An Investigation into the Roles of Photosynthesis and Respiration in H^+ Efflux from Aerated Suspensions of *Asparagus* Mesophyll Cells[1]

Received for publication October 22, 1981 and in revised form May 10, 1982

ALAN W. BOWN
Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1 Canada

ABSTRACT

Aerated and stirred suspensions of mechanically isolated *Asparagus sprengeri* Regel mesophyll cells were used to investigate the roles of respiration and photosynthesis in net H^+ efflux. Rates varied between 0.12 and 1.99 nanomoles H^+ per 10^6 cells per minute or 3 and 40 nanomoles H^+ per milligram chlorophyll per minute. The mean rate of H^+ efflux was 10% greater in the dark. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea, an inhibitor of noncyclic photophosphorylation, did not inhibit H^+ efflux from illuminated cells. Bubbling with N_2 or addition of oligomycin, an inhibitor of mitochondrial ATP production, resulted in rapid and virtually complete inhibition of H^+ efflux in light or dark. In the absence of aeration, H^+ efflux came to a halt but resumed with aeration or illumination. When aeration was switched to CO_2-free air, rates of H^+ efflux were reduced 43% in the dark and 57% in the light. Oligomycin eliminated dark CO_2 fixation but not photosynthetic CO_2 fixation. It is suggested that H^+ efflux is dependent on respiration and dark CO_2 fixation, but independent of photosynthesis.

The membrane potential of plant cells is usually greater than can be accounted for by outward diffusion of accumulated ions. Evidence suggests that an ATP requiring electrogenic H^+ efflux is involved in creating the additional separation of charge across the membrane (8, 14, 26, 27). Reports on the influence of light-dark transitions on membrane potentials and net rates of H^+ flux in photosynthetic cells are conflicting and difficult to interpret. In some cases, changes in illumination cause only transient changes in the resting membrane potential (25) in others, photosynthetically active light causes hyperpolarization of the resting potential (5, 11). Light-dependent alkalinization and acidification of the medium bathing leaf slices has been detected (9, 16, 19, 23, 25). The apparent proton influx could be passive and reflect H^+ fluxes within the chloroplast (25) or photosynthetic reduction of CO_2 levels in the medium (9, 11, 16). In slices of pea and *Atriplex* leaves, light stimulated net proton efflux (19, 23). It is not clear, however, that the energy required for active H^+ efflux in light is derived from chloroplasts (11). Unlike mitochondria which can export ATP directly chloroplasts export triosephosphate which may be used for ATP synthesis in the cytosol (7, 28).

Limited gaseous diffusion may have resulted in conflicting literature data. In some reports, the medium bathing the leaf slices is aerated (25) in others the tissue is perfused (5), placed on a shaker (19) or placed in a closed system (9, 16). In all cases diffusion of O_2 or CO_2 between the medium and the intact tissue could be limiting for respiration or photosynthesis. Diffusion of gases in water-filled air spaces will be significantly slower than diffusion in air, and photosynthetic rates in submerged leaf slices are significantly reduced when compared to intact leaves (20, 21). Thus, reports that photosynthesis can drive membrane transport processes may arise indirectly through a stimulation of respiration resulting from photosynthetic O_2 production.

In the present study, the influence of light on net rates of H^+ efflux was investigated using a suspension of mechanically isolated mesophyll cells. The cell suspension was stirred and aerated to reduce diffusion distance to a minimum. The unbuffered suspension medium of 5 mM KCl, 5 mM NaCl, and 0.1 mM $CaCl_2$ was chosen to reflect ion concentrations in the leaf free space (18).

MATERIALS AND METHODS

Asparagus sprengeri Regel was grown at 20°C in vermiculite in a growth chamber under a 12-h photoperiod and cool white Sylvania fluorescent tubes. Cladophylls 1.5 to 2.5 cm long and less than 3 months old were washed and 3 g of tissue cut into 0.5-cm long sections. Cells were isolated at room temperature using gentle grinding in 20 mM (pH 7.2) K-phosphate buffer (4). Released cells were filtered through muslin and centrifuged twice at 400*g* for 2 min before resuspension in 15 ml unbuffered salt solution (5 mM KCl, 5 mM NaCl, 0.1 mM $CaCl_2$). Cells were stored on ice in the dark and used the same day. Chl content of the cell suspension was determined by the method of Arnon (1), and cell numbers measured using a hemocytometer and light microscope. The fraction of cells with intact protoplasts was determined through their ability to exclude Evans blue (13).

Recordings of pH changes with time were made using Radiometer (Copenhagen) equipment. A PHM 64 pH meter with a combination electrode was coupled to an REC 61 recorder through an REA 100 pH meter interface. Full scale deflection of the recorder pen (25 cm) corresponded to a pH change of 0.7. The electrode tip was immersed in 7 ml of the unbuffered salt solution and recordings of pH initiated prior to the addition of 10 to 12 × 10^6 cells contained in 3 ml of the cell suspension. The 10-ml volume was maintainted at 30°C in an open water-jacketed glass vessel with a tapered inside wall (taper stopper 34/45). The vessel, which was treated with Sigma Sigmacote (Sigma) to prevent cells sticking to the glass, was placed on a magnetic stirrer and the cells were maintainted in suspension with a spin bar and aeration at 500 ml min^{-1} through an hypodermic needle (Yale 22). Conversion into a stirred but closed system was achieved by sliding a 6-cm high, tapered, polyethylene cone into the glass taper until a 13-ml volume remained. The electrode and an hypodermic needle fitted snugly into holes drilled into the cone to allow pH measurements and addition of reagents. The apparatus was surrounded

[1] Supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC).

