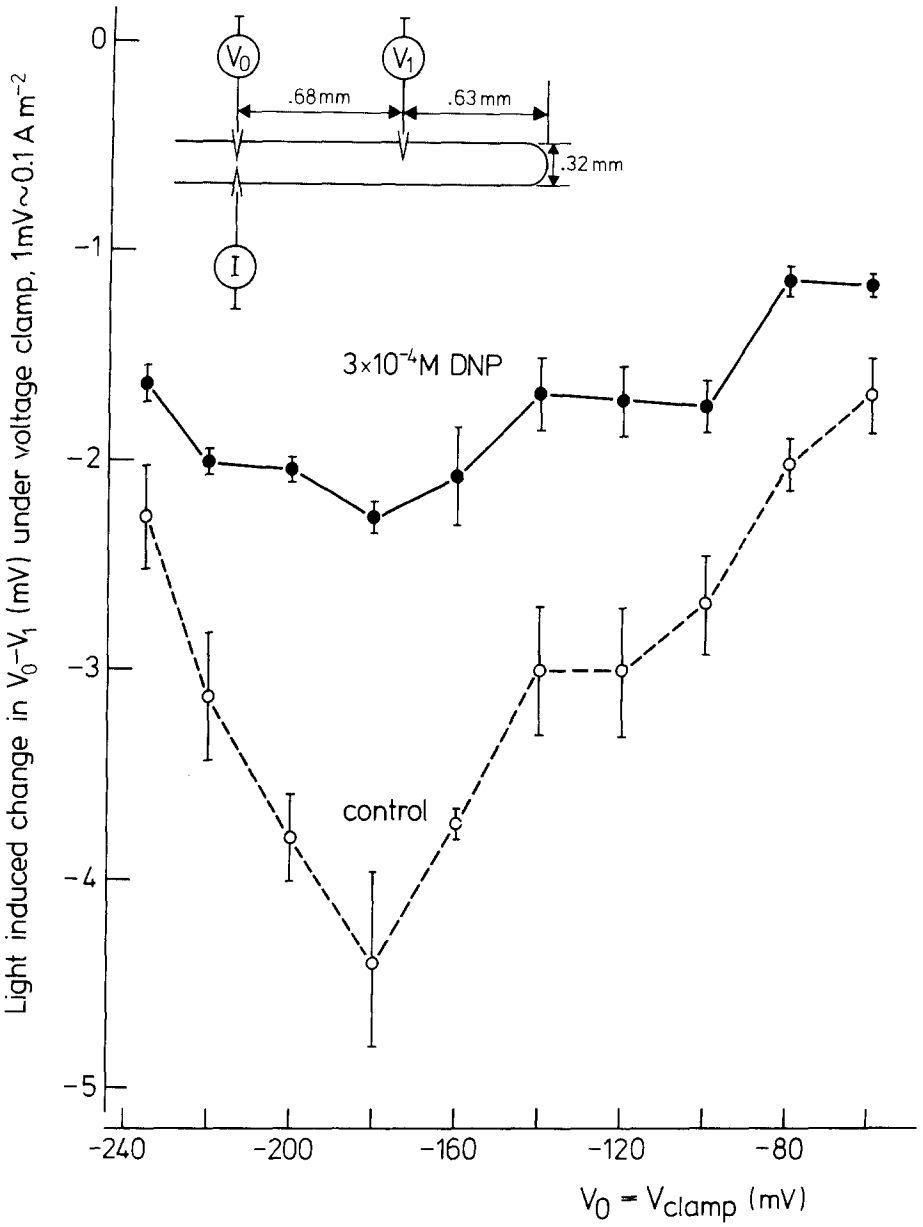

Fig. 11. Comparison of the current-voltage relationship of the fast, light-induced channel under inhibition of the energy metabolism by 3×10^{-3} M 2,4-dinitrophenol and under control conditions; each point at least 3 measurements, ±SEM, all measurements from one cell. Inset: electrode arrangement

Metabolic Dependence

For reasons which will be obvious in the discussion, we were interested in the role of the energy metabolism for our fast photoelectric

response. Therefore, we compared the current-voltage relationship of the fast photoelectric effect under normal conditions with the relationship under inhibition of the energy metabolism by the uncoupler 2,4-dinitrophenol (DNP). The corresponding set of experiments (Fig. 11) is basically similar to those shown in Figs. 9 and 10; but with a smaller voltage range and finer voltage intervals. It is evident that under high energy conditions the light-induced currents are much larger than in a low energy state of the cell.

