1 L. ERNSTER AND S. ORRENIUS, *Federation Proc.*, 24 (1965) 1190.
2 G. C. MUELLER AND I. A. MILLER, *J. Biol. Chem.*, 202 (1953) 579.
3 M. L. DAS, S. ORRENIUS AND L. ERNSTER, *European J. Biochem.*, 4 (1968) 519.
4 K. ICHIHARA, E. KUSUNOSE AND M. KUSUNOSE, *Biochim. Biophys. Acta*, 176 (1969) 704.
5 H. S. MASON, *Advan. Enzymol.*, 19 (1957) 79.
6 D. Y. COOPER, S. NARASIMHULU, O. ROSENTHAL AND R. W. ESTABROOK, in T. E. KING, H. S. MASON AND M. MORRISON, *Oxidases and Related Redox Systems*, Wiley, New York, 1965, p. 838.
7 R. SATO, T. OMURA AND H. NISHIBAYASHI, in T. E. KING, H. S. MASON AND M. MORRISON, *Oxidases and Related Redox Systems*, Wiley, New York, 1965, p. 861.
8 M. RAFTELL AND P. PERLMANN, *Exptl. Cell Res.*, 57 (1969) 119.
9 O. H. LOWRY, N. I. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
10 I. BOOTH AND E. BOYLAND, *Biochem. J.*, 66 (1957) 73.
11 S. ORRENIUS, J. L. E. ERICSSON AND L. ERNSTER, *J. Cell. Biol*, 25 (1965) 627.
12 G. DALLNER, P. SIEKEVITZ AND G. E. PALADE, *J. Cell Biol.* 30 (1966) 97.
13 G. DALLNER, *Acta Pathol. Microbiol. Scand. Suppl.*, (1963) 166.
14 U. LUNDKVIST AND P. PERLMANN, *Immunology*, 13 (1967) 179.
15 S. ORRENIUS, A. BERG AND L. ERNSTER, *European J. Biochem.*, 11 (1969) 193.

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Application of the voltage-clamp technique for measuring the quantum efficiency of light-induced potential changes in *Nitella translucens*

It has been shown[1] that in algal cells, like *Nitella translucens*, the negative potential across the two cellular membranes and the cell wall reversibly changes upon illumination, and that these changes are caused by photosynthetic energy conversion in the two pigment systems of the chloroplast-localized photosynthetic apparatus. It has been suggested[1] that the potential changes primarily are caused by changes in the cytoplasmic proton concentration due to electron transport coupled uptake of protons by the chloroplast in the cytoplasm.

This communication reports on experiments with *Nitella translucens*, energized by monochromatic light, under conditions at which the membrane potential is continuously clamped at the potential in the dark. This voltage-clamp technique, widely applied in research on changes in ionic conductance underlying action potentials in excitable membranes of various organized structures of living organisms (*cf.* refs. 2–5), enables the measurement of the efficiency of light quanta for changing the membrane potential under controlled steady-state light conditions.

Nitella translucens was collected from a fresh water pool in the Netherlands. Cells were bathed in artificial pond water, containing 1.0 mM NaCl, 0.1 mM KCl and 0.1 mM $CaCl_2$ and were kept growing at 15° in a conditioned growth room under weak light in a light–dark regime of 12h each. For the experiments, young fresh grown cells about 5–8 cm in length and 0.5 –0.8 mm in diameter were used. Measurements were carried out at room temperature. Fig. 1 shows a schematic diagram of the voltage-clamp arrangement. An increase in membrane potential (ΔV) brought about by actinic illumination causes a current (I) which flows in the feed-back

circuit, *i.e.* an inward current passing across the cellular membranes. This current controls the dark potential by causing a compensating potential difference ($-\Delta V$) across the membranes. The tips of the internal electrodes were in the central vacuole of the algal cell which means that the responses measured are those of the complex series membrane system, *i.e.* cell wall, plasmalemma and tonoplast. An area of 8 mm in length of the central part of the cell was illuminated by a monochromatic 435-nm light beam, transmitted by an interference filter (band width 10–15 nm). Light intensities were measured by a photocell, calibrated with a thermopile.