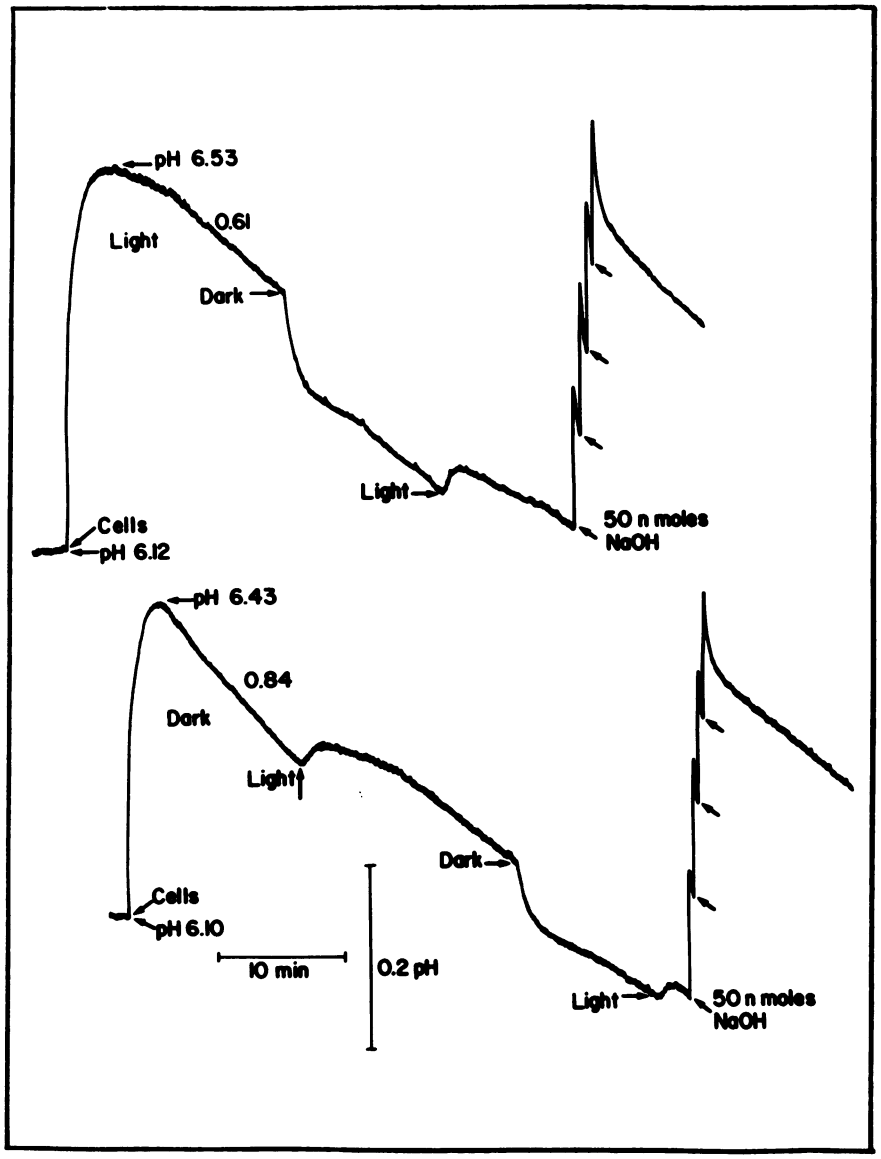

FIG. 1. The influence of light-dark transitions on the rate of acidification. Upper curve experiment started in the light, lower curve experiment started in the dark. Rates indicated are expressed as nmol $H^+/10^6$ cells · min.

Table I. *Effect of Light on Net Rates of H^+ Efflux*

Mean values obtained from a minimum of eight measurements. Results expressed ± SD

	Light	Dark
nmol $H^+/10^6$ cells·min	0.77 ± 0.45	0.85 ± 0.42
nmol H^+/mg Chl·min	18.0 ± 11.4	20.2 ± 9.6

by a light tight box with two removable opposite sides to allow illumination of the cells from two directions, and light tight apertures for the electrode, air line, and a syringe needle. Back titration of the cell suspension with standard alkali allowed calculation of the net rate of H^+ efflux as nmol $H^+/10^6$ cells · min or nmol H^+/mg Chl · min. Cell suspensions were illuminated with a 300 w reflector lamp (Sylvania) which gave an irradiance at the surface of the vessel of $1{,}100 \times 10^{-5}$ w cm^{-2} as measured with an Alphametrics radiometer (model 1010) equipped with a broad-band (400–100 nm) probe.

Dark CO_2 fixation experiments were conducted in the same water-jacketed glass vessel as used for pH measurements using 15 $\times 10^6$ cells suspended in 5 ml 5 mM K-phosphate buffer (pH 7.2). A tapered 7-cm polyethylene cone with aperture for a syringe needle converted the open vessel into a closed vessel of 5 ml capacity. A stirring bar maintained the cells in suspension. At time zero, 20×10^6 dpm of ^{14}C labeled $H^{14}CO_3^-$ (58.2 mCi $mmol^{-1}$) was added. At set times, 0.1-ml samples of cell suspension were removed to scintillation vials containing 1 ml 80% (v/v) ethanol and dried overnight in a fume hood, prior to scintillation counting.

Rates of O_2 production in the light and consumption in the dark were measured with a calibrated Clark O_2 probe (YSI 4004) connected to a recorder. To the water-jacketed glass vessel was added a stirring bar, 6.5 ml 5 mM K-phosphate buffer (pH 7.2), and 4 to 6×10^6 cells contained in 1 ml of suspension. The vessel was converted into a closed system of 7.5 ml capacity using a polyethylene cone with apertures for the electrode and a syringe needle. Rates of O_2 production were measured at an irradiance of $1{,}000 \times 10^{-5}$ w cm^{-2}, after addition of 100 μl of sodium bicarbonate (100 mM) to ensure saturation with CO_2 (4). Rates of O_2 consumption in the dark were measured immediately the cells were added to the glass vessel.