Discussion

Specific Conclusions

Before proposing and discussing a model for the fast, light-sensitive channel in *Acetabularia*, some direct interpretations of the results should be stated:

The velocity of the phenomenon (Figs. 4–6) suggests that the photoreceptor is located in, or very close to, the plasmalemma membrane.

The overshoot reaction of the electrogenic pump after depolarization with green light (Figs. 2 and 3) points to an inhibition of the pump by green light (550 nm). During this inhibition, energy equivalents (perhaps ATP) are accumulated, which normally fuel the electrogenic pump; after release of this inhibition by green light, these accumulated energy equivalents are now available for a temporary overshoot reaction of the pump. The amount of this overshoot (Fig. 3*B*) depends, of course, on the energy metabolism; therefore, its action spectrum goes well with the action spectrum of photosynthesis.

The requirement of 10^3 photons per transfer of one elementary charge is a maximum estimate. However, without an idea about the concentration and the molecular weight of the receptor in the membrane, further conclusions would be premature. The situation with the striking proportionality of the dose-effect relationship up to very high intensities is similar; qualitatively, we can assume a very short lifetime of the excited state of the receptor, probably in the range of a μsec.

From the current-voltage relationship of the light-induced pathway we can obtain more information than from our dose effect curves. The maximum light-induced current occurs at about the same voltage as the electromotive force of the electrogenic pump (-190 mV); at this voltage the pump also has maximum slope conductance. It is therefore likely that the electrogenic pump and the fast, light-induced channel are closely linked.

The equilibrium potential of the fast, light-induced pathway must be considerably more positive than +200 mV; however, none of the ions of seawater (including H^+ or OH^-) seem to be essentially involved. If the ions outside the membrane are apparently not involved, we must assume the role of ions "just-inside" the membrane, like lipophilic ions, which can move in the membrane but not leave it.

General Hypothesis

All these findings point to the role of the electrogenic pump in the membrane. Therefore, we need to examine actual models of electrogenic pumps to find a consistent interpretation of our results. A model of the action of the electrogenic Cl^- pump in *Acetabularia* has been proposed [4]. This model is given in Fig. 12, slightly modified according to present conventions for such models of membrane transport [14]. This model should work as follows: The electro-neutral complex, $X\,Cl$, between the carrier X^+ and the substrate Cl^- is able to pass the membrane. Inside the cell, $X\,Cl$ changes into the unstable form $Y\,Cl$ by ATP hydrolysis. This form decomposes to Cl^-, which enters the intracellular compartment, and the unoccupied, charged carrier in the excited state Y^+, which can pass the membrane. Outside, Y^+ falls back to the unexcited state X^+, which will bind another Cl^- from the extracellular compartment. The reverse reaction should, of course, also be possible. The described direction of the cycle only represents the normal operation of the pump. The hypothesis, which should explain our phenomena, is as follows.

In the *Acetabularia* membrane, light of 550 nm wavelength directly causes a permeability increase for the unoccupied, positively charged carrier molecules of the electrogenic pump in their Cl^- binding low energy state X^+.

Qualitatively, this would explain our findings: If the unoccupied carrier passes the membrane from outside to inside, the electrogenic cycle is interrupted; inside, a deficit of $X\,Cl$ will occur; so that energy equivalents, which are not used any more to energize $X\,Cl$ to $Y\,Cl$, can be accumulated. After closing of the electrogenic cycle by light-off, these accumulated energy equivalents can cause a transient overshoot of the pump action.

In the simple model of Fig. 12, X^+ is found only on the outside; inside it is not defined but assumed to be at a very low concentration. Such a large concentration difference would yield a very positive equilib-

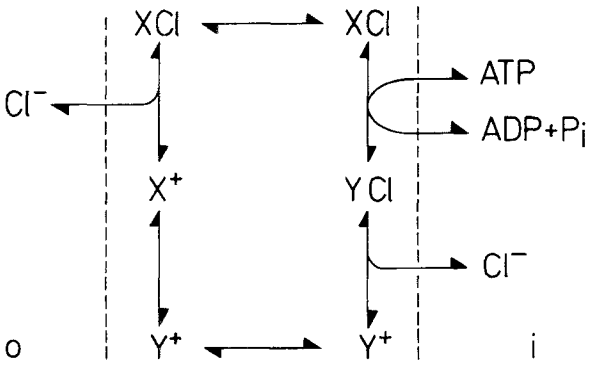

Fig. 12. Simplified model of a possible electrogenic Cl^- pump in the *Acetabularia* membrane according to [4]

rium potential, E_{X^+}. The only other charged particle of the model which passes the membrane is Y^+. The equilibrium potential of Y^+, however, must be rather negative in order to permit the flux of Y^+ from inside to outside under normal operation of the electrogenic pump. Therefore, only X^+ is left to cause the fast, light-induced charge transport.

