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Effects of Ultraviolet Radiation on the Membranes of *Chara corallina*

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Summary. The effects of 253.7 nm ultraviolet (UV) radiation on the membrane properties of *Chara corallina* have been studied. UV irradiation caused depolarization of the membrane potential (p.d.) and a decrease in membrane resistance. These effects were largely reversible with steady values being obtained within 40 minutes after the UV was turned off. The effects on ionic fluxes of Na^+, K^+ and Cl^- have also been studied using radioactive tracer techniques. The influxes were unchanged by irradiation. The chloride efflux was increased sevenfold during the irradiation period but recovered to the pre-irradiation value within 30 minutes after the irradiation period. The potassium efflux was also increased and reached a maximum 10 minutes after irradiation. The resting potential and the average depolarized p.d. reached during irradiation were in good agreement with those calculated from permeability coefficients indicated by the observed passive fluxes, using the Goldman equation for p.d. However, the plasmalemma resistance and its change due to irradiation did not match the values calculated from the same permeability coefficients used to estimate p.d. This disagreement, and an apparent imbalance in the charge transferred across the resting or irradiated plasmalemma, suggest the participation of another ion species as well as K^+, Na^+ and Cl^-.

There have been few studies on the effects of radiation at the membrane level. The giant algal cells are an ideal system for such studies. Certain modifications of the electrophysiological properties in charophyte cells, due to irradiations of various sorts, have already been reported. Thus, action potentials have been initiated in *Nitella* by intense flashes of ultraviolet (UV) radiation (Harvey, 1942) and by α-particles (Gaffey, 1972). Esch, Miltenburger and Hug (1964) found that X-irradiation of *Nitella* caused depolarization of the membrane potential (p.d.) and increased the duration of the action potential. γ-ray irradiation also caused depolarization of the membrane potential (Esch, 1966). Hansen (1967) exposed *Nitella flexilis* to X-radiation and also observed depolarization as well as a decrease in the membrane resistance. In none of these studies was the effect of the radiation

connected unequivocably with a particular change in cell or membrane function.

The present investigation is concerned with the effects of UV radiation on some membrane properties of *Chara corallina*, namely the resting electrical characteristics and ion fluxes.

We chose UV radiation because of the absence of "indirect effects" (Giese, 1950) and *C. corallina* because the electrical properties and fluxes in the cytoplasmic membranes are reasonably well known.

Materials and Methods

Cells of *Chara corallina* were cultured in tanks in the laboratory (*see* Hope & Aschberger, 1970). The cells used throughout were internodal cells 20 to 40 mm in length and 0.8 to 1.0 mm in diameter. The bathing medium in the experiments was Flinders Pond Water (FPW) consisting of 0.2 mM KCl, 2.0 mM NaCl, 0.05 mM $CaCl_2$. After cutting, the cells were usually soaked overnight in this medium before use.

The vacuole p.d. was recorded continuously from an electrode inserted into the vacuole. A pulse generator, which passed a measured current through a second micro-salt-bridge in the vacuole, was switched on electronically for 1 to 2 sec approximately every minute. The superimposed changes in vacuole p.d. enabled the cell resistance to be calculated. The p.d. and resistance usually became stable about 1 hr after insertion of the two electrodes. In some experiments a few temporary insertions into the cytoplasm were made using finer electrodes (*cf.* Findlay & Hope, 1964).

The measurements of fluxes across the plasmalemma were based on procedures already described in the literature (Hope & Walker, 1960; Hope, Simpson & Walker, 1966; Findlay, Hope, Pitman, Smith & Walker, 1969).

The fluxes of Na^+, K^+ and Cl^- were determined at room temperature (20 °C) and under white fluorescent lights at an incident energy flux in the visible wavelengths of about 13 W m^{-2}. Influxes were generally estimated from the radioactivity in cells after a 10-min soaking in radioactive solution, followed by a 1-min rinse in an inactive medium: FPW, or for some experiments ice-cold 5 mM $CaSO_4$, which would have eluted more thoroughly the radioactivity in the cell wall without removing significant radioactivity from the cytoplasm. UV treatment usually coincided with the 10 min in radioactive solution, but variations on this were made; these are noted in Table 2.