Total dissolved inorganic carbon was determined using the

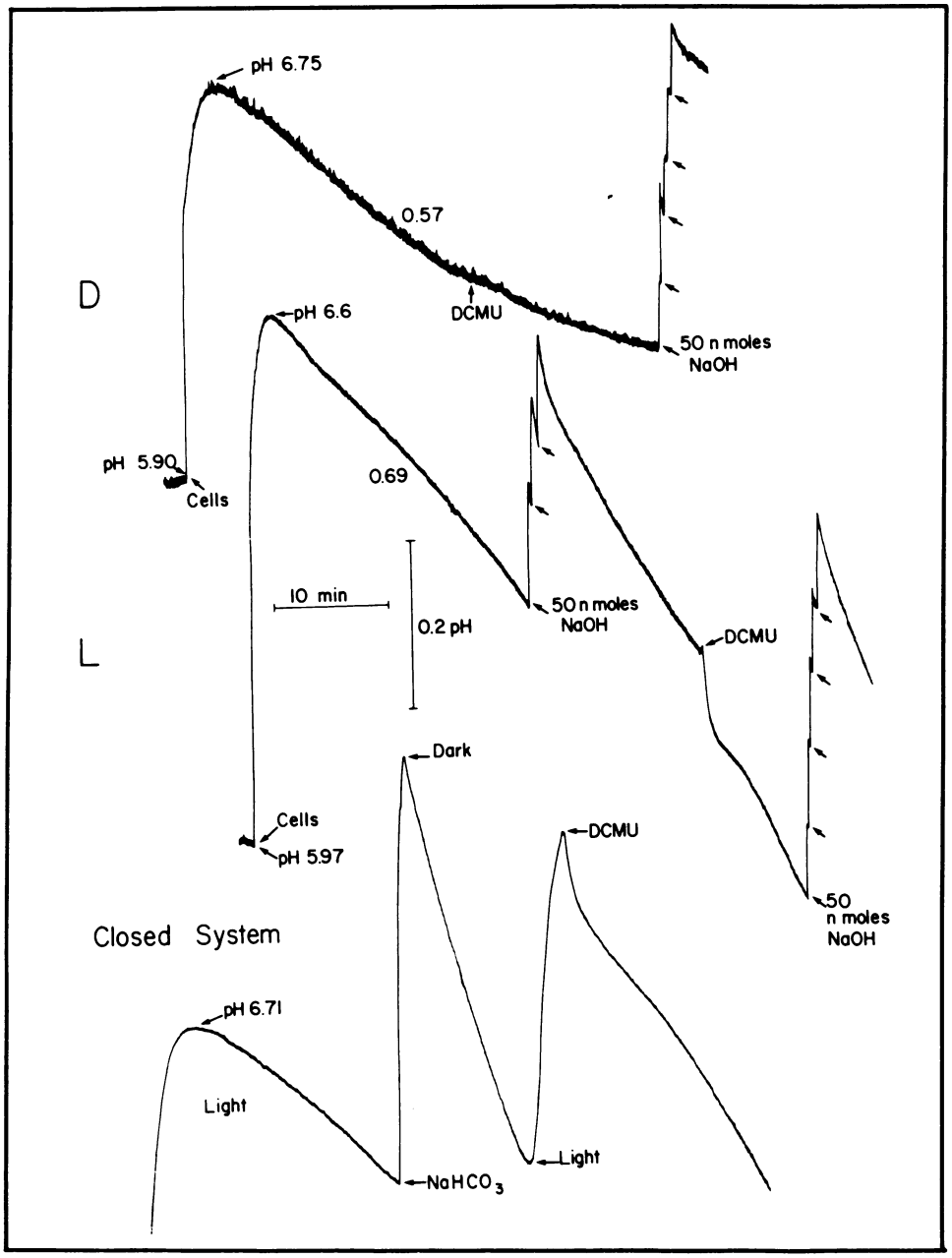

FIG. 2. The influence of DCMU on rates of acidification and light-driven alkalinization. Upper and middle curves derived from experiments with aeration throughout; the lower curve from an experiment which was started in the light using a closed system. The final concentration of DCMU was 1 μM, $NaHCO_3$ addition resulted in a concentration increase of 70 μM. The rates of acidification indicated are expressed in nmol $H^+/10^6$ cells · min. L, light; D, dark.

method of Birmingham and Colman (2). Stock solutions of DES,[2] FCCP, DCMU, and oligomycin were made up in 80% (v/v) ethanol and 0.1-ml volumes added to the cell suspension as indicated. All concentrations quoted are final concentrations after dilution with the cell suspension.

RESULTS

Addition of cells to the stirred and aerated unbuffered salt solution invariably resulted in a rapid pH rise to around 6.3 to 6.8. Typically, the increase was around 0.3 to 0.6 units and greatest when cells were illuminated. After approximately 4 min the pH started to decline (Figs. 1–4, and 6). Acidification continued at a diminishing rate until a constant pH between 4.0 to 5.0 was attained (data not shown). Initial rates of net H^+ efflux varied between 0.12 and 1.99 nmol $H^+/10^6$ cells · min^{-1} or 3 to 40 nmol H^+/mg Chl · min. However, the mean value for net H^+ efflux in the dark was approximately 10% higher than the rate in the light (Table I), suggesting that light does not result in a significant stimulation of H^+ efflux.

The influence of light on the rate of net H^+ efflux was investigated further using light to dark transitions. When illumination began, a short-lived pH increase interrupted the pH decline; when illumination stopped, there was a transient increase in the rate of pH decline (Fig. 1). These rapid changes indicated that photosynthesis reduced the steady-state CO_2 concentration in the stirred and aerated cell suspension. The relationship between increasing

[2] Abbreviations: DES, diethylstilbestrol; FCCP, carobonyl cyanide *p*-trifluoromethoxyphenyl hydrazone.