A theory for a carrier mediated electrogenic pump [3] yields a current-voltage relationship with saturated current for large voltage displacements from its intrinsic electromotive force, because of limited sites of the carrier. Maximum slope conductance of the pump indicates a maximum of unoccupied carrier sites. This property is found for the element $P1$ in the analog circuit of the *Acetabularia* membrane; the maximum slope conductance here is at a membrane voltage of -190 mV, which is also the electromotive force of the pump. It is, in fact, at the very membrane voltage where the fast, light-induced membrane current is maximum.

Quantitative Treatment

For final discussion of our model, we must know whether it works not only on the basis of plausibility, as we have treated it so far, but on a quantitative basis, as well. For this purpose, we wrote the model from Fig. 12 explicitly with all necessary compounds and reaction constants (Fig. 13). In this case we have to define the concentration N'_{X^+} (mol m^{-2}) of the unoccupied carrier for the inside of the cell, as well. We also must regard the possible spontaneous transition between X^+ and Y^+ inside and the dissociation (K_{DX}) respectively association (K_{ClX}) between Cl^- and X^+ inside.

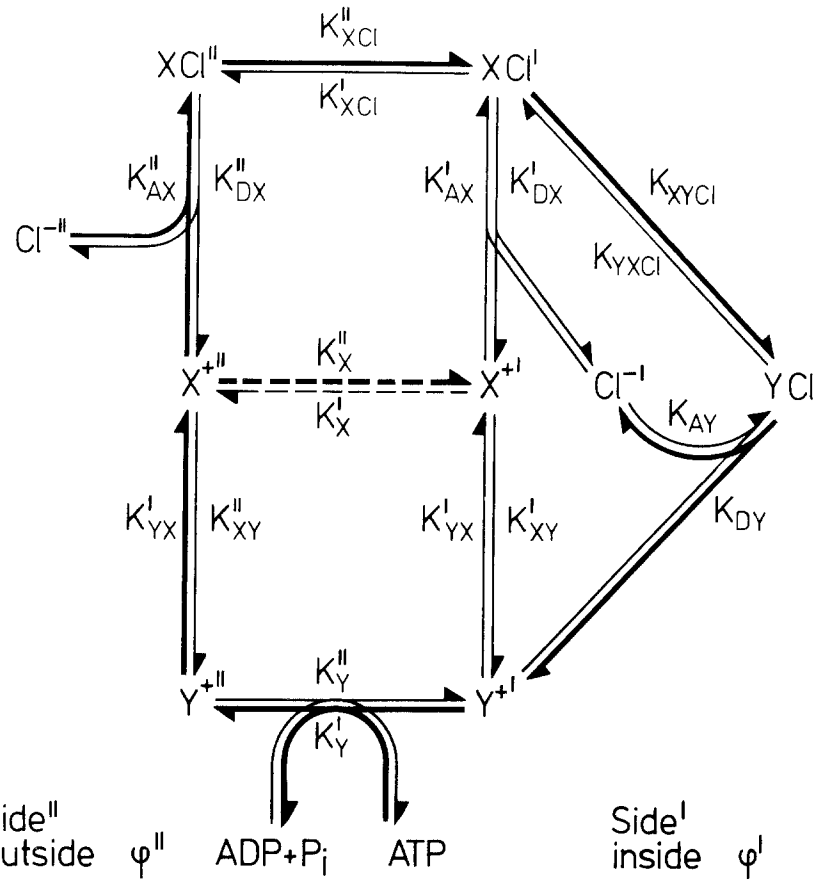

Fig. 13. Detailed model of the electrogenic pump in the *Acetabularia* membrane. Fat arrows: ordinary direction; active reaction here is voltage-dependent

The transport of a substance from one side of the membrane to the other can also be characterized by a rate constant [11]. For electrically charged particles, in our case X^+ and Y^+, the rate constants become sensitive to the membrane voltage $V_m = uF/RT$, where R, T and F have their usual meaning and u is an expression without dimension for the membrane voltage. If $\overline{K}'_X$, for instance, is the rate constant for the transition of X^+ from inside to outside at zero voltage, the voltage sensitivity of K'_X could be $\overline{K}_X \exp(u/2)$ [11]. The formal rate constants of those reactions which involve another compound (like ATP or Cl^-) contain the particular concentration (mol m^{-3}) as a factor. In the case of a steady state, the concentrations N (mol m^{-2}) of the seven states of the carrier (XCl'', XCl', YCl, $Y^{+'}$, $Y^{+''}$, $X^{+''}$ and $X^{+'}$) do not change in