The effluxes of K^+, Na^+ or Cl^- were estimated from the appearance of radioactivity in unlabeled FPW following a loading period in the appropriate radioactive solution, and a long rinse to obtain cells with approximately uniform internal specific activity. The loading period was as long as seven days to obtain high enough internal radioactivity, in the case of Cl^- and Na^+. The efflux was followed during several periods of 30 min or occasionally, 10 min, and then UV irradiation was given during a single 10-min period. Finally, the efflux was followed after irradiation for several further periods of 10 min.

The ultraviolet source was a germicidal lamp with 90% of the output at 253.7 nm. The height of the lamp above the exposed cells was 0.5 m. The lamp was regulated with an auto-transformer usually set at 100 V; the dose-rate at the cells was about 4.7 W m^{-2}, measured by a Cambridge thermopile and a microvoltmeter. The cells were situated in experimental solution about 1 to 2 mm below quartz coverslips, in which situation there would have been negligible attenuation of the UV just above the cells. However, the

absorbance of the cells was such that the UV was probably fully absorbed by the layer of cytoplasm including chloroplasts, at a depth of about 10 μm (Jagger, 1967). Therefore, in the present experimental arrangement, it is expected that only the top halves of the cells were affected by irradiation, unless there were effects propagated by cytoplasmic streaming. This is discussed below.

Results

Membrane Potential Difference

The resting p.d. of the cells measured between the vacuole and the bathing solution of FPW was usually between −140 mV and −150 mV. Exposure of the cells to 253.7 nm UV radiation caused rapid depolarization of the membrane potential, as shown in Fig. 1. When the UV was turned off, the membrane potential immediately began to repolarize but then depolarized to a secondary minimum before repolarizing again to a steady value 40 to 60 min later. The degree of depolarization caused by the UV depended on the time of exposure and hence dose. For some cells, the depolarization was immediate but for others it began only after 1 to 2 min of exposure. The recovery of the p.d. following a single irradiation was to a value 2 ± 1 (21) mV more positive than the initial steady resting potential. Depolarization after a second irradiation with the same dose caused a variable response, sometimes smaller, sometimes greater than with the first dose; this aspect has not been further studied.

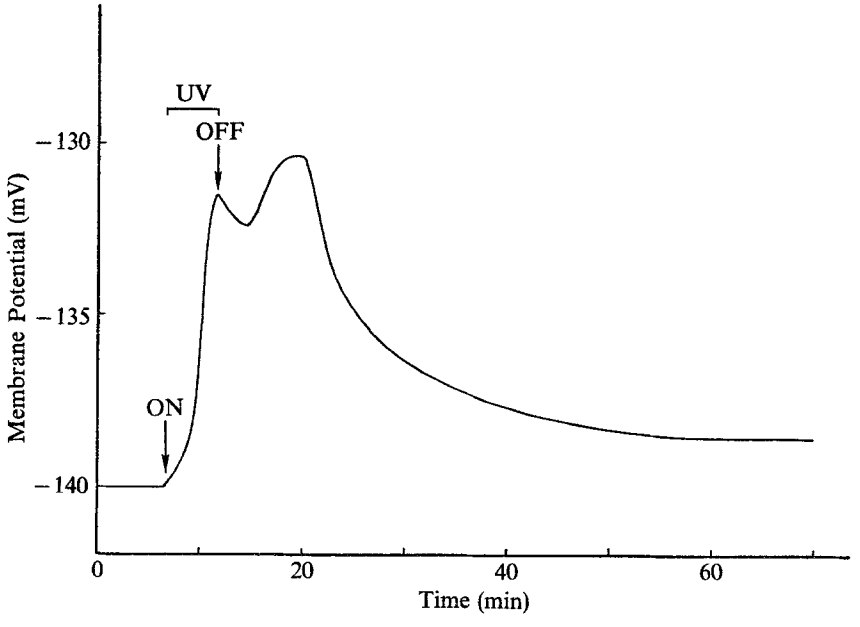

Fig. 1. The effect of 5 min of UV irradiation on the membrane p.d.

Both the initial depolarization due to UV irradiation and the secondary depolarization depended on the initial resting membrane p.d. Details of this will be left for a further paper. Cells in a bathing medium of unbuffered FPW at pH about 5.5, showed 8 mV depolarization in the first wave and a total depolarization of about 9 mV in the second, as a result of 5-min irradiation (Fig. 1). Insertion of microelectrodes into the cytoplasm and the vacuole enabled the p.d. across the plasmalemma and the tonoplast to be measured simultaneously (Findlay & Hope, 1964). It was found that UV irradiation caused depolarization of the plasmalemma p.d. but the p.d. across the tonoplast (about +10 mV) was unchanged.