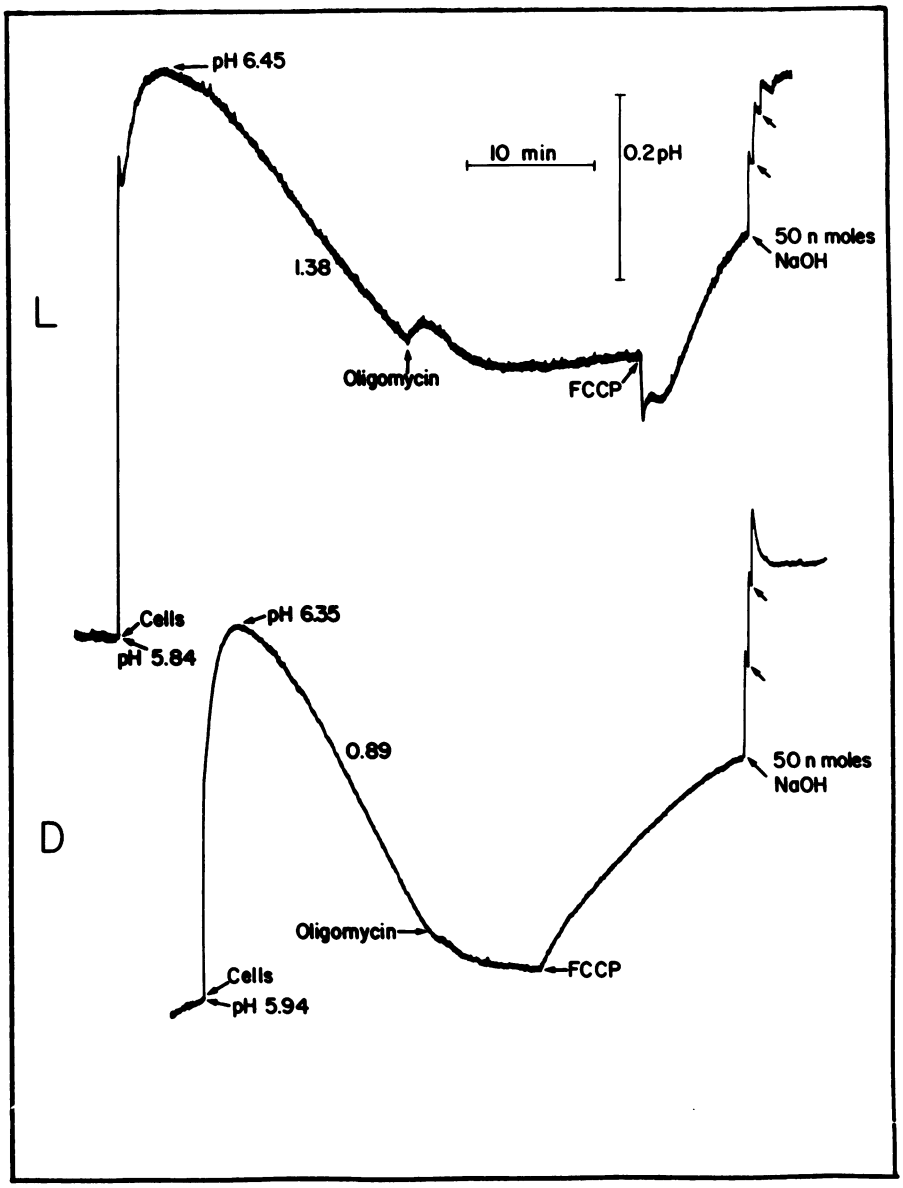

FIG. 3. The influence of oligomycin on rates of acidification in light or dark. The final concentration of oligomycin was 10 μg/ml, and of FCCP 0.8 μM. Rates of acidification are expressed in nmol $H^+/10^6$ cells · min. L, light; D, dark.

pH and decreasing CO_2 concentration was confirmed by removing aliquots of illuminated suspension medium during the pH rise following addition of cells to stirred but nonaerated medium. For each pH value, the total dissolved inorganic carbon was measured and CO_2 and bicarbonate concentrations calculated. As the pH rose from 5.6 to 6.0, the CO_2 level dropped from 35.0 to 5.6 μM and the bicarbonate concentration dropped from 6.1 to 2.5 μM. Alkalization stopped at approximately the same time as did the decrease in CO_2 and bicarbonate concentrations. When the cell suspension was aerated, the concentration of CO_2 at pH 6.54 was found to be 5.9 μM, and that of the bicarbonate 9.0 μM. When air was replaced with CO_2-free air, the pH rose within 4 min to 6.93 and the CO_2 and bicarbonate concentrations were reduced to 1.8 and 6.9 μM, respectively.

Apart from immediate transient effects, switching from light to dark diminished the rate of acidification by an average of 10% and switching from dark to light decreased acidification by 23%. Thus, the decline in acidification rate was greater in the presence of light (Fig. 1). In some experiments, the rate of acidification was stimulated by up to 25% when illumination ceased. The Chl content of the cell suspension varied between 0.02 and 0.11 mg Chl/10^6 cells. However, no correlation could be found between net rates of H^+ efflux in the light and Chl content per cell. This further suggests the absence of a direct relationship between photosynthesis and H^+ efflux. Support for this view was obtained using DCMU, an inhibitor of noncyclic photophosphorylation. DCMU did not influence acidification in the dark but actually stimulated acidification in the light by approximately 50% (Fig. 2). Experiments were conducted with the closed system to determine whether DCMU inhibited photosynthesis. In the light, cells maintained in suspension with vigorous stirring acidified the medium at a reduced rate. Alkalinization resulted on the addition of $NaHCO_3$, and was reversed immediately when illumination stopped. Reintroduction of light resulted in photosynthetically driven CO_2 uptake and alkalinization which was reversed immediately 10^{-6} M DCMU was added to the cells (Fig. 2). Thus, DCMU inhibited photosynthetic CO_2 fixation without inhibiting acidification.

The role of respiration in H^+ efflux was investigated using two inhibitors of oxidative phosphorylation oligomycin and 100% N_2.