time. For this case we can write seven equations of the type:

$$0=\frac{dN''_{X\mathrm{Cl}}}{dt}=-K''_{X\mathrm{Cl}}N''_{X\mathrm{Cl}}+K'_{X\mathrm{Cl}}N'_{X\mathrm{Cl}}-K''_{DX}N''_{X\mathrm{Cl}}+K''_{AX}N''_{X} \tag{1.1}$$

$$0=\frac{dN'_{X\mathrm{Cl}}}{dt}=-K'_{DX}N'_{X\mathrm{Cl}}+K'_{AX}N'_{X}-K'_{X\mathrm{Cl}}N'_{X\mathrm{Cl}}+K''_{X\mathrm{Cl}}N''_{X\mathrm{Cl}} \tag{1.2}$$

$$\vdots$$

$$-K_{XY\mathrm{Cl}}N'_{X\mathrm{Cl}}+K_{YX\mathrm{Cl}}N_{Y\mathrm{Cl}}. \tag{1.7}$$

We can assume numerical values for the 18 rate constants; then we have a homogeneous system of 7 equations and 7 unknown concentrations. With the assumption that the sum of all concentrations N is constant, we can eliminate one of the seven equations (we took $dN_{Y\mathrm{Cl}}/dt$) and obtain an inhomogeneous system of 6 equations, which can be solved numerically by computer.

The electrical current across our model is then:

$$i_m=F(K'_XN'_X-K''_XN''_X+K'_YN'_Y-K''_YN''_Y) \quad \text{ind A m}^{-2}. \tag{2}$$

The voltage dependence of the 4 rate constants involved has been given before. So we can obtain a current-voltage relationship of the system for any set of the 18 rate constants (the voltage sensitive ones first set for zero voltage).

In order to simulate first the S-shaped current-voltage relationship of the element $P1$, we started with choosing the same value for all the rate constants of the normal electrogenic cycle (marked by fat arrows in Fig. 13) and for the reverse reactions; the transfer of X^+ is not allowed for control conditions; and for X' the rate constants are chosen to yield a low concentration N'_X. In this case we obtain a sigmoid $I-V$ relationship, which is symmetrical to the origin. The limiting current is determined by the scaling factors $\sum 1/K_i$ and the total concentration of the carrier.

If we set the active step (rate constants for both directions unequal) for a voltage insensitive reaction, like $K_{XY\mathrm{Cl}}$, the transition from $X\mathrm{Cl}$ to $Y\mathrm{Cl}$ inside, as in the preliminary model in Fig. 12, the current-voltage relationship is shifted along the current axis out of the origin. This is not, however, the current-voltage relationship of the electrogenic pump in *Acetabularia;* this current-voltage relationship is found to be shifted along the voltage axis out of the origin (by -190 mV). This property can be obtained in our model if we choose the active reaction to be voltage-sensitive; for the normal operation of the electrogenic pump, this must be

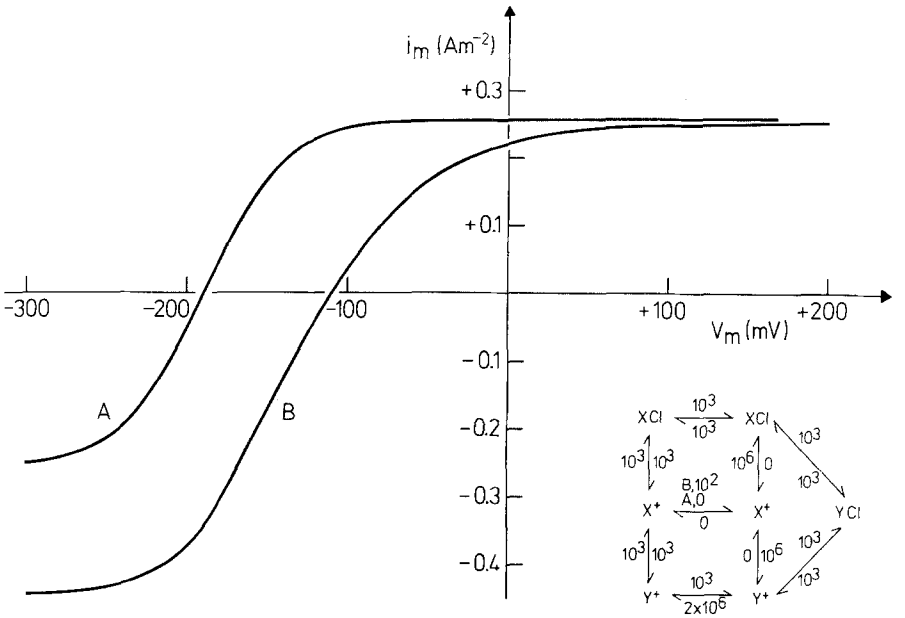

Fig. 14. Current-voltage relationship of the model (Fig. 13) of the electrogenic pump with the rate constants as given by the inset. Total concentration of the carrier: 4×10^{-9} mol m^{-2}. (*A*): control; (*B*): under illumination

the transfer of Y^+ from inside to outside (K'_Y) as formulated in the explicit model in Fig. 13.