Membrane Resistance

The resting membrane resistance was between 0.8 $\Omega\,m^2$ and 1.2 $\Omega\,m^2$. This is the resistance of the plasmalemma and tonoplast in series but closely approximates to that of the plasmalemma. When the cells were exposed to UV radiation, the resistance decreased and continued to do so after the UV was turned off as shown in Fig. 2. However, after approximately 5 min, the resistance reached a minimum value, about 50 to 60% of the resting value, and then began to increase to a steady value 30 to 40 min later.

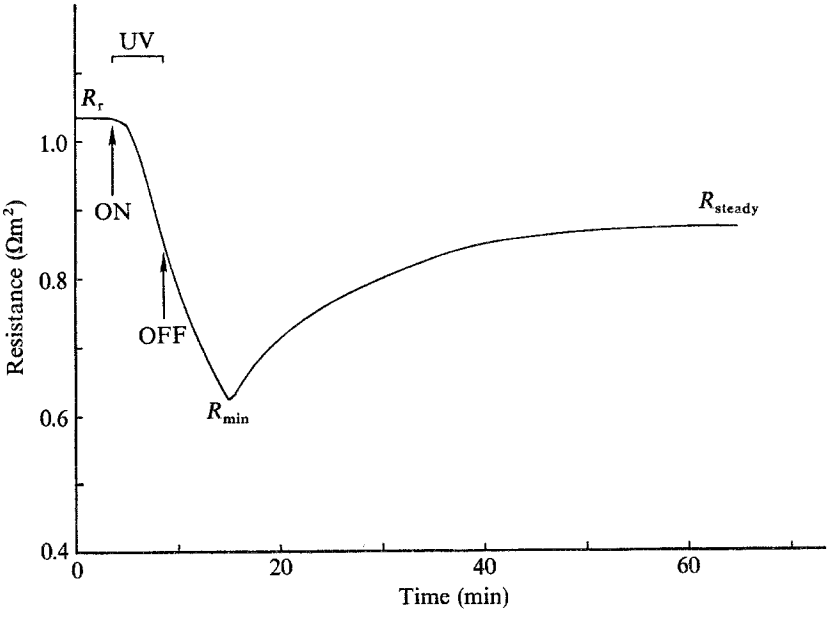

Fig. 2. The effect of 5 min of UV irradiation on the membrane resistance. R_r refers to the resting resistance, R_{min} to a minimum and R_{steady} to a steady resistance following UV irradiation

Table 1. Resistance of *Chara* before and after UV irradiation (Ωm^2)

Exposure time (min)	R_r[a]	R_{min}	R_{steady}	R_{min}/R_r (%)
2.5 (8)	0.91 ± 0.07	0.52 ± 0.05	0.72 ± 0.05	57.3 ± 2.1
5 (3)	0.94 ± 0.15	0.55 ± 0.10	0.89 ± 0.13	59.0 ± 1.4
7.5 (5)	0.95 ± 0.12	0.49 ± 0.05	0.88 ± 0.13	52.4 ± 3.4

[a] *See* Fig. 2 for the meaning of R_r, R_{min} and R_{steady}. Numerals in parentheses signify the number of cells used. Errors shown are the SEM.

Increasing the time of exposure from 2.5 min to 7.5 min had no effect on the minimum value reached, as shown in Table 1. The minimum resistance occurred at a time corresponding to the second peak of potential (Fig. 1) and no discontinuity was observed corresponding to the first p.d. peak. As with the p.d., the steady value of resistance after recovery was almost equal to that of the resting state, but somewhat less (Table 1).

Fluxes

No effects of UV irradiation on ion influx were found (*see* Table 2). The influxes were similar in size to those found by Findlay *et al.* (1969) for *C. corallina* except that our sodium influxes were higher (occasionally much higher) and potassium, lower. No explanation for these higher influxes can be offered at present.