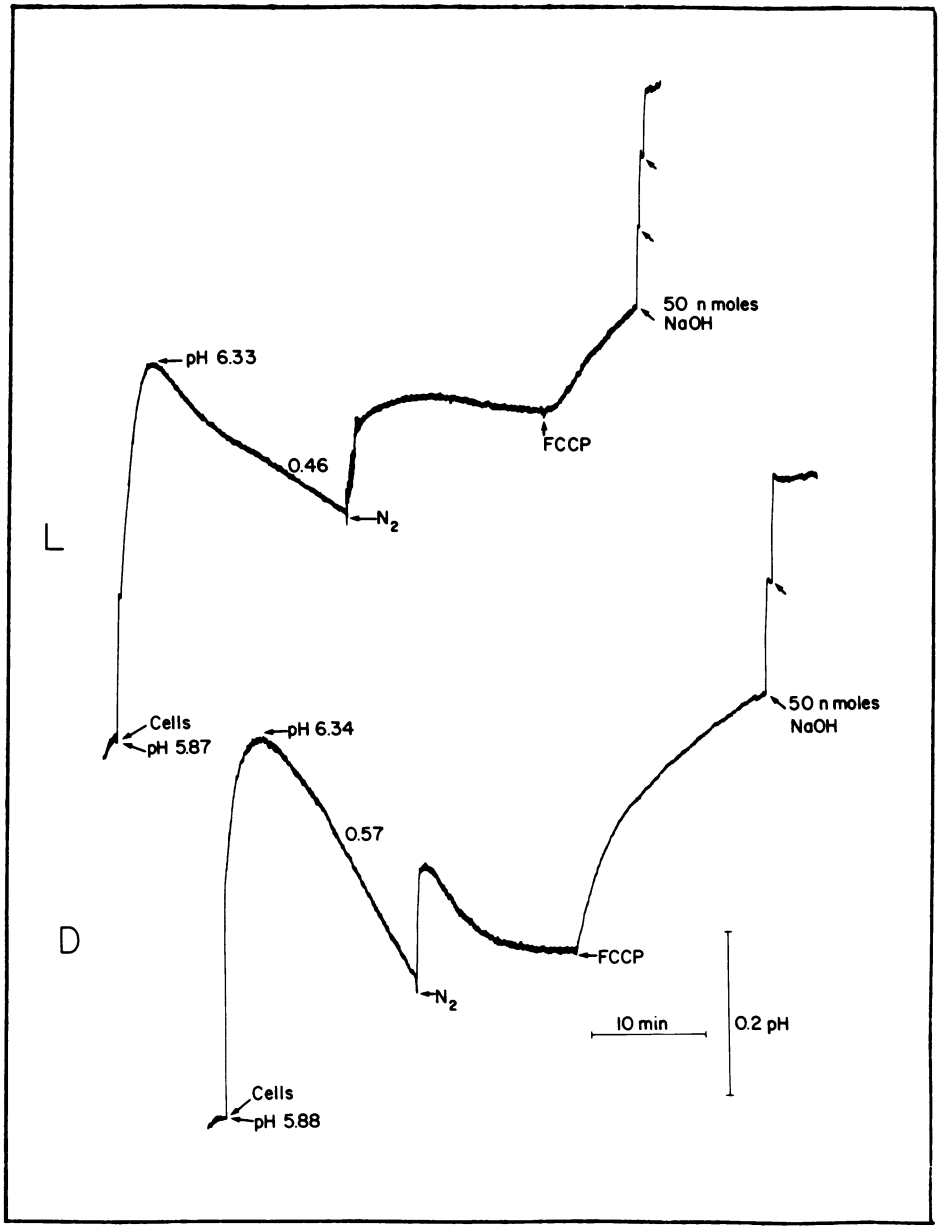

FIG. 4. The influence of N_2 on rates of acidification in light or dark. Bubbling was changed from air to 100% N_2 at the times indicated. Rates of acidification are expressed in nmol $H^+/10^6$ cells · min. The final concentration of FCCP was 0.8 μM. L, light; D, dark.

The volatile nature of cyanide and its acid base properties precluded its use in an aerated nonbuffered system. Both inhibitors resulted in rapid virtually complete inhibition of acidification in light or dark (Figs. 3 and 4). The replacement of air by N_2, however, also resulted in a rapid pH increase resulting from reduction in the CO_2 level of the cell suspension medium (Fig. 4). Inhibition by N_2 was reversed completely on the reintroduction of air (data not shown). It appears that H^+ efflux is dependent on respiration and this view is supported by findings that, in the absence of aeration, net H^+ efflux was eliminated (Fig. 5). After acidification was inhibited by oligomycin or N_2 addition of the protonophore, FCCP resulted in rapid alkalinization, indicating back diffusion of H^+ into the cells (Figs. 3 and 4).

When the nonilluminated cell suspension was stirred slowly without aeration, the majority of cells settled to the bottom of the glass vessel and acidification came to a halt (Fig. 5). The introduction of aeration or light resulted in a period of alkalinization followed by restoration of acidification. Alkalinization can be attributed to the removal of accumulated respiratory CO_2, acidification to the introduction of O_2 via aeration or photosynthesis.

The influence of CO_2 on rates of H^+ efflux was investigated in light or dark by switching aeration between air and CO_2-free air. On changing to CO_2-free air, the reduced CO_2 level invariably led to a pH increase and acidification then resumed at a reduced rate. Acidification in the light was inhibited by a mean value of 57%, whereas the value in the dark was 43%. Reintroduction of air rapidly reduced the pH and often resulted in a stimulated rate of acidification, even though the rate of acidification normally decreased with lower pH (Fig. 6). To investigate the possible relationship between dark fixation of CO_2 and H^+ efflux, the influence of known inhibitors of net H^+ efflux on rates of dark fixation of $H^{14}CO_3^-$ was examined. Oligomycin (26 μg/ml) DES (8 μM) and FCCP (20 μM) all inhibited dark fixation completely within 5 min. In addition, DES and FCCP inhibited photosynthetic fixation (data not shown), whereas oligomycin did not (Fig. 7). In the absence or presence of oligomycin the rate of CO_2 fixation in the