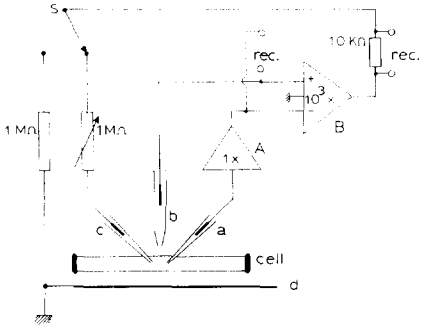

Fig. 1. Diagram of the experimental arrangement for measuring light-induced changes in membrane potential and in membrane current when the membrane potential is continuously clamped at the potential in the dark. The membrane potential is measured using a 3 M KCl-filled glass capillary microelectrode (a) inserted transversely into the Nitella cell at its midpoint, in contact with an Ag–AgCl wire *via* an agar–KCl bridge, and an identical Ag–AgCl wire in agar–3 M KCl, bathed in the external medium, acting as reference electrode (b). The electrode voltage is fed into a high-input impedance unity gain solid-state amplifier (A, IL Picrometric Amplifier, Model 181). The output of amplifier A is compensated by an adjustable d.c. voltage and is connected either to a recorder (Beckman, Model 1005), or when the membrane potential is brought under feed-back control (voltage clamp), to the differential input of a high gain ($10^3 \times$) d.c. oscilloscope amplifier (B, Tektronix 502). The output of amplifier B is connected *via* a series resistor of 10 kΩ and a variable resistor of 1 MΩ to an Ag wire in a 3 M KCl-filled micro-glass capillary, inserted into the cell at a distance of about 1–2 mm of the internal voltage electrode and acting as internal current electrode (c). A grounded Ag wire placed along the length of the cell in the external medium acts as the external current electrode (d). The current is measured as the voltage drop across the 10-kΩ resistor on the recorder. The accuracy of the feed-back control is adjusted by the variable 1-MΩ resistor. The response time of the system is about 20 msec, but in the present experiments, it is limited by the 1-sec responce time of the recorder. When recording the change in membrane potential, the output of amplifier B is grounded *via* a 1-MΩ resistor by means of the switch S.

Fig. 2 shows the recordings of the change in membrane potential (a) and the controlling inward current when the membrane potential is clamped at the dark potential (b) upon illumination with 435 nm light of a young fresh cell suspended in artificial pond water (pH 6) with 1 mM $CaCl_2$ added (further referred to as Ca^{2+}-containing artificial pond water). At intensities below 2 nEinsteins/cm^2·sec, the rate and extent of the changes are linearly dependent on the intensity of the activating light. From such experiments, the quantum requirement for the number of equiv which need to be transported per sec across the cellular membranes to keep the membrane potential in the light at the dark level, can be calculated. In the experiment illustrated in Fig. 2, a cell with a diameter of 0.06 cm was illuminated with 435-nm light of 0.33 nEinstein/cm^2·sec intensity over a length of 0.8 cm.

The efficiency of 435-nm quanta relative to that of 663-nm quanta was estimated to be about 0.55, and was somewhat higher than had been reported previously[1]. The absorption of this cell at 435 nm was approx. 0.5. Thus, an energy flux of 4.3 pEinsteins/sec was effective in the energy conversion process. The number of transported equiv/sec in steady-state light conditions is equal to I/F, in which I is the controlling current in A and F, the Faraday constant equal to $9.6.10^4$ C/equiv. The experiment illustrated shows a transport of $5.7.10^{-13}$ equiv/sec in the steady state. The quantum requirement then is calculated to be 7.5 per equiv. For a large

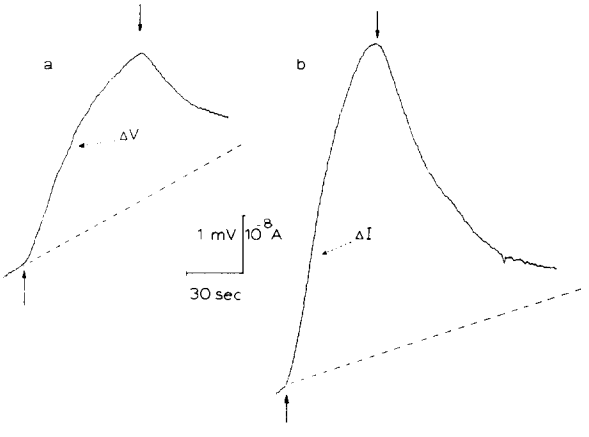

Fig. 2. Kinetics of the light-induced change in membrane potential (a), and in inward current under voltage clamp (b) of a single internodal cell of *N. translucens*, bathed in Ca^{2+}-containing artifical pond water. The dark potential of the cell was −115 mV. Upward and downward pointing arrows mark the beginning and the end, respectively, of an illumination period. The intensity of the light of wavelength 435 nm was 0.33 nEinstein/cm^2·sec.