The effluxes during and after irradiation have been calculated, for the purposes of comparison, on the assumption that the whole cell has a uniform

Table 2. Influxes (nmoles m^{-2} sec^{-1}) before and after UV irradiation

Ion	Conditions	Control	+UV
Cl^-	Nodes discarded, remainder counted	34 ± 4 (9)[a]	29 ± 3 (14)
	Whorl cells, all counted	20 ± 4 (10)	30 ± 4 (9)
Na^+	Wall discarded, contents counted 30 min uptake time	16 ± 2 (5)	
	(*i*) UV at 0–10 min		17 ± 2 (5)
	(*ii*) UV at 20–30 min		16 ± 1.3 (5)
K^+	rinsed in ice cold 5 mM $CaSO_4$	2.2 ± 0.3 (10)	2.5 ± 0.2 (10)

[a] Errors shown are SEM with the number of cells used in parentheses. None of the differences (UV minus control) is statistically significant.

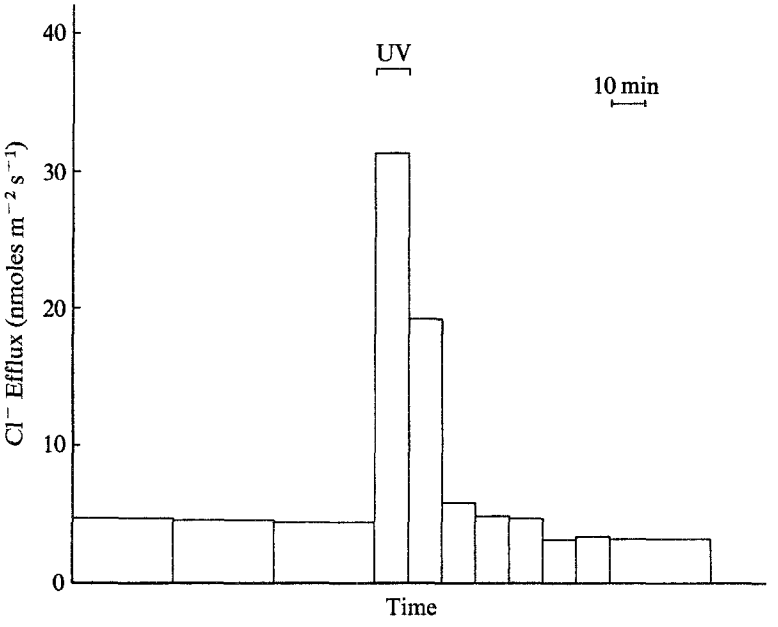

Fig. 3. The effect of 10 min of UV irradiation on the chloride efflux from a single cell, calculated assuming the whole cell was uniformly affected

efflux. This is not correct if the nonirradiated half of the cell has an efflux about equal to the resting value while the irradiated half has a changed efflux (*see* Discussion). Fig. 3 shows a typical result for the efflux of chloride. The mean Cl^- efflux before irradiation was 5.4 ± 0.6 (6) nmoles m^{-2} sec^{-1}. In the 10 min of UV irradiation, the mean efflux from the whole cell was increased about 6 or 7 times, but rapidly decreased to the control value within 30 to 40 min after irradiation. The efflux of chloride into a medium containing sulfate instead of chloride was only about 1 nmole m^{-2} sec^{-1}, in agreement with Findlay *et al.* (1969) but the UV-stimulated efflux was up to 35 nmoles m^{-2} sec^{-1}.

The control value for the Na^+ efflux in nine cells was 4.8 ± 0.6 nmoles m^{-2} sec^{-1}. The 10 min of UV radiation caused only a moderate increase in the efflux as shown in Fig. 4, and this occurred after the UV was turned off, reaching an average maximum of about 8 nmoles m^{-2} sec^{-1} within 30 min. The efflux then gradually decreased over the next hour.

Fig. 5 shows the effect of 10-min UV exposure on the K^+ efflux from a single cell. For 10 cells the control K^+ efflux was 32 ± 2 nmoles m^{-2} sec^{-1} and the UV irradiation caused an increase which reached a maximum of