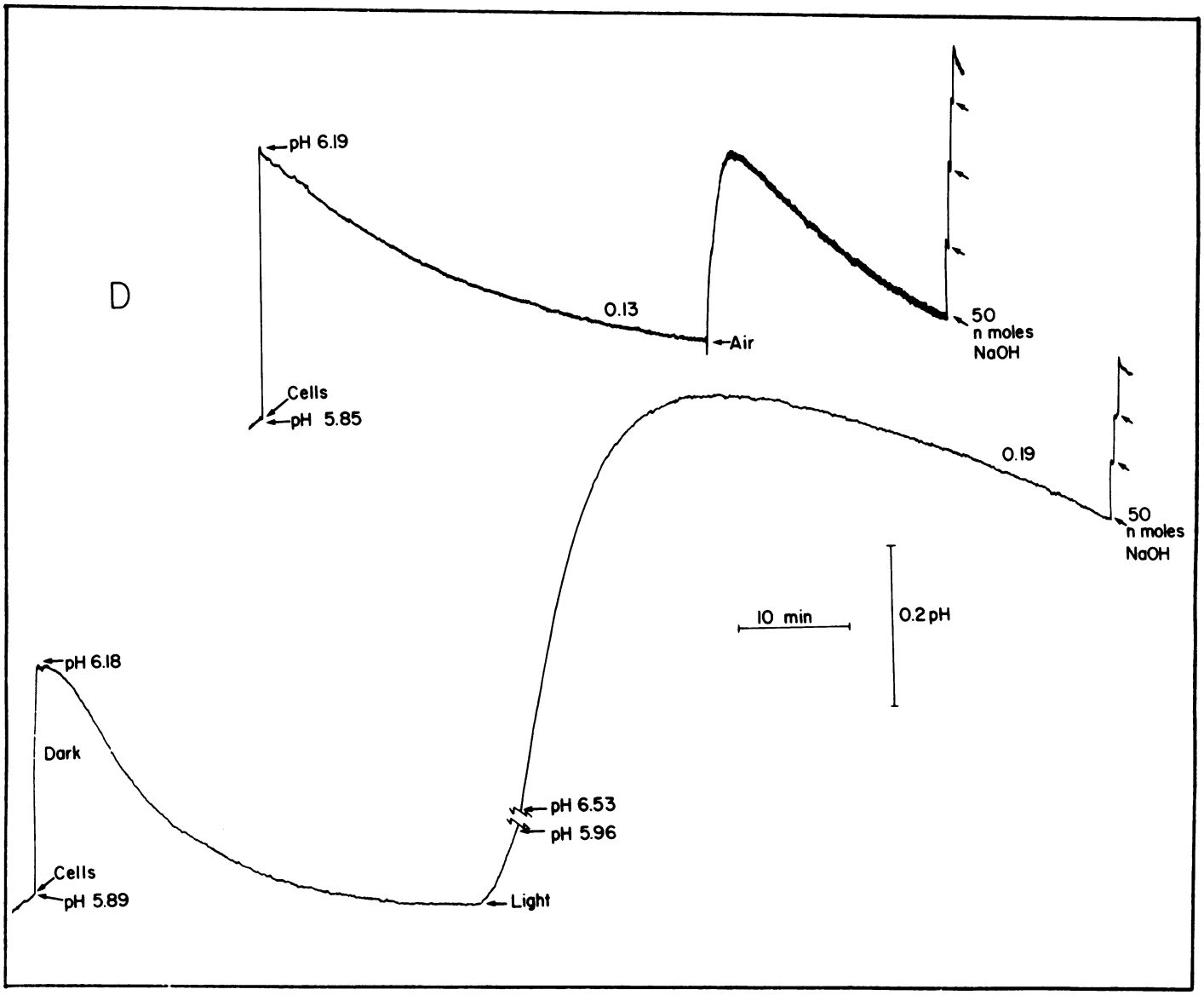

FIG. 5. The influence of aeration or light on rates of acidification from cells stored in the dark with stirring but without aeration. Aeration or light introduced at the times indicated. Rates of acidification are expressed in nmol $H^+/10^6$ cells · min. D, dark.

light was 15 to 20 times greater than the rate in the dark.

Using nine different cell preparations the relative rates of photosynthesis, respiration, and net H^+ efflux were determined on a Chl basis. The mean rate of photosynthetic O_2 production was 34.4 μmol O_2 mg^{-1} Chl h^{-1} (±12.4 SD), the rate of respiratory O_2 consumption 6.9 μmol O_2 mg^{-1} Chl h^{-1} (±2.8 SD), and the net rate of H^+ efflux 1.02 μmol H^+ mg^{-1} Chl (±0.46 SD). DCMU (10^{-6} M) completely inhibited photosynthetic O_2 production.

DISCUSSION

Acidification of the medium could result from (a) the release of organic acids, (b) HCO_3^- uptake, (c) the formation of carbonic acid from respiratory CO_2, and (d) H^+ efflux. Titration of the suspension medium after acidification did not reveal any buffering capacity, indicating that acidification was not due to excretion of organic acids (data not shown). *Asparagus* cells take up CO_2 not HCO_3^- during photosynthesis (personal communication, Dr. B. Colman, York University). Experiments in which medium in an open beaker system was gently agitated on a gyratory shaker demonstrated that CO_2 escapes to the atmosphere too rapidly to allow significant acidification (24). In this study, the medium was both stirred and vigorously bubbled to ensure rapid equilibration with the gas mixture being used. The small but rapid increases in pH which occurred when normal air containing CO_2 was replaced with CO_2-free air or N_2 demonstrates that equilibration occurred within 4 min in this system (Figs. 4 and 6). Similar small rapid changes in pH were obtained when medium without cells was subjected to changes in the aerating gas. It is particularly difficult to argue that acidification in the light results from CO_2 accumulation when the cells photosynthetic capacity to remove CO_2 appears to be five times greater than the cells respiratory activity. The rapid alkalinization which occurs on the addition of the protonophore FCCP (Figs. 3 and 4) is a strong indication that acidification results from H^+ efflux against a proton electrochemical gradient.

Thus, the evidence indicates that long-term acidification results from energy-dependent H^+ efflux. However, the rapid changes in pH which occur within 4 min in response to changes in aeration or illumination are consistent with changes in steady-state levels of CO_2 in the medium (Figs. 1, 4, and 6). Analysis of dissolved inorganic carbon demonstrated that CO_2 in the medium diminished when a transient increase in pH occurred in response to a