With this model we can now simulate the sigmoid current-voltage relationship of the electrogenic pump under control conditions (Fig. 14 *A*), when the transfer of X^+ from outside to inside is impossible ($K''_X = 0$) and for the assumed case, that light enables the transfer of X^+ from outside to inside ($K''_X > 0$); the resulting current-voltage relationship for this case is given as curve *B* in Fig. 14. The current difference between the two curves should give the current-voltage relationship of the light-induced channel. As shown by the experiments and theoretical curve in Fig. 15, there is a good coincidence between the measured data and the theoretical curve.

In order to check the possibilities and limits of our model, we did our experiments under low energy conditions, i.e., under 3×10^{-4} mol m^{-3} of the uncoupler 2,4-dinitrophenol in the medium. For this situation we expect a smaller intracellular ATP concentration. So the rate constant of K'_Y should be smaller. We repeated the simulation procedure with exactly the same parameters as for the control but K'_Y reduced by the factor 200. The dashed curve in Fig. 15 is the theoretical result for the current-voltage relationship of the fast, light-induced channel under low energy

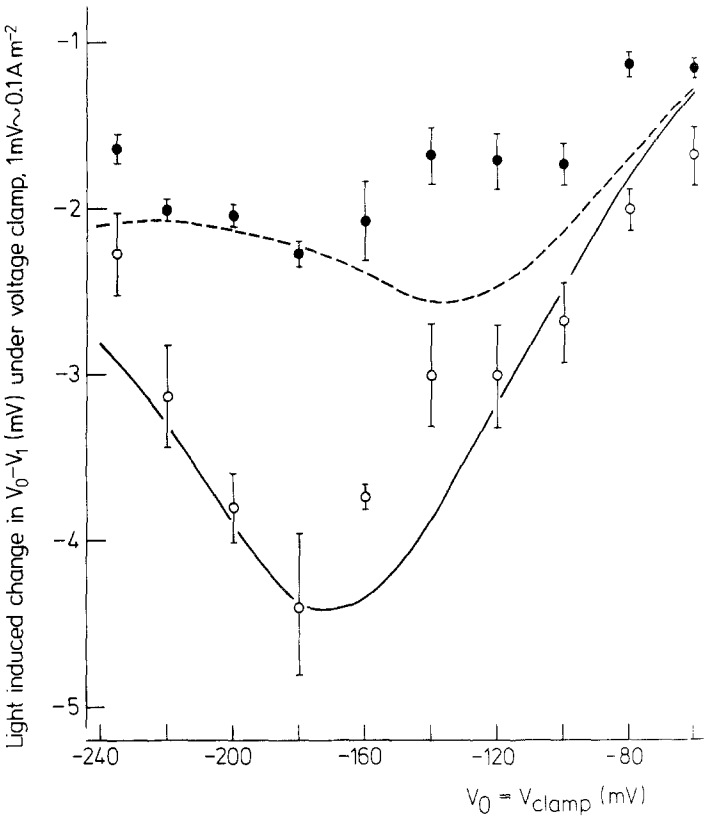

Fig. 15. Open and filled symbols ±error bars, data from Fig. 10. *Solid curve:* difference between the two curves $B-A$ from Fig. 14; theoretical current-voltage curve for the fast, light-induced pathway under normal state of the energy metabolism. *Dashed curve:* same curve as solid one but $\bar{K}'_Y = 10^4\,s^{-1}$ instead of 2×10^6, for simulation of low energy state

conditions; the corresponding measured data are given by the filled symbols with error bars.

The coincidence for the low energy conditions is fair but not as good as for the control experiment. The theoretical curve shows a shift of the current peak to more positive voltage. This shift cannot be seen in the experimental curve where the current peak appears at about the same voltage as in the control experiment. The experimental results under DNP look rather like a decrease by a scaling factor which can arise from about any pair of rate constants. Therefore, we would not be surprised, if the real mechanism should turn out not to be as simple as described in this first approach. The basic results, however, give rise to a feeling of being on the right track.

This paper is dedicated to Noe Higinbotham on the occasion of his 65th birthday.

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