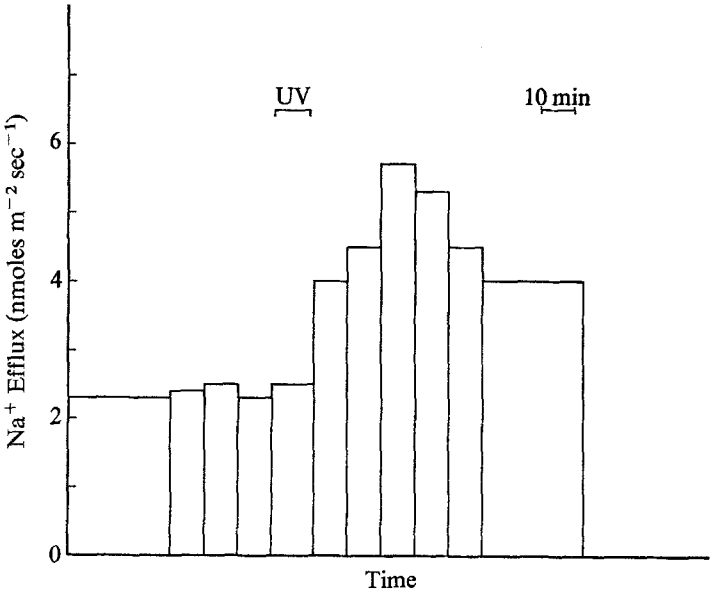

Fig. 4. The effect of 10 min of UV irradiation on the sodium efflux from a single cell, calculated assuming the whole cell was uniformly affected

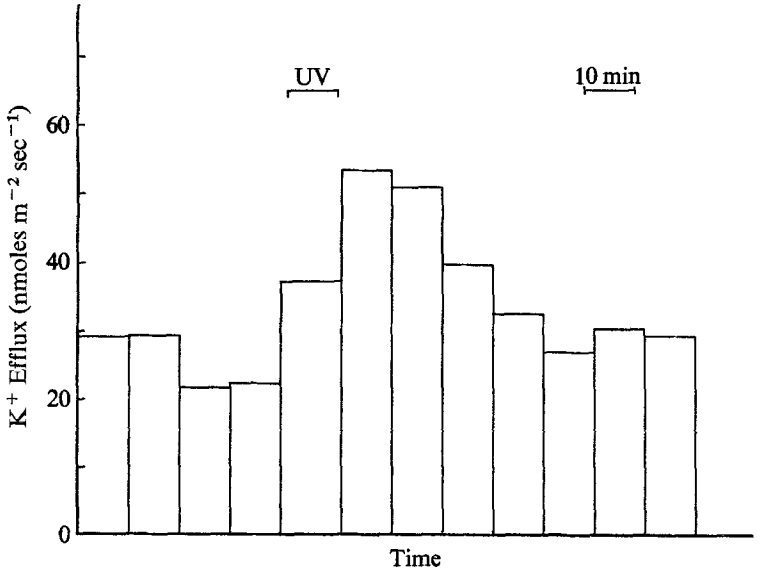

Fig. 5. The effect of 10 min of UV irradiation on the potassium efflux from a single cell, calculated assuming the whole cell was uniformly affected