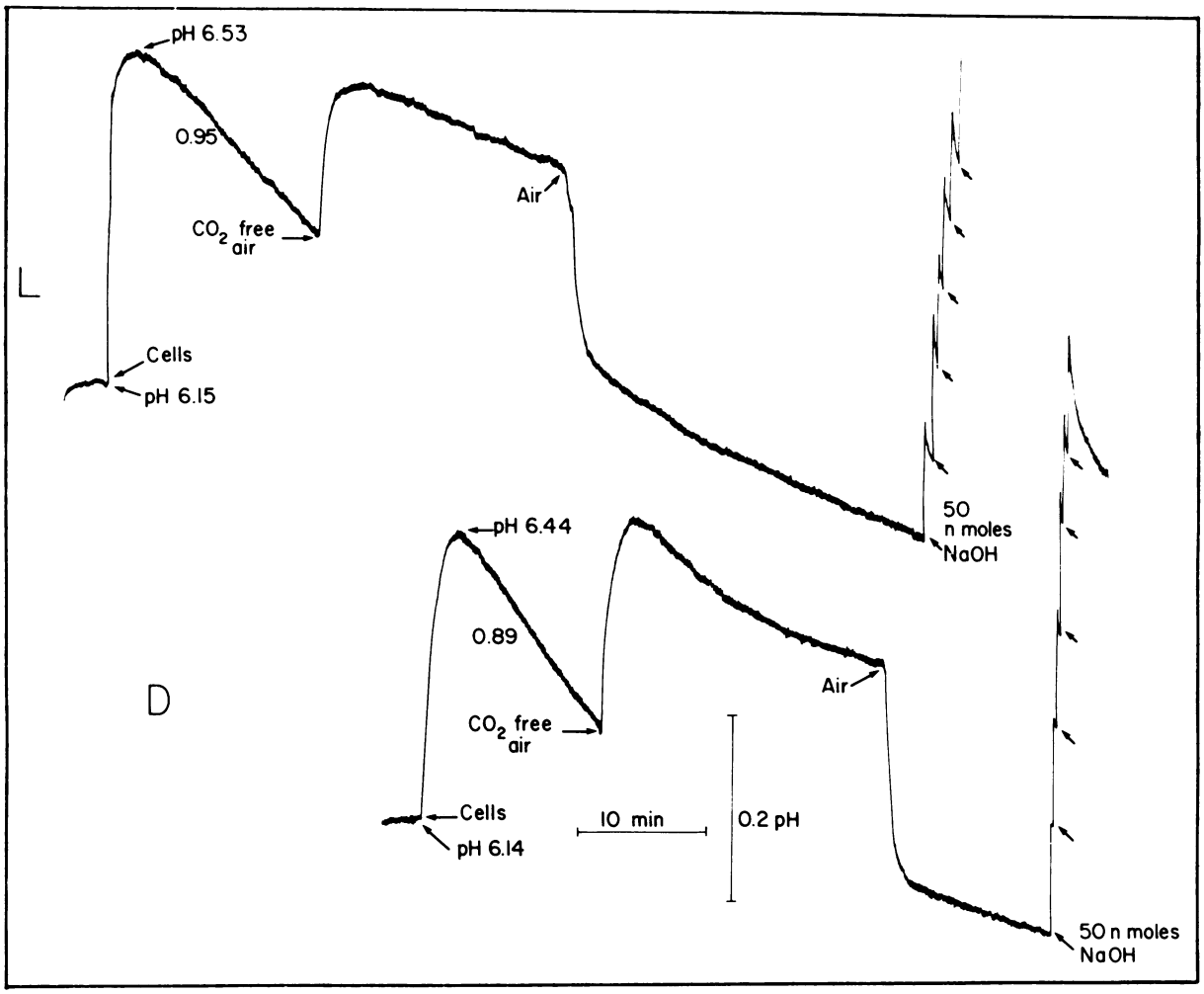

FIG. 6. The influence of CO_2 free air on rates of acidification in light or dark. CO_2-free air, or air were introduced at the times indicated. Rates of acidification are expressed as nmol $H^+/10^6$ cells · min. L, light; D, dark.

switch from air to CO_2-free air. The rapid acidification seen on termination of illumination (Fig. 1) may well indicate a change in the steady-state level of CO_2 when photosynthetic CO_2 assimilation ceases and a postillumination CO_2 burst occurs. The rest of this discussion is concerned with the long-term acidification process.

The inhibition of acidification in cells exposed to light and N_2 suggests that either oxidative phosphorylation, which requires O_2, or noncyclic photophosphorylation, which requires CO_2, are required for net H^+ efflux. Cyclic photophosphorylation, however, requires neither O_2 nor CO_2 and inhibition of acidification by N_2 demonstrates that this process cannot drive H^+ efflux. However, the stimulation of H^+ efflux by DCMU in illuminated aerated cells demonstrates that noncyclic photophosphorylation is not a requirement for H^+ efflux (Fig. 2). The ability of DCMU to inhibit noncyclic photophosphorylation was indicated by its rapid and complete inhibition of light-driven CO_2 uptake (Fig. 2) and photosynthetic O_2 production. Oligomycin inhibition of H^+ efflux in illuminated cells (Fig. 3) without inhibition of light-dependent CO_2 fixation (Fig. 7) suggests that noncyclic photophosphorylation can not be used as an energy source for H^+ efflux.

Conversely, the demonstration that H^+ efflux is not inhibited when cells are transferred from light to dark suggests that oxidative phosphorylation is in some manner coupled to H^+ efflux (Fig. 1). This suggestion is supported by the observation that two inhibitors of mitochondrial ATP production N_2 and oligomycin inhibit acidification rapidly and completely in light or dark (Figs. 3 and 4). Whereas this interpretation appears clear for N_2, oligomycin may also inhibit H^+ efflux through inhibition of a plasma membrane ATPase that functions in H^+ efflux (22). The conclusion that oxidative phosphorylation, not photosynthesis, is required for net H^+ efflux is consistent with reports demonstrating that levels of ATP and rates of transport processes in green cells are often independent of illumination (10, 17). If H^+ efflux is coupled to ATP hydrolysis, then the inability of chloroplasts to export ATP directly (7, 28) may be significant in this context.

The vast majority of papers on the influence of light-dark transitions on ion transport processes ignore the possibility that O_2 maybe limiting in submerged green tissue. Thus, any stimulation of a process attributed to photosynthesis could in fact be caused by increased O_2 levels and rates of oxidative phosphorylation. This suggestion was tested by experiments in which cells were stirred in the dark in the absence of aeration. Under those circumstances, H^+ efflux came to a halt, indicating the O_2 levels were limiting. When aeration or illumination was initiated, alkalinization due to removal of CO_2 resulted and then acidification resumed. This experiment demonstrates that photosynthetic release of O_2 may be important in determining rates of ion transport processes in submerged green tissue. A recent preliminary report suggests that light stimulated H^+ efflux from submerged nonaerated protoplasts of mesophyll cells is mediated via photosynthetic O_2 production (15). Although the K_m for O_2 uptake during leaf respiration is low and approximately 2.5×10^{-6} M (20) it seems clear that the O_2 concentration will eventually become

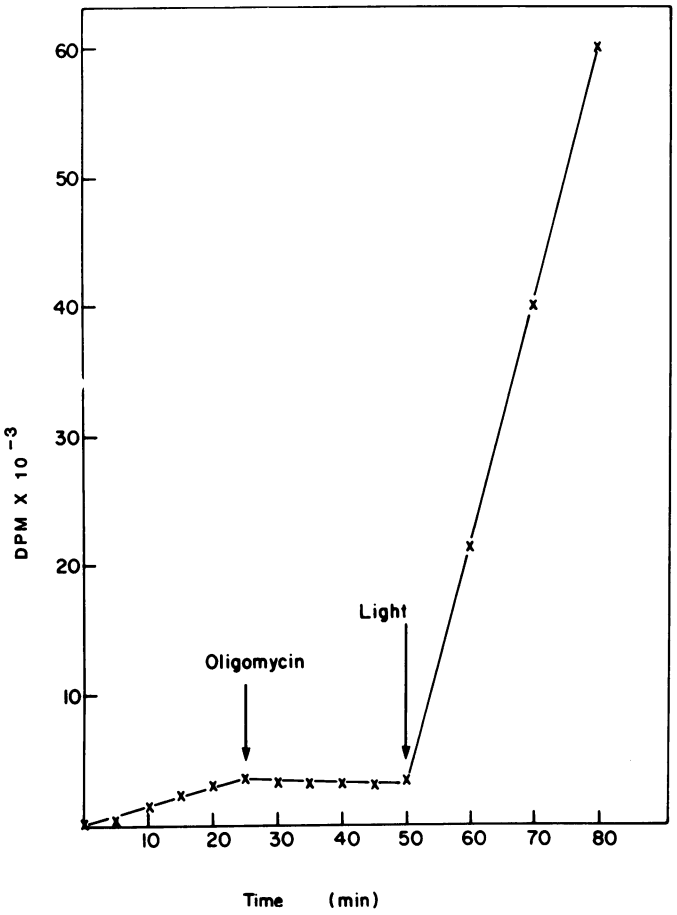

FIG. 7. The influence of oligomycin on the rate of dark CO_2 fixation. Oligomycin, final concentration 20 μg/ml, and light were introduced at the times indicated. At time zero, 20×10^6 dpm of ^{14}C-labeled $NaHCO_3$ (58.2 mCi/nmol) was added.

limiting if respiration removes O_2 faster than it can be replaced by diffusion through water.