number of different cells, values of the quantum requirement in the range between 5 and 15 were found. These results give support for the hypothesis that the light-induced potential changes are primarily caused by, or are related to, changes in cytoplasmic ion concentration(s) due to light-driven ion uptake by the chloroplasts. It is reasonable to assume, in view of current knowledge on light-induced ion uptake in isolated chloroplasts[6], that protons are primarily involved. It has been established[7,8] that in isolated chloroplasts, electron transport coupled H^+ uptake proceeds with an H^+/e ratio of 2, which indicates a quantum requirement of 1. There is as yet no direct experimental proof that the membrane potential responds to changes in cytoplasmic H^+ concentration. It has been reported[9,10] that in the presence of Ca^{2+} in the external medium the plasmalemma of *N. translucens* behaves much less as a K^+-diffusion barrier than it apparently does in Ca^{2+}-depleted or low Ca^{2+} media. Recently, it has been shown for Nitella[11] that in a Ca^{2+}-enriched medium, in the pH range below 7, the membrane predominantly behaves as an apparent H^+-diffusion barrier. Our finding that a 10-fold increase in $CaCl_2$ concentration in the usually used artificial pond water (containing 0.1 mM $CaCl_2$) caused an improvement in the efficiency of the light reaction by a factor of 2–3 is consistent with this. For a few cells bathed in Ca^{2+}-containing artificial pond water, the change in the membrane potential in the dark upon changing the external pH from 6 to 5, and *vice*

versa, was measured simultaneously with the quantum requirement for the light-induced ΔV. The quantum requirement, although different in different cells, was found to be constant in the pH range 5–6. The results indicate that under the experimental conditions, the relatively large variations in the quantum requirement between different cells were due to variations in the property of the membranes to respond to changes in the pH of the external Ca^{2+}-containing artificial pond water medium. The experiments showed that the lowest quantum requirements were measured under conditions at which the change in the dark potential per unity change in pH was at best 14 mV. At 20°, a ratio of 58 mV/pH unit could be predicted if the membrane would behave predominantly as an apparent H^+-diffusion barrier. Values in this range have been found by KITASATO[11] and by ANDRIANOV *et al.*[12] with other Nitella species. In view of the present experimental results, it is reasonable to assume that the true quantum requirement of the energetic process giving rise to the potential changes is close to 1. Values of the quantum requirement as low as 5, measured under nonoptimal conditions, suggest that the changes are due to, or are closely associated with, a reaction which is efficiently coupled to light-driven photosynthetic electron transport. Such an efficient coupling has been shown to exist for the light-driven H^+ uptake by isolated chloroplasts.

In summary, the voltage-clamp method as described offers a means to study the energetics of electron transport-coupled ion transport in living plant cells, under conditions at which the membrane potentials respond to changes in the concentration of the ions involved.

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Department of Physiology and Biophysics,
University of Illinois,
Urbana, Ill. 61801 (U.S.A.)

W.J. VREDENBERG*

REFERENCES

1 W. J. VREDENBERG, *Biochem. Biophys. Res. Commun.*, 37 (1969) 785.
2 A. L. HODGKIN, A. F. HUXLEY AND B. KATZ, *J. Physiol.*, 116 (1952) 424.
3 F. A. DODGE AND B. FRANKENHAEUSER, *J. Physiol.*, 143 (1958) 76.
4 U. KISHIMOTO, *J. Cellular Comp. Physiol.*, 66 (1965) 43.
5 E. J. WILLIAMS AND J. BRADLEY, *Biochim. Biophys. Acta*, 150 (1968) 626.
6 M. AVRON AND J. NEUMANN, *Ann. Rev. Plant Physiol.*, 19 (1968) 137.
7 M. SCHWARTZ, *Nature*, 219 (1968) 915.
8 B. RUMBERG, E. RAINWALD, H. SCHRÖDER AND U. SIGGEL, in H. METZNER, *Progress in Photosynthesis Reesarch*, Vol. 3, 1969, p. 1374.
9 A. B. HOPE AND N. A. WALKER, *Australian J. Biol. Sci.*, 14 (1961) 26.
10 R. M. SPANSWICK, J. STOLAREK AND E. J. WILLIAMS, *J. Exptl. Botany*, 18 (1967) 1.
11 H. KITASATO, *J. Gen. Physiol.*, 52 (1968) 60.
12 V. K. ANDRIANOV, I. A. VOROB'EVA AND G. A. KURELLA, *Biophysics U.S.S.R.* (Engl. translation), 13 (1968) 396.

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* On leave of absence from Centre for Plant Physiological Research, P.O. Box 52 Wageningen, The Netherlands.