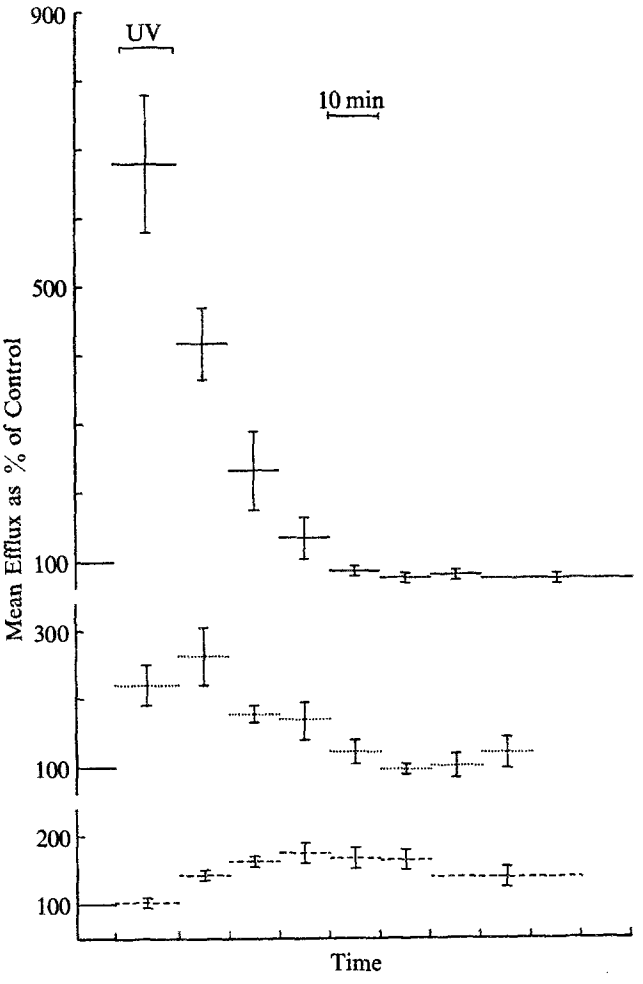

Fig. 6. A summary of the efflux experiments, with the mean effluxes during and after UV irradiation expressed as percentages of the average pre-irradiation (control) values for the appropriate ion. The chloride results are indicated by the full lines; the sodium results by the dashed lines; and the potassium results by the dotted lines. The errors bars are SEM

about 83 nmoles m^{-2} sec^{-1} 10 min after the UV had been turned off. The efflux then gradually decreased to the control value within 40 min.

The results of the efflux experiments are summarized in Fig. 6 in which the effluxes are expressed as a percentage of the average values before irradiation. It is seen that except for sodium, the increases in efflux due to UV were reversed within 40 min but that the sodium efflux had not reached the resting value 70 min after irradiation.

Discussion

The Effects of UV on C. corallina

A possible equation to describe the potential across the plasmalemma in *C. corallina* is of the form:

$$\psi_{co}=58\log_{10}\frac{P_K[K]_o+P_{Na}[Na]_o+P_H[H]_o+P_{Cl}[Cl]_c}{P_K[K]_c+P_{Na}[Na]_c+P_H[H]_c+P_{Cl}[Cl]_o}+\psi_{AT} \qquad (1)$$

[*cf.* equation (6.1) of Hope (1971)], where symbols P refer to permeability, o to the medium, c to the cytoplasm, and ψ_{AT} is a component due to electrogenic active transport. There is evidence that extrusion of protons may give rise to such an effect (Kitasato, 1968). Many of the concentrations are known, and the permeabilities may be calculated from observed passive fluxes, if some assumptions are made. In FPW, pH 5.5, the importance of the term $P_H[H]_{o,c}$ is probably not very great, nor is the electrogenic component, though it becomes much more significant at pH 6 to 8 (Richards & Hope, 1972, *unpublished data.*)

Changes in membrane p.d., which have been identified as changes in ψ_{co}, and which occur as a result of UV irradiation, may be due to changes in any of the permeabilities, or in ψ_{AT}. Changes in cytoplasmic ion concentrations following permeability changes are unlikely in the times under consideration (5 to 10 min – Fig. 1). Changes due to products of ionization do not seem possible with UV irradiation of the wavelength used, the effects usually being thought of as direct and not due to ionization and free radicles. Consideration of the Nernst potentials (Table 3) shows that depolarization of the plasmalemma would be expected for an increase in P_{Na} or P_{Cl} but not in P_K. In either case, an increase in membrane conductance would be expected to accompany the depolarization. If P_{Na} increased, the driving force on Na^+ is such that an increase in influx or decrease in efflux should

Table 3. Nernst potentials of the ion species K^+, Na^+ and Cl^- for the plasmalemma of *C. corallina*[a]

Ion (j)	Ext conc (mM)	Cyt conc (mM)	ψ_j (mV)
K^+	0.2	100	−156
Na^+	2.0	40	−75
Cl^-	2.25	15	+48

[a] The cytoplasmic concentrations adopted are those measured for *C. corallina* and other charophyte cells. For K^+, *see* Vorobiev (1967); for Na^+, Richards (*unpublished data*); for Cl^-, *see* Coster (1966) and Lefebvre and Gillet (1971). The observed mean vacuolar p.d. in these experiments was −145 mV. The p.d. across the tonoplast is usually 10 mV (Findlay & Hope, 1964; this study) and hence $\psi_{co}\approx -155$ mV, close to ψ_K.

occur, especially the former. If P_{Cl} increased, an increase in chloride efflux should occur. The passive influx of Cl^-, because these ions are so far from equilibrium, is probably very small indeed, the total influx comprising an active and an exchange diffusion component only (Findlay *et al.*, 1969).