Net H^+ efflux was inhibited in light or dark when aeration was switched from air to CO_2-free air (Fig. 6). Many reports have demonstrated a correlation between rates of H^+ efflux and dark fixation and models of H^+ efflux suggest that dark fixation of CO_2 into malate is the process which replaces the H^+ excreted (6, 12). Inhibitors of H^+ efflux, such as oligomycin, may inhibit dark fixation through a pH-stat mechanism in which cytoplasmic pH and rates of dark fixation decrease when H^+ efflux is inhibited (6, 12). To my knowledge, this is the first report which demonstrates that the rate of H^+ efflux can be regulated by changing CO_2 levels in the bathing medium. The increase in rate of H^+ efflux when cells are treated with DCMU, may indicate that the rate of H^+ efflux is diminished when photosynthetic and dark CO_2 fixation processes compete for CO_2. Phosphoenolpyruvate carboxylase activity in *Asparagus* mesophyll cells has been detected (personal communication, Dr. B. Colman, York University).

Acknowledgments—The author wishes to thank Eunice Fong for expert technical assistance, and George Espie and Brian Colman for measurements of dissolved inorganic carbon and critical reading of the manuscript.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BIRMINGHAM BC, B COLMAN 1979 Measurement of carbon dioxide compensation points of freshwater algae. Plant Physiol 64: 892–895
3. BOWN AW 1981 H^+ excretion in mechanically isolated mesophyll cells from *Asparagus* cladophylls. Plant Sci Lett 22: 155–160
4. COLMAN B, BT MAWSON, GS ESPIE 1979 The rapid isolation of photosynthetically active meosphyll cells from *Asparagus* cladophylls. Can J Bot 57: 1505–1510
5. FELLE H, FW BENTRUP 1974 Light-dependent changes of the membrane potential and conductance in *Riccia fluitans*. *In* J Dainty, U Zimmermann, eds., Membrane Transport in Plants. Springer-Verlag, Heidelberg, pp 120–125
6. HASCHKE H, U LÜTTGE 1975 Stoichiometric correlation of malate accumulation with auxin-dependent K^+-H^+ exchange and growth in *Avena* coleoptile segments. Plant Physiol 56: 696–698
7. HELDT HW 1976 Metabolite carriers of chloroplasts. *In* U Lüttge, MG Pitman, eds, Encyclopedia of Plant Physiology New Series, Vol 3. Springer-Verlag, Heidelberg, pp 137–143, 235–254
8. HIGINBOTHAM N, WP ANDERSON 1974 Electrogenic pumps in higher plant cells. Can J Bot 52: 1011–1021
9. HOPE AB, U LÜTTGE, E BALL 1972 Photosynthesis and apparent proton fluxes in *Elodea canadensis*. Z Pflanzenphysiol 68: 73–81
10. JEANJEAN R 1976 The effect of metabolic poisons on ATP Level and on active phosphate uptake in *Chlorella pyrenoidosa*. Physiol Plant 27: 107–110
11. JESCHKE WD 1976 Ionic relations of leaf cells. *In* U Lüttge, MG Pitman, eds, Encyclopedia of Plant Physiology. New Series, Vol 2, Part B. Springer-Verlag, Heidelberg, pp 160–194
12. JOHNSON KD, DL RAYLE 1976 Enhancement of CO_2 uptake in *Avena* coleoptiles by fusicoccin. Plant Physiol 57: 806–811
13. KANAI R, GE EDWARDS 1973 Purification of enzymatically isolated mesophyll protoplasts from C3, C4 and Crassulacean acid metabolism plants using an aqueous dextran-polyethylene glycol two phase system. Plant Physiol 52: 484–490
14. KEIFER DW, RM SPANSWICK 1979 Correlation of ATP levels in *Chara corallina* with the activity of the electrogenic pump. Plant Physiol 64: 165–168
15. KELLY B, B RUBINSTEIN 1981 Light and oxygen stimulated proton efflux. Plant Physiol 67: S–51
16. LÜTTGE U 1973 Proton and chloride uptake in relation to the development of photosynthetic capacity in greening etiolated barley leaves. *In* WP Anderson, ed, Ion Transport in Plants. Academic Press, New York, pp 205–221
17. LÜTTGE U, E BALL 1976 ATP Levels and energy requirements of ion transport in cells of slices of greening barley leaves. Z Pflanzenphysiol 80: 50–59
18. LÜTTGE U, N HIGINBOTHAM 1979 Transport in Plants. Springer-Verlag, New York, pp 322–323
19. LÜTTGE U, CK PALLAGHY, CB OSMOND 1970 Coupling of ion transport in green cells of *Atriplex spongiosa* leaves to energy sources in the light and dark. J Membrane Biol 2: 17–30
20. MACDONALD IR 1975 Effect of vacuum infiltration on photosynthetic gas exchange in leaf tissue. Plant Physiol 56: 109–112
21. MACDONALD IR, AES MACKLON 1975 Light enhanced chloride uptake by wheat laminae. A comparison of chopped and vacuum infiltrated tissue. Plant Physiol 56: 105–108
22. MALONE CP, JJ BURKE, JB HANSON 1977 Histochemical evidence for the occurrence of oligomycin sensitive plasmalemma ATPase in corn roots. Plant Physiol 60: 916–922
23. NOBEL SP 1969 Light dependent uptake by *Pisium sativum* leaf fragments. Plant Cell Physiol 10: 597–605
24. OBERBAUER S, D RAYLE, K JOHNSON, R CLELAND 1978 Auxin induced acidification is not due to the hydration of respiratory CO_2. Plant Sci Lett 11: 317–322
25. PALLAGHY CK, U LÜTTGE 1970 Light induced H^+ fluxes and bioelectric phenomena in mesophyll cells of *Atriplex spongiosa*. Z Pflanzenphysiol 62: 417–425
26. SPANSWICK RM 1974 Hydrogen ion transport in giant algal cells. Can J Bot 52: 1029–1934
27. SPANSWICK RM 1981 Electrogenic ion pumps Annu Rev Plant Physiol 32: 267–289
28. STOCKING CR, S LARSON 1969 A chloroplast cytoplasmic shuttle and the reduction of extraplastic NAD. Biochem Biophys Res Commun 37: 278–282