J. Membrane Biol. 27, 153–170 (1976)

Effect of Light upon Membrane Potential, Conductance, and Ion Fluxes in *Riccia fluitans*

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Received 20 May 1975; revised 2 October 1975

Summary. The membrane potential E_m, slope conductance g_m, and fluxes of $^{86}Rb^+/K^+$ and $^{36}Cl^-$ have been measured on cells of the aquatic liverwort *Riccia fluitans* in artificial pond water of pH 4 to 8 and 0.1 to 10 mM K_o^+, in the dark and 1 mW/cm^2 of white light. In the dark, E_m reflects a passive diffusion potential according to the Goldman equation with relative ionic permeabilities, $P_H/P_K/P_{Na} = 10:1:0.02$. In the light, E_m of -200 to -240 mV exceeds the most negative ion diffusion potential, i.e. E_K, by more than 100 mV, is more sensitive to H_o^+ than K_o^+, and is reduced to the dark level by uncouplers of phosphorylation. At 1 mM K_o^+, g_m is 33 and 50 μS/cm^2 in the light and dark, respectively. g_m is sensitive to H_o^+ only in the light, and more sensitive to K_o^+ in the dark. Current-voltage relationships are given. Light increases the influx at the plasmalemma of $^{86}Rb^+/K^+$ and $^{36}Cl^-$ less than would be expected from the increase of E_m. It is concluded that the electrogenic pump operates in the dark as a constant current source which is shunted by the diffusive channels, whereas in the light E_m approaches the H^+-dependent electromotive force of the electrogenic pump.

The origin of the common notion that electrogenesis at the cell membrane of green plants utilizes photosynthetic energy may be dated back to the days of the founder of membrane biology, W. Pfeffer, whose coworker Haacke (1892) recorded light-induced voltages from green tissues of higher plants. In current hypotheses the plant cell membrane is represented by an electrical equivalent circuit which contains ion-specific passive diffusive elements in parallel with an ATP-powered electrogenic proton pump (*see* Slayman, 1974; Bentrup, 1975). However, the complex influence of light upon ion transport (*see* MacRobbie, 1971; Higinbotham, 1973), and furthermore, the intimate linkage of energy metabolism of the chloroplasts to that of the other cellular compartments (*see* Heber, 1974), renders unlikely that light acts only via ATP-consuming pumps, the passive elements being coupled only electrically to them. The regulation of cytoplasmic pH as outlined by Raven and Smith (1973), requires a more refined mode of control. In fact, Spanswick (1972), and Vredenberg and

Tonk (1973) showed that in *Nitella* membrane potential and resistance are different functions of light. In *Acetabularia* (Hansen & Gradmann, 1971) and *Nitella* (Hansen, Warncke & Keunecke, 1973) the action of frequency-modulated light implied that light controls the membrane potential *via* different parallel pathway.

Riccia fluitans is an aquatic liverwort. Light-dependent changes of its membrane potential strikingly resemble those of aquatic higher plants, notably *Elodea* (Umrath, 1934; Jeschke, 1970; Spanswick, 1973), and *Vallisneria* (Bentrup, Gratz & Unbehauen, 1973). Contrary to these species, however, *Riccia* lends itself favorably to the techniques of both, ion flux analysis and electrophysiology, developed on animal and algal cells. Particularly, therefore, we were able to measure the current-voltage relationship of, in a sense, higher plant cells.

The purpose of this paper is to analyze the effect of light upon individual passive, and the active electrogenic membrane element. A quantitative treatment is given for the steady-states of membrane potential, conductance and fluxes of K^+ and Cl^- in the dark and light. Transient phenomena will be dealt with only briefly. A preliminary report of this work has been communicated by Felle and Bentrup (1974); the complete body of data is given in Felle (1974).

Materials and Methods

Riccia fluitans from a greenhouse pond was transferred 24–48 hr before the experiments into the following test solution (mM): KCl(0.1–10), NaCl(0–10), $CaCl_2$(0.1), Na_2HPO_4(5.9), NaH_2PO_4(13.9). This solution permitted isoosmolar changes of K^+ and Na^+, as well as changes of the pH from 4.5 to 8. The ionic content of the thalli was determined under the different experimental conditions. Cell sap was assayed by flame photometry for K^+ and Na^+, by titration for Cl^- (*see* Bentrup *et al.*, 1973). Experiments were carried out at (24 ± 1) °C. White light of about 1 mW/cm^2 was delivered by quartz-iodine lamps.

Electrophysiological Measurements

Pieces of *Riccia* thalli were arrested in a Plexiglas chamber which was perfused by the test solution and mounted on a microscope stage. Membrane potentials were recorded from thallus or rhizoid cells by means of conventional glass microelectrodes of < 1 μm tip diameter, pen chart or tape recorder, and oscilloscope. Membrane resistances were measured on the transparent, cylindrical 20–30 μm thick and up to 3 mm long rhizoid cells which were vacuolated except for the growing tip. Some recordings from this tip showed that E_{co} is not significantly different from E_{vo}. Hence $E_m \simeq E_{co}$ will be assumed throughout this paper.

Flux Measurements

Thalli were incubated with $^{86}Rb^+$ and $^{36}Cl^-$, respectively, under the particular external conditions. For efflux experiments loading time was 12 to 24 hr. The released radioactivity

was sampled at 30 or 60 sec intervals for at least 3 hr. Dioxan-scintillator was used for influx samples, unisolve-1 (Zinsser) for efflux samples. For flux calculations the area of the tissue was estimated microscopically from cross-sections (*see* Etherton, 1967). In contrast to ordinary higher plant tissue, most of the highly lobed thallus of *Riccia fluitans* does not exceed three cell layers and contains large intercellular spaces further increasing the surface-to-volume ratio.

Frequently Used Symbols and Abbreviations

APW = artificial pond water; NPW = natural pond water; E_m = membrane potential (mV); E_j = Nernst equilibrium potential for the ion j (mV); g_m = electrical slope conductance ($\mu S/cm^2$); $r_m = 1/g_m$ ($M\Omega\,cm^2$). Indices: (L) = light, (D) = dark, i = inside, o = outside, c = cytoplasmic, v = vacuolar.

Results

The Effect of Light upon Membrane Potential and Resistance

During a change of illumination light finally hyperpolarizes, darkness depolarizes E_m of thallus and rhizoid cells of *Riccia fluitans* (Fig. 1). Depending on K_o^+, E_m varied from -165 to -230 mV in the light and -50 to -135 mV in the dark. Also r_m undergoes light-dependent changes; in the experiment of Fig. 1, r_m drops from 14 $k\Omega\,cm^2$ in the light to below 10 $k\Omega\,cm^2$ in the dark. The change of E_m after light-on and -off showed a

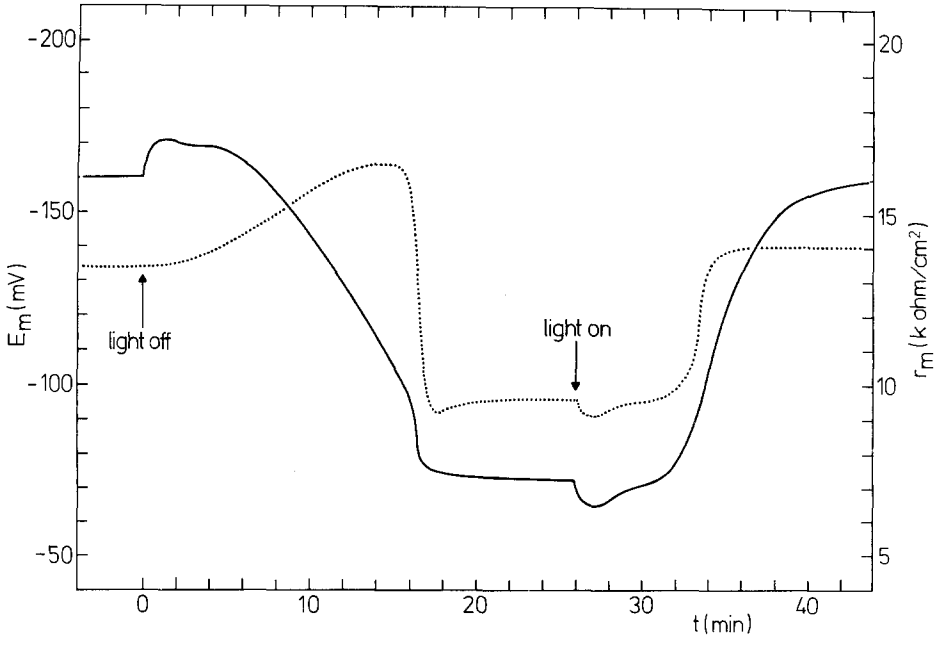

Fig. 1. Membrane potential (*solid line*) and resistance (*dotted line*) of a *Riccia* rhizoid cell under a light/dark regime in APW of pH 5.5; 10 mM K_o^+

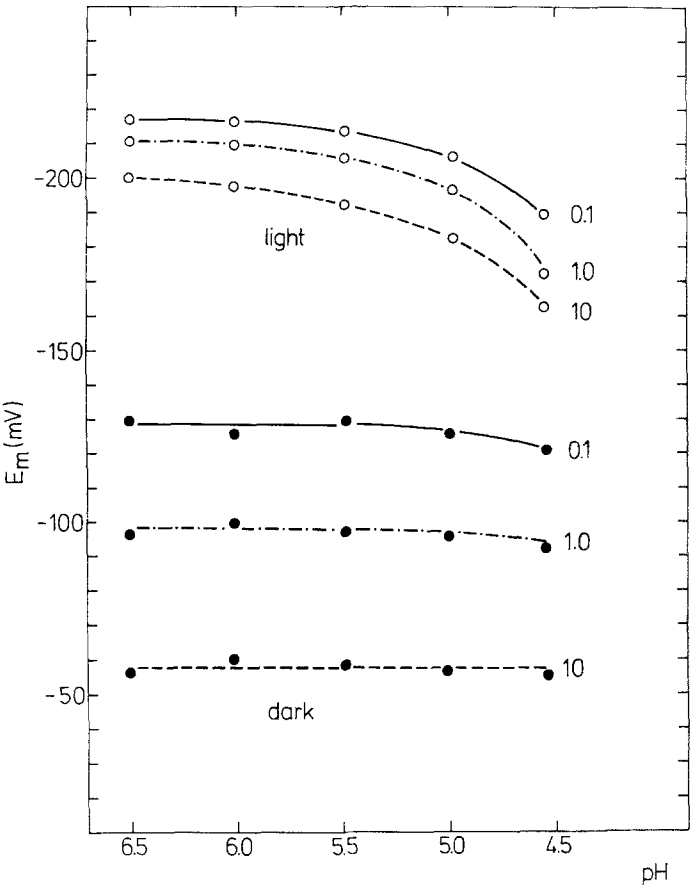

Fig. 2. Membrane potential of *Riccia* thallus cells as a function of the external pH in the light (○) and dark (●) for three different K_o^+ (figures in mM). Mean values; SEM is about ±1 mV

latency of 1.3 and 0.6 sec in 10 mM K_o^+, respectively. The values were found to decrease with K_o^+: in 0.1 mM K_o^+ 0.7 and 0.5 sec, respectively, were measured.

The Response of E_m to Changes in External K^+, Na^+, H^+, *and* Cl^-

Changes of K_o^+ between 0.1 and 10 mM caused E_m to depolarize by 30–40 mV in the dark and 10–15 mV in the light per tenfold increase of K_o^+ (Fig. 2). K_o^+ was replaced by Na_o^+. E_m did not significantly respond to changes of Cl_o^-, if Cl^- was replaced by SO_4^{2-}. Fig. 2 shows that H_o^+ substantially changes E_m in the light, but only slightly, at pH ≦ 5, in the dark.

The Resistance of the Rhizoid Membrane

The rhizoid cells, if irradiated separately, did not respond to light. However, the photoelectric responses of E_m and r_m in Fig. 1 can be recorded provided some of the green thallus cells receive light. In the dark all thallus and rhizoid cells have the same value of E_m, whereas in the light E_m attenuates exponentially along the rhizoid from its base in the thallus towards the tip (Felle, 1974). Using three intracellular electrodes, on 15 cells a *space constant* for the light-induced signal λ_{light} of 450 to 980 (mean 760) μm has been calculated.

Similarly, from application of electric current pulses of 2 to 5 sec and <0.3 μA/cm^2 density to the vacuole of 20 rhizoid cells, $\lambda_{\text{electrical}}$ was found in the range from 250 to 730 (mean 370) μm. Presumably, $\lambda_{\text{light}} > \lambda_{\text{electrical}}$ (the difference is significant at the 0.5% level) because the light-induced signal is generated in the cytoplasm, while the electrical signal is fed to the vacuole.

The membrane-specific resistance r_m (Ωcm^2), has been calculated using the formula for an infinite linear cable given by Hogg, Williams and Johnston (1969). Fig. 3 shows that $r_m(D)$ does not respond to changes in

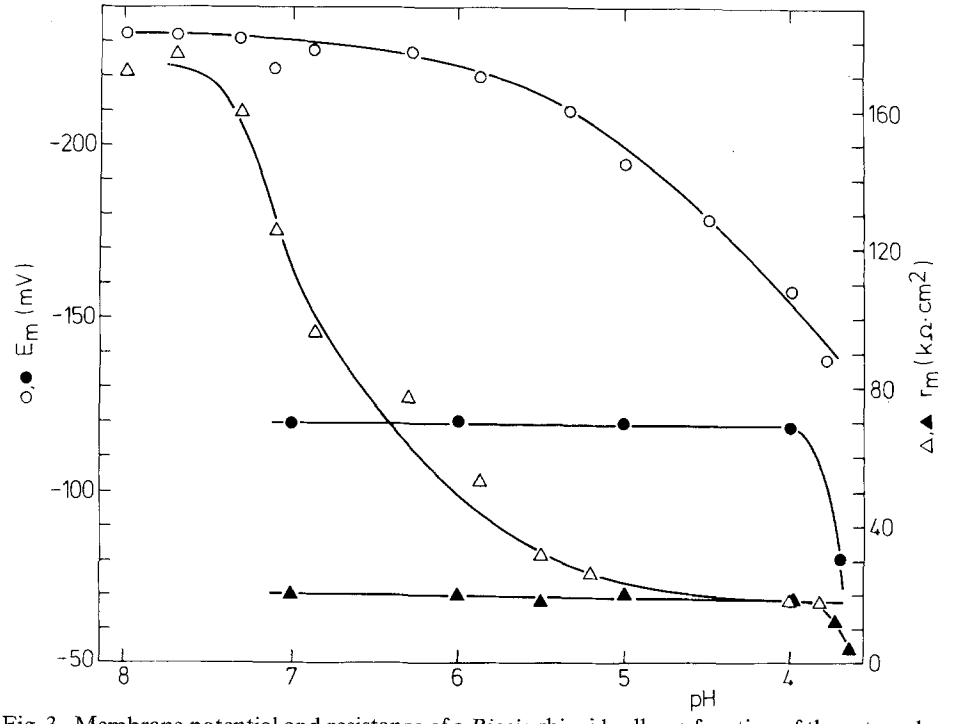

Fig. 3. Membrane potential and resistance of a *Riccia* rhizoid cell as a function of the external pH in the light (*open symbols*) and dark (*filled symbols*) in APW of 1 mM K_o^+ at pH ≦ 7 and in NPW of 0.06 mM K_o^+ at pH > 7, respectively

Table 1. Electrical resistance of the rhizoid membrane of *Riccia fluitans* in the light, $r_m(L)$, and the dark, $r_m(D)$, at different K_o^+; pH 5.5[a]

K_o^+ (mM)	$r_m(L)$ (kΩ cm^2)	$r_m(D)$ (kΩ cm^2)
0.1	35±0.8 (24)	24±0.3 (21)
1.0	30±0.9 (25)	20±0.7 (20)
10.0	13±0.6 (19)	5±0.9 (17)

[a] Figures are mean values ±SEM from the number of cells given in parentheses.

pH between 4 and 8, whereas $r_m(L)$ decreases with decreasing pH. The strikingly similar response of E_m (*see* Fig. 2) has been included for comparison. Table 1 shows that both, $r_m(D)$ and $r_m(L)$ depend upon K_o^+. Obviously, $r_m(L)$ is larger than $r_m(D)$.

Effect of Metabolic Inhibitors upon E_m and r_m

In the presence of 3×10^{-8} to 10^{-6} M CCCP (carbonylcyanide 3-chlorophenylhydrazone), $E_m(L)$ drops within 3–5 min to the dark level or some mV more positive than $E_m(D)$. At $\geqq10^{-6}$ M CCCP the transients of E_m after light-on and -off disappear. 10^{-7} to 3×10^{-6} M DCMU (3,4 dichlorophenyl-1, 1-dimethylurea) also reduced $E_m(L)$ to the dark level.

Application of 10^{-7} to 2×10^{-6} M DCMU causes $r_m(L)$ to increase from 15 to 20 kΩ cm^2. However, 2,4-dinitrophenol decreased $r_m(L)$ from 16 to 10 kΩ cm^2, if its concentration was stepped up from 2×10^{-6} to 10^{-5} M. No further changes occur in the tested range of 10^{-5} to 3×10^{-5} M.

Current-Voltage Relationships of the Rhizoid Membrane

Current pulses I_o up to ±10 nA were fed to the midpoint of rhizoid cells and the change in E_m, V_o, was measured under the current electrode. The recorded I_o/V_o input data have been subjected to Cole's theorem to obtain the current density at the voltage electrode. The theorem applies to infinite cables. This condition has been considered fulfilled because $\lambda_{\text{electrical}}$ of 370 μm never exceeded 30% of the length of a given rhizoid. The membrane current per cm length of the cell, i_m, nA/cm, is given by Cole (1968):

$$i_m=(dI_o/dV_o)\,I_o\,(R_i/4), \tag{1}$$

where dI_o/dV_o is the slope of the input *I-V* curve at V_o, and R_i, Ω/cm, denotes the resistance of the cell interior. The thus obtained i_m/E_m rela-

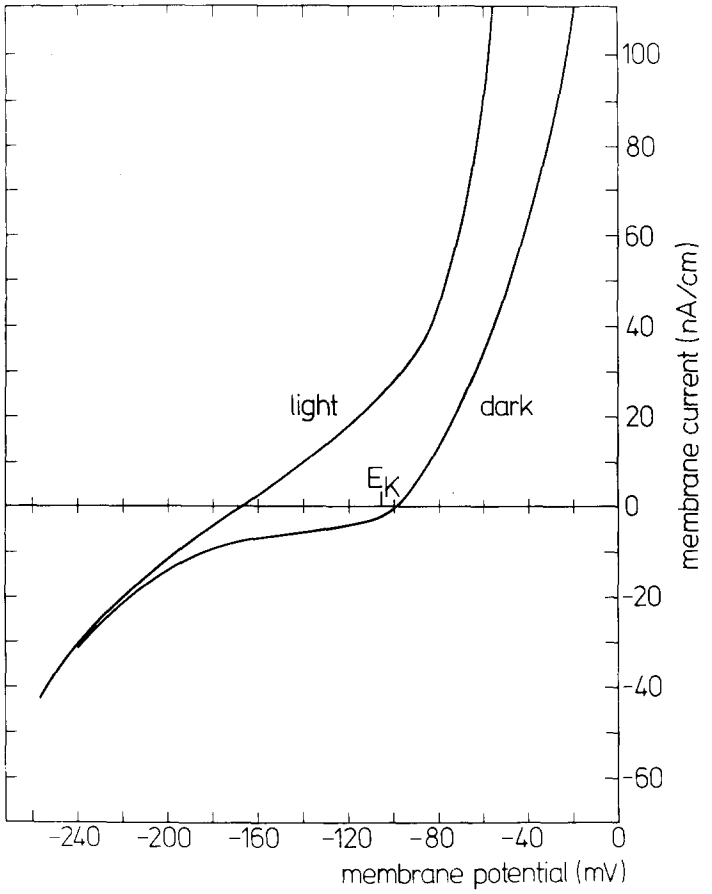

Fig. 4. Current-voltage response from a *Riccia* rhizoid cell in the light and dark at 1 mM K_o^+. The ordinate denotes the membrane current (outward = positive) per cm length of the cell. *See text*

tionship is given in Fig. 4. Division of the i_m values by the cell circumference of 7.5×10^{-3} cm yields the current density, i.e., 1 nA/cm $\triangleq$ 0.13 μA/cm^2 for the cell of Fig. 4.

In the range of ± 10 nA/cm the *I-V* relationship is different in the dark and light: the former displays rectification, the latter represents a linear conductance. At larger currents the differences disappear.

The time course of the light-dependent change of r_m under low currents is illustrated by an original recording in Fig. 5. Clearly visible is the asymmetrical voltage response in the dark. The symmetrical response in the light is gradually developed within 6–7 min after light-on. Similarly gradual is the transition after light-off.

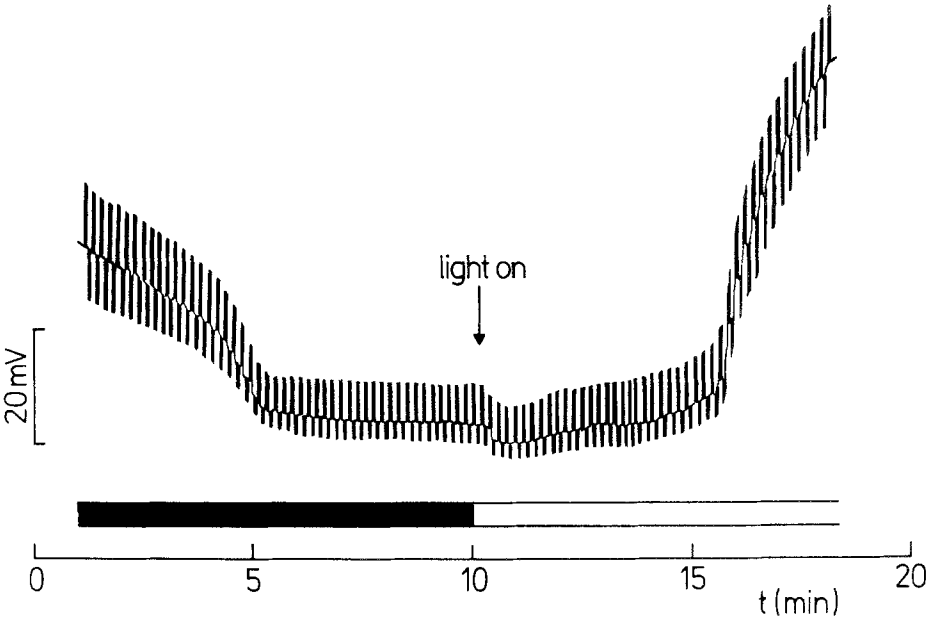

Fig. 5. Membrane potential of a *Riccia* rhizoid cell responding to injected square current pulses of (1±0.2) nA and 10 sec duration of either polarity. The area under the voltage signal has been filled in black for better visibility

Efflux Experiments

By compartmental analysis (*see* Cram, 1968; MacRobbie, 1971; Higinbotham, 1973) the unidirectional fluxes of $^{86}Rb^+/K^+$ and $^{36}Cl^-$ across the plasmalemma (φ_{oc}, φ_{co}) and the tonoplast (φ_{cv}, φ_{vc}) have been determined. The fluxes have been calculated from efflux rate constants, k_c and k_v, respectively, from the content of radioactivity, I_c and I_v, respectively, and from the loading time, t_{in}, using the following set of equations (*see* Cram, 1968):

$$\varphi_{oc} = k_c I_c + I_v/t_{in} \tag{2}$$

$$\varphi_{co} = k_c I_c + k_v Q_v \tag{3}$$

$$\varphi_{cv} = \varphi_{co} I_v/t_{in} k_c I_c \tag{4}$$

$$\varphi_{vc} = \varphi_{cv} - (\varphi_{oc} - \varphi_{co}) \tag{5}$$

$$Q_c = (\varphi_{co} + \varphi_{cv})/k_c. \tag{6}$$

The ion content of the vacuole Q_v was assumed to be Q_{total}, the ion content of the cell sap.

Typical efflux kinetics of $^{86}Rb^+/K^+$ are shown in Fig. 6; of $^{36}Cl^-$ in Fig. 7. Mean values of several experiments of k and I have been summarized

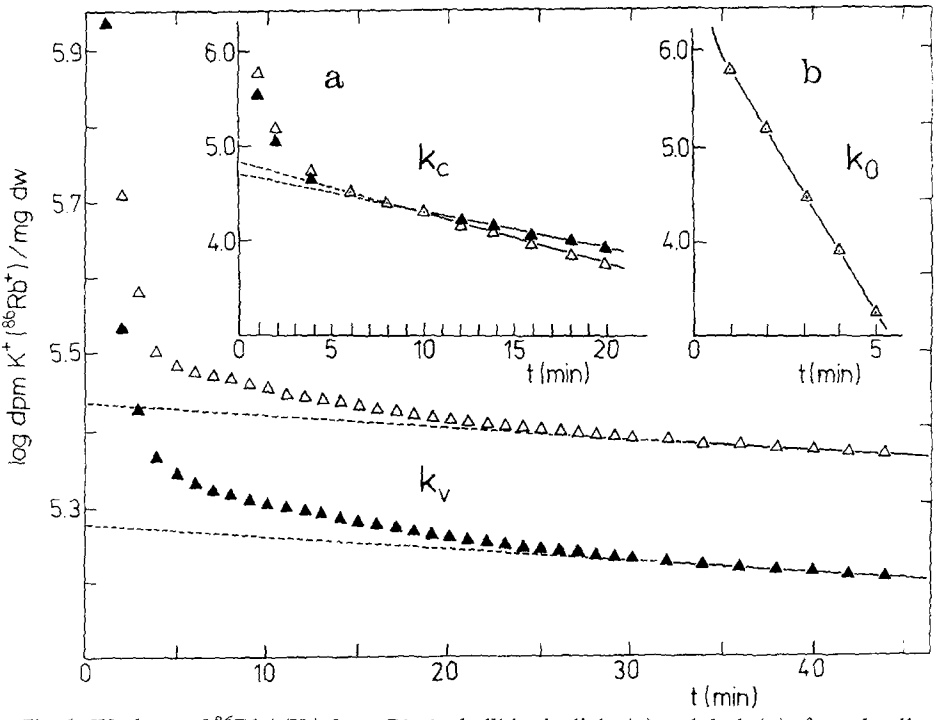

Fig. 6. Washout of $^{86}Rb^+/K^+$ from *Riccia* thalli in the light (△) and dark (▲) after a loading time of 12 hr. pH 5.5, 1 mM K_o^+. The dotted line indicates k_v. *Insets:* Replotted data to indicate (*a*) k_c, and (*b*) the rate constant of the apparent free space, k_o

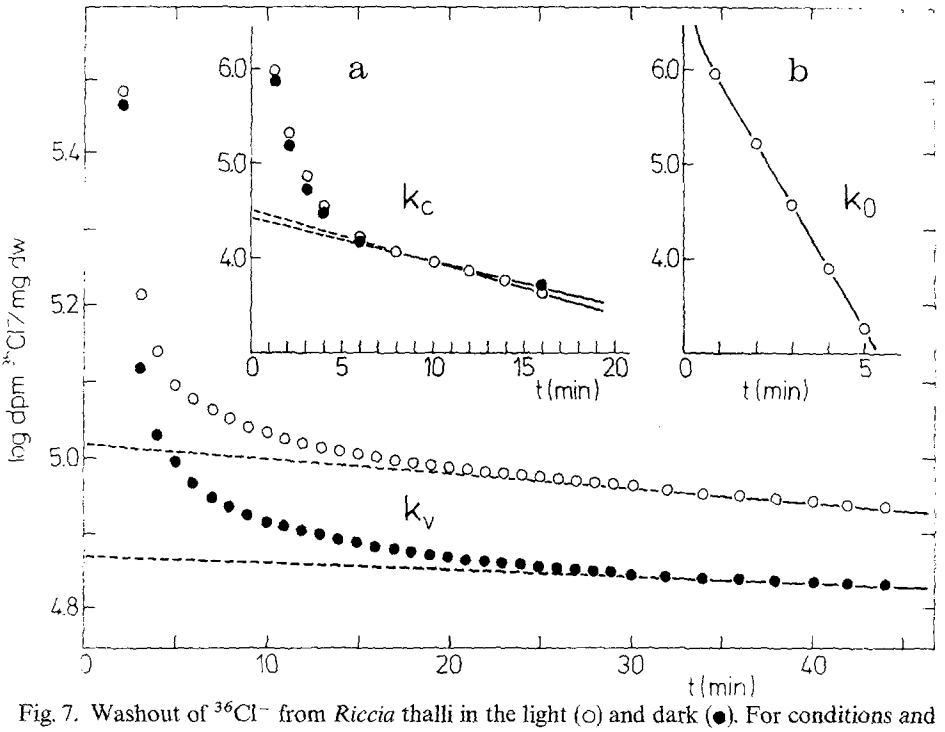

Fig. 7. Washout of $^{36}Cl^-$ from *Riccia* thalli in the light (○) and dark (●). For conditions and explanation *see* Fig. 6

Table 2. Efflux of $^{86}Rb^+/K^+$ and $^{36}Cl^-$ from thalli of *Riccia fluitans* in the dark and light; 10 mM K_o^+, pH 5.5[a]

	Dark		Light	
	$^{86}Rb^+/K^+$	$^{36}Cl^-$	$^{86}Rb^+/K^+$	$^{36}Cl^-$
k_o	1.17×10^{-2}	1.13×10^{-2}	1.17×10^{-2}	1.13×10^{-2}
k_c	6.2×10^{-4}	8.0×10^{-4}	7.9×10^{-4}	8.7×10^{-4}
k_v	1.7×10^{-5}	1.6×10^{-5}	2.0×10^{-5}	2.5×10^{-5}
I_c	4.8×10^{4}	2.9×10^{4}	6.0×10^{4}	3.3×10^{4}
I_v	1.8×10^{5}	7.7×10^{4}	2.7×10^{5}	1.1×10^{5}

[a] Given are rate constants, k_o etc., sec^{-1}, and content of radioactivity at $t=0$, I_c and I_v, dpm per mg d.w. Mean values from four ($^{86}Rb^+$) and three experiments ($^{36}Cl^-$), respectively

Table 3. Fluxes and concentrations of K^+ and Cl^- in *Riccia fluitans* in the light and dark[a]

		Flux (pM cm^{-2} sec^{-1})				Concentration (mM)	
		φ_{oc}	φ_{co}	φ_{vc}	φ_{cv}	Q_c	Q_v
$^{86}Rb^+/K^+$	(*D*)	6.86±0.2	6.19±0.13	1.0 ±0.04	0.97±0.03	22	89
	(*L*)	10.37±0.17	10.0 ±0.14	1.45±0.06	1.82±0.05	26	109
$^{36}Cl^-$	(*D*)	4.3 ±0.31	4.22±0.28	0.32±0.03	0.42±0.02	10	33
	(*L*)	5.02±0.31	5.14±0.27	0.44±0.03	0.32±0.02	11	30

[a] Calculated from Table 2; Figures are mean values ±SEM

in Table 2. Both isotopes evade with half-times ($t_{1/2}$), of 10–15 min from the cytoplasm and 10–15 hr from the vacuole. The calculated values of φ and Q have been listed in Table 3. The plasmalemma fluxes of $^{86}Rb^+/K^+$ show a light-dependent increase of about 50%, those of $^{36}Cl^-$ an increase of less than 20%. Tonoplast flux data are less satisfactory, because only $^{86}Rb^+/K^+$ shows flux equilibrium.

Efflux of $^{86}Rb^+/K^+$ and $^{36}Cl^-$ during a Change of Illumination

Between 5 and 35 min after onset of an efflux experiment illumination was suddenly changed. When light was turned on after 9 min of washout in the dark, a significant stimulation of efflux was found for both isotopes (Fig. 8). Consistently, three discrete peaks of efflux could be distinguished; in the particular experiment of Fig. 8 they appear at 10, 14.5, and 18 min after light-on. The first peak coincides with the transient membrane depolarization as indicated.

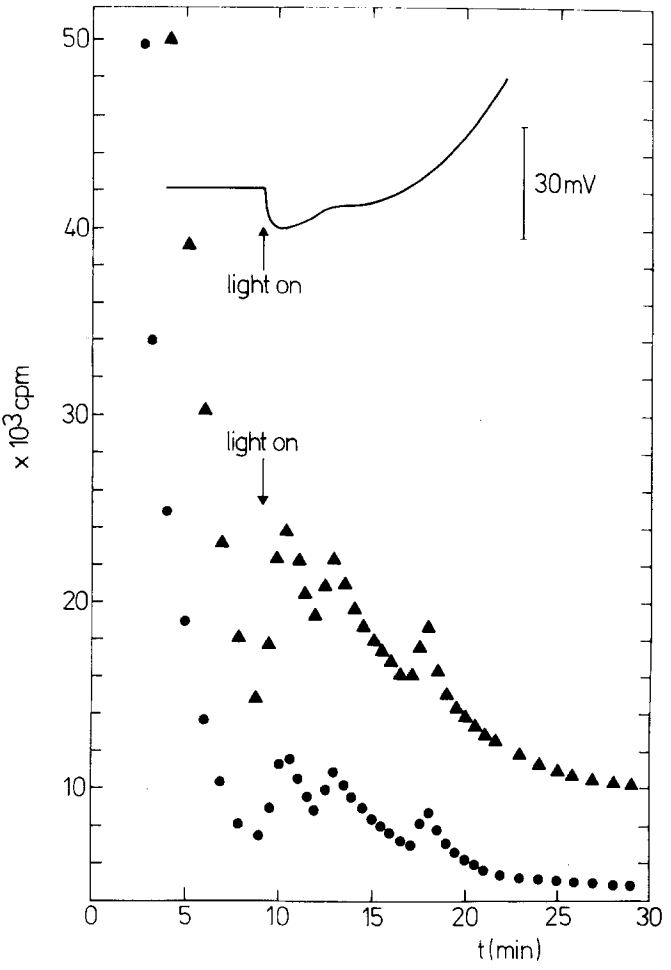

Fig. 8. Kinetics of the efflux of $^{36}Cl^-$ (●) and $^{86}Rb^+/K^+$ (▲) from *Riccia* thalli in the dark from $t=0$ until $t=9$, and the following 21 min of light. Loading period was 12 hr, preincubation in the dark 2 hr. Samples were collected at intervals of 30 sec. pH 5.5, 1 mM K_o^+

The Light-on Response of the Membrane Potential

The data presented so far suggest that $E_m(D)$ is somewhat more positive than E_K. The question arises what equilibrium potential, if any, E_m tends to approach when it becomes even more positive right after light-on (*see* Fig. 1). It can be calculated that both, E_{Na} and E_{Cl} range between zero and about +20 mV, if computed for both membranes in series. The experiment in Fig. 9 was performed to indicate whether E_m transiently approaches E_{Na}, E_{Cl}, or E_H. When the steady-state $E_m(D)$ was lowered by increasing K_o^+ to 35 mM, E_m did not attain positive values but transiently hyperpolarized after light-on. This strongly argues against E_{Na} or E_{Cl} controlling

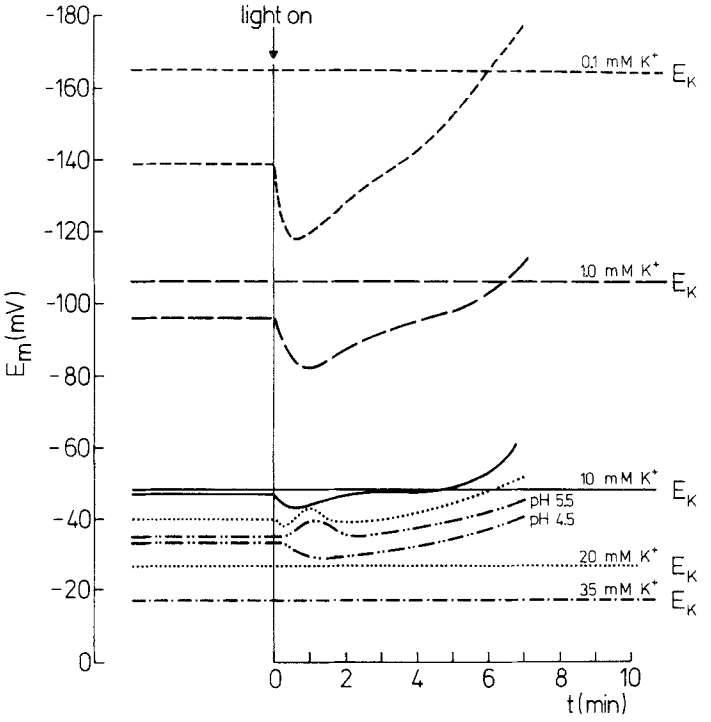

Fig. 9. Membrane potential of a *Riccia* thallus cell after light-on at $t=0$ for five different K_o^+ as indicated. Horizontal lines indicate the respective values of E_K between vacuole and the outside medium. The pH was 5.5 except for 35 mM K_o^+ where another track shows E_m also at pH 4.5

E_m. Moreover, when the pH was lowered from 5.5 to 4.5, the transient depolarization reappeared. Provided both plasmalemma and tonoplast are predominantly permeable to the proton during this transient state, the critical voltage of -40 ± 5 mV in Fig. 9 might indicate E_H, hence a vacuolar pH of 4.6.

Discussion

$E_m(L)$ is by 50 to 150 mV more negative than the most negative diffusion potential, E_K; *see* Figs. 2 and 9. Currently ATP-powered electrogenic proton export is generally inferred to account for the hyperpolarization of plant cell membranes beyond conceivable diffusion potentials (*see* Slayman, 1974; Bentrup, 1975). Hitherto the evidence for this inference is still incomplete; so it is in *Riccia*: (a) After light-on *Riccia* thalli acidify a weakly buffered medium (Felle, 1974); (b) Inhibitors of oxidative and

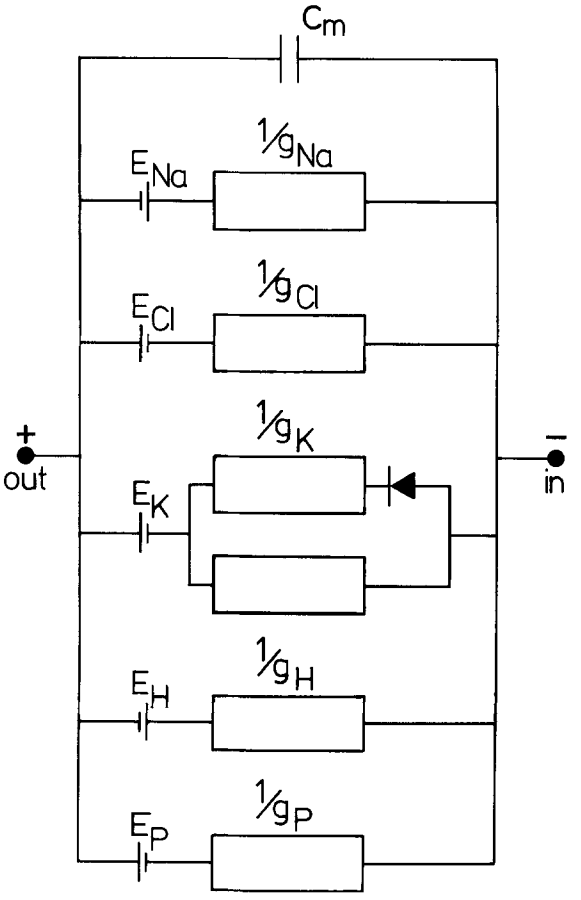

Fig. 10. Equivalent circuit for the membrane potential of the plasmalemma of *Riccia fluitans*. $C_m = 0.6$ to 1.9 μF/cm². *See text*

photophosphorylation invariantly cause E_m to drop to the diffusional level; (c) The effect of H_o^+ upon E_m and g_m.

At this point the equivalent circuit in Fig. 10 is introduced to assess the role of the proton in the light-dependent behavior of the *Riccia* membrane. The circuit shows the passive diffusion channels for Na^+, K^+, Cl^-, and H^+, in parallel with the electrogenic pump having the emf E_P and the conductance g_P. The diffusive channels may be summarized by E_D and g_D, respectively. This type of circuit has been used, for instance, to describe the membrane potential of *Neurospora* by Slayman (1965), of *Acetabularia* by Gradmann (1970), of *Nitella* by Kitasato (1968) and Spanswick (1972). The circuit yields $g_m = g_P + g_D$, and

$$E_m = E_D + \frac{g_P}{g_D + g_P}(E_P - E_D). \tag{7}$$

Eq. (7) favorably lends itself to assess possible modes of control of E_m by the circuit in Fig. 10. It must be kept in mind that the conductance elements are nonohmic.

Kitasato (1968) considered E_P to be a current source ($g_D \gg g_P$), and hence concluded that in *Nitella* $E_m \simeq E_D$.

On the other hand, following theoretical work of Rapoport (1970), results on *Nitella translucens* lead Spanswick (1972, 1974) to argue that most of g_m is provided by the pump, that is, $g_P \gg g_D$. Hence Eq. (7) yields $E_m \simeq E_P$. The effect of H_o^+ upon E_m then would be through E_P, rather than E_D as postulated by Kitasato (1968). Recently, Spanswick's view gained strong support from a study by Walker and Smith (1975) who measured the cytoplasmic pH of *Chara corallina*, then calculated E_H, and entered it into Eq. (8):

$$E_P = \Delta G/n \cdot F - E_H. \tag{8}$$

On the basis of values of the molar free energy change of ATP-hydrolysis, ΔG, derived from data on *Griffithsia* by Lilley and Hope (1971), they found that the maximum E_P which could be produced by the electrogenic ATPase across the plasmalemma of *Chara* by extrusion of 2 H^+ per ATP split approximates the value of $E_m(L)$ and $E_m(D)$, respectively, observed by Richards and Hope (1974).

In the following the available data of *Riccia* will be argued to suggest that in the light E_m and g_m are governed by emf and conductance of the electrogenic proton pump; in the dark, however, they are governed by the diffusive elements, i.e. E_D and g_D, respectively.

1. In the light, E_P of the *Riccia* membrane can be roughly estimated from Eq. (8) as follows: Using the values of ΔG of both, *Griffithsia* (taken from Walker & Smith, 1975) and *Neurospora* (Slayman, Long & Lu, 1973), of 50–55 kJ/mole, the term $\Delta G/n \cdot F$ yields about -270 mV for $n=2$. In *Riccia*, the steady-state level of ATP in the light and dark, respectively, is not significantly different from a mean value of (300 ± 9) ng ATP/mg dry weight. From the cytoplasmic pH of another archegoniate cell, *Phaeoceros laevis* (Davis, 1974), $E_H(L)$ and $E_H(D)$ of the *Riccia* plasmalemma of $+59$ and $+65$ mV, respectively, can be estimated. Then Eq. (8) yields $E_P(L) = -211$ mV to be compared with $E_m(L)$ at pH 5.5 and 0.1 mM K_o^+ of -215 mV (Fig. 2). In the dark, $E_m(D)$ of -135 mV is 70 mV more positive than $E_P(D)$ of about -205 mV from Eq. (8).

2. If $E_m(D)$ is essentially governed by the diffusive regime, the well-known constant field equation for the membrane potential (Goldman, 1943; Hodgkin & Katz, 1949) may be applied to $E_m(D)$ using terms for

Table 4. K^+ influx and K^+ permeability coefficient of the plasmalemma of *Riccia fluitans* in the light and dark as functions of K_o^+; pH 5.5[a]

K_o^+ (mM)	E_m (mV)	φ_{oc}^K (pM cm^{-2} sec^{-1})	P_K (cm · sec^{-1})
A. Dark			
0.1	-133 ± 1.1 (52)	0.47 ± 0.02	0.89×10^{-6}
1.0	-100 ± 0.6 (52)	2.70 ± 0.15	0.68×10^{-6}
10.0	-56 ± 1.2 (38)	5.84 ± 0.20	0.24×10^{-6}
B. Light			
0.1	-215 ± 1.0 (56)	0.56 ± 0.08	0.13×10^{-6}
1.0	-210 ± 1.0 (61)	4.05 ± 0.19	0.50×10^{-6}
10.0	-195 ± 1.2 (40)	10.02 ± 0.24	0.66×10^{-6}

[a] P_K values have been calculated from Eq. (9). E_m values are the mean ± SEM of the number of cells in parentheses. Flux figures are the mean ± SEM from six experiments

H^+, K^+, and Na^+. From the response of $E_m(D)$ to changes of H_o^+, K_o^+, and Na_o^+ (Fig. 2) relative ionic permeabilities have been obtained from this equation, i.e. $P_H/P_K/P_{Na} = 10:1:0.02$. Richards and Hope (1974) inferred from their data on *Chara corallina* that at pH ≦ 6 the ratio P_H/P_K is about 25.

Absolute ionic permeability coefficients according to the Goldman-Hodgkin-Katz equation can be calculated from unidirectional passive ion fluxes, if independence of ion movement can be assumed. P_K is of special interest because of the light-dependent sensitivity of E_m and g_m to K_o^+ (Fig. 2; Table 1). The K^+ influx at the plasmalemma always occurs down the electrochemical gradient: at 10 mM K_o^+, the difference, $E_m - E_K$, is 36 mV in the dark, and 170 mV in the light. Table 3 shows that light increases φ_{oc}^K 1.5-fold, whereas a threefold increase due to the increase of E_m should occur, if φ_{oc}^K were purely passive and independent of other ion fluxes. Presently no evidence exists to show which, if any, of both K^+ fluxes satisfies the independence criterion. Hence, no reliable estimate of P_K can be given to settle the problem of light-dependent changes of this entity. Nevertheless, from K^+ influx measurements at various K_o^+ we could calculate tentative P_K values according to (Goldman, 1943):

$$P_K = -\frac{RT}{F} \cdot \varphi_{oc}^K \frac{1 - \exp(E_m F/RT)}{K_o^+ E_m}. \tag{9}$$

R, T, and F have their usual meanings. All pertinent data have been listed in Table 4. (The φ_{oc}^K values at 10 mM K_o^+ are slightly below those from the efflux experiments given in Table 3.) Obviously, these tentative P_K values

range from 0.1 to 0.9×10^{-6} cm/sec, slightly vary with K_o^+, but fail to show a consistent effect of light.

3. The current-voltage data support our idea of a different origin of $E_m(L)$ and $E_m(D)$. The dark I–V curve is proposed to reflect mainly a diode characteristic of the K^+ diffusion channels (*see* Fig. 10). The ratio of asymptotic conductances for low de- and hyperpolarizing currents ($\leqq 20$ nA/cm in Fig. 4), respectively, is about 10–15 and roughly compares with the K^+ concentration gradient, K_c^+/K_o^+ of 20. This fact reminds one of the K^+ diode model for a K^+-selective membrane by Cole (1968, p. 156). The conclusion of Kitasato (1973), that depolarizing currents increase the number of K^+ channels of the *Nitella* membrane in its K^+-sensitive state, seems equivalent.

In the light, the I–V curve is essentially linear in the considered current range of $\leqq 20$ nA/cm. A carrier model for an electrogenic pump by Finkelstein (1964) predicts this feature, i.e. g_P should be independent of the sign of currents passing through the pump and highest near its emf, E_P. Also, the high sensitivity of $g_m(L)$ to H_o^+ (Fig. 3) is plausible from Eq. (8), if in this low current range $g_m(L)$ were in fact the conductance of a proton pump, g_P. At large displacements of E_m from E_P the pump model postulates a minute slope conductance because of the finite number of carrier molecules per unit area of membrane. This property could be concealed in the *Riccia* circuit, if large depolarizing currents pass through the diffusive limbs, mainly the K^+ channels, and hyperpolarizing currents lead to membrane punch-through, i.e. a large chloride conductance as observed on *Chara* by Coster (1969).

4. A detailed analysis of the transient phenomena will be necessary to elucidate the causal relationship between the photosynthetic signals and the individual membrane elements in the circuit of Fig. 10. Briefly, since at least the steady-state values of E_P and E_H apparently do not undergo substantial light-dependent changes, g_H might be suspected as an immediate target of photosynthetic signals. Also g_P might be controlled this way, but could likewise be controlled through E_m according to the Finkelstein model. A control of g_D through E_m is suggested by the dark I–V curve: g_D rises/drops due to the assumed substantial contribution of g_K as E_m moves below/above E_K. (Note that in Fig. 1 the fast changes of r_m occur around -130 mV, i.e. E_K.) Also the result of Fig. 5 that g_m does not change before E_m rises about 6 min after light-on suggests that the light-induced changes of g_m in fact are partly voltage-controlled.

This paper is dedicated to Professor E. Bünning on the occasion of his seventieth birthday. We are grateful to Dr. D. Gradmann for many stimulating discussions. This work was supported by the Deutsche Forschungsgemeinschaft.

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