Replacement of cellular potassium by caesium in *Chlorella emersonii*: differential sensitivity of photoautotrophic and chemoheterotrophic growth

SIMON V. AVERY, GEOFFREY A. CODD and GEOFFREY M. GADD*

Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, UK

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Photoautotrophic growth of *Chlorella emersonii* in the presence of 1 mM-CsCl resulted in a 34% increase in cell doubling time and an 83% reduction in the final cell yield as compared to growth in the absence of Cs⁺. In contrast, the presence of 1 mM-Cs⁺ had no effect on chemoheterotrophic growth in the dark with glucose. These observations were correlated with the stage of growth at which Cs⁺-induced cellular K⁺ loss was evident. For photoautotrophically growing cells this occurred during the exponential growth phase (after 4 d); for chemoheterotrophically growing cells this was during stationary phase (after 10 d). Inhibition of chemoheterotrophic cell division occurred after 2 d in 50 mM-Cs⁺ or 5 d in 20 mM-Cs⁺, and coincided with a decline in intracellular K⁺ to ~2 nmol (10⁶ cells)⁻¹. Accumulation of Cs⁺ ceased after 2 d in both of these cases. Cell doubling times during chemoheterotrophic growth remained approximately constant at internal K⁺ levels between 7 and 28 nmol (10⁶ cells)⁻¹. In contrast, a decline in intracellular K⁺, from 42 to 19 nmol (10⁶ cells)⁻¹, after 4 d photoautotrophic growth in the presence of Cs⁺, was concurrent with the 34% increase in cell doubling times. Photosynthesizing cells accumulated approximately 2-fold more Cs⁺ than respiring cells after incubation for 12 h in HEPES buffer, pH 8. Culture age and intracellular K⁺ levels had little effect on the ability of *C. emersonii* to accumulate Cs⁺. Externally supplied K⁺ inhibited Cs⁺ accumulation to a greater extent in photosynthesizing cells (75% inhibition at 10 mM-K⁺, 1 mM-Cs⁺) than in respiring cells (50% inhibition at 10 mM-K⁺, 1 mM-Cs⁺). Greatly elevated Na⁺ concentrations (50 mM) were required to inhibit Cs⁺ accumulation in both cases. Incubation of cells in buffer in the light in the absence and presence of 10 mM-Cs⁺ resulted in decreases in cellular K⁺ of 44% and 77% respectively and a concomitant 66% reduction in the rate of photosynthesis in both cases. A Cs⁺-induced K⁺ loss of 71% from respiring cells had no effect on the rate of respiration. These results indicate that it is not the presence of Cs⁺ in cells that is growth inhibitory, but rather the resulting loss of K⁺ and that photosynthesis and photoautotrophic growth are more sensitive to this K⁺ loss than respiration and chemoheterotrophic growth.

Introduction

Caesium is a nuclear fission product that is of particular environmental importance due to the long half-life of the radioisotope (approximately 30 years) and the high water solubility and bioavailability of the monovalent caesium ion, Cs⁺ (Greenwood & Earnshaw, 1984). For the latter reason, *in vivo* studies on the accumulation of ¹³⁷Cs into living biomass have been used as a means of determining natural levels of the isotope and such studies have intensified since the Chernobyl accident in 1986 (Cambray *et al.*, 1987; Elstner