The results reported here are qualitatively consistent with UV radiation causing an immediate specific increase in passive permeability to Cl^-, since a substantial, reversible increase in membrane conductance accompanied the depolarization; also, in parallel experiments, a very large reversible increase in passive efflux of chloride, as shown by the tracer studies, occurred at times corresponding to the electrical changes.

Whether the observed increase in chloride permeability is quantitatively consistent with the depolarization may be tested by means of Eq. (1), simplified to take into account only the ions K^+, Na^+ and Cl^-, and omitting any component of electrogenic active transport, for the present. A serious objection to the use of such an equation is that implicit assumptions of independence of ion movements, and of a linear potential gradient in the membrane, have been made. At present, no suitable alternative theory seems to exist. The permeability coefficients in the present treatment should be regarded as parameters that may be useful to describe the membrane properties under defined conditions. Table 4 gives the values of permeabilities calculated from the observed passive fluxes of ions in the present experiments. We have assumed the efflux of chloride (into sulfate media), the efflux of potassium and the influx of sodium are passive fluxes for this purpose, although some of the sodium influx may be linked to light-stimulated chloride influx (Findlay *et al.*, 1969). We have made two alternative assumptions about the area of the plasmalemma that has been affected by the UV radiation. On the one hand, approximately one-half of the cell area may be uniformly affected (though this is not exact due to the changing angles of incidence between the top and side of the cell). On the other hand, if the effects of UV radiation on permeability were carried via the streaming cytoplasm or diffusion, it is possible that the whole cell area becomes uniformly changed within a few minutes. We favor the first alternative. In either case, the p.d. of the plasmalemma for the purpose of calculating P_j from the Goldman flux equation (*see* Table 4) is taken as the average p.d. observed during rest or irradiation. Though the p.d. of the irradiated portion may change more than the average cell p.d. indicates, the conclusions we draw do not depend strongly on the p.d.'s used for these calculations.

Using these permeabilities, it may be seen that the resting value of ψ_{co} would be -146 mV, which is fairly close to the mean observed, -155 mV.

Table 4. Changes in the permeability coefficients after UV irradiation[a]

Ion	Conc (mole liter^{-1})		Passive flux (nmoles m^{-2} sec^{-1})			Permeability (m sec^{-1})		
	o	*c*	Control	UV (whole cell area)	UV (half cell area)	Control	UV (whole cell area)	UV (half cell area)
Na^+	2.0×10^{-3}	4.0×10^{-2}	16	17	18	1.3×10^{-9}	1.5×10^{-9}	1.5×10^{-9}
K^+	2.0×10^{-4}	1.0×10^{-1}	32	71	110	2.4×10^{-8}	4.1×10^{-8}	6.4×10^{-8}
Cl^-	2.2×10^{-3}	1.5×10^{-2}	1	37	73	10.8×10^{-12}	4.2×10^{-10}	8.3×10^{-10}

[a] The permeabilities were calculated from the following equations for passive fluxes (after Goldman, 1943)

$$\text{influx} = \vec{\phi}_j = -\frac{P_j z_i F \psi_{co}}{RT} \frac{c_j^o}{1-\exp(z_j F \psi_{co}/RT)}$$

$$\text{efflux} = \overleftarrow{\phi}_j = -\frac{P_j z_j F \psi_{co}}{RT} \frac{c_j^c \exp(z_j F \psi_{co}/RT)}{\exp(z_j F \psi_{co}/RT)-1}$$

where P is permeability, z is valency and ψ_{co} is the p.d. across the plasmalemma. The only passive fluxes for *C. corallina* were assumed to be K^+, Cl^- efflux and Na^+ influx. The permeability values for irradiated cells were calculated using the average p.d. of -147 mV during UV irradiation since the observed fluxes were averages for the 10-min irradiation period.

Using next the mean values for the increased fluxes during the period of UV irradiation (Fig. 6), a value for the depolarized p.d. may be calculated. It will be approximate because the flux can be determined only as an average in the 10-min period whereas the p.d. has a complicated time course during that time (Fig. 1). This calculation leads to either -137 or -138 mV (depending on the area used for permeability calculations), a mean depolarization of 9 or 8 mV, in agreement with the observation of an average depolarization of 8 mV during 10 min of UV irradiation. During the 10-min time interval following the irradiation, the p.d. had passed its second wave of depolarization and was returning toward the resting level (Fig. 1). In this interval also the chloride efflux was declining and the potassium efflux was still higher, both factors tending in the expression for ψ_{co} to return it to the resting value.

When we come to examine the changes in plasmalemma resistance, it is impossible (as many authors have noted) to get agreement between the calculated and observed values, using either the resting or UV-increased permeabilities. Walker and Hope (1969) interpreted this lack of agreement as indicating nonindependent ion migration and the inappropriateness of

the Goldman equation, which assumes independence. While this latter is probably a factor, some degree of proton permeability and possibly a conductance inherent in an active proton pump (Spanswick, 1972) can also lead to an observed resistance much less than calculated. To calculate resting resistance use may be made of the following equation:

$$r_0 = -R^2 T^2 (1/C_o - 1/C_c)/(F^3 \psi_{co}) \quad (2)$$

(Hope & Walker, 1961), where $C_o = P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_c$, $C_c = P_K[K]_c + P_{Na}[Na]_c + P_{Cl}[Cl]_o$. Using parameters already defined, $r_o = 5.6\ \Omega\ m^2$, without UV irradiation, compared with the observed average of $1\ \Omega\ m^2$. The UV-induced increases in permeability suggest that r_o should decrease to about 1.6 or 2.6 $\Omega\ m^2$ (depending on the area used), whereas 0.8 is observed (the mean of the 10-min period including UV irradiation – *see* Fig. 2).

If the inclusion of proton fluxes is the only way to resolve the disagreement over the resting resistance then it follows that UV radiation may affect proton fluxes, either through alteration in active transport or passive permeability. For the present, it is clear that one of the prompt effects on *Chara* of nonlethal doses of UV radiation of wavelength 253.7 nm is a reversible increase in passive permeability to chloride ions, and later, potassium ions.

Another indication that another ion species may contribute to ion fluxes, and hence conductance, comes from consideration of the net flux of charge across the plasmalemma in the resting or irradiated condition. Calculations made from the fluxes given in the Results show that in the resting state there is an equivalent efflux of positive charge of 33 ± 5 nmoles $m^{-2}\ sec^{-1}$, and that during the 10-min UV irradiation this has increased to 49 ± 7 nmoles $m^{-2}\ sec^{-1}$ (mean flux for whole cell area). If the cells are to stay electrically neutral, there must be an equivalent influx of some positive ions or efflux of negative ions as yet unidentified. The direction of the electrochemical potential gradient for protons would allow a net passive influx of protons provided the plasmalemma is proton permeable. The possibility that UV radiation also inhibits a putative active efflux of protons is clearly worth investigation.

Comparison with Other Work

Changes in passive permeability after irradiation have been inferred in a number of studies with erythrocytes and nerve axons, as well as plant cells. Ozerskii (1969) has reviewed some of the evidence from the use of ionizing

radiation making the point that inactivation of an active transport system is also a possible effect, having in mind particularly the Na^+ efflux pump that is ubiquitous in animal cells. It has been suggested that permeability changes may be the primary lesions that eventually lead to cell death (Bacq & Alexander, 1961).

Esch *et al.* (1964) and Esch (1966) explained the depolarization of the resting potential of *Nitella*, after irradiation with X-rays, as a temporary reduction in potassium permeability. The present results are inconsistent with this interpretation, which was made solely on the basis of the changes in p.d. in *Nitella*. Hansen (1967) also observed an X-ray-induced depolarization of *Nitella* and by reference to an equation similar to (1) above showed that an increase in permeability to chloride might explain the effect. Gaffey (1972) suggested that since action potentials could be invoked in *Nitella* by 60 MeV α-particles, the radiation caused the same permeability changes as those responsible for an action potential stimulated by an electric pulse. In charophyte cells the action potential is caused by a transient, large increase in chloride permeability. However, we have some evidence (Findlay, Hope & Sydenham, *unpublished data*) that increases in the resting chloride permeability may occur, with the addition of nystatin, while the cells become inexcitable. Thus, the channels for the transient increase in chloride flux may be independent of those for the resting flux.

Future work will aim at answering the following questions: What are the effects of UV irradiation on transient permeability changes during the action potential, both at the plasmalemma and tonoplast? Are there different effects at these membranes and on organelle membranes? What is the target molecule(s) in the plasmalemma? What is the action of UV irradiation on the hyperpolarization of the plasmalemma observed in solutions of higher pH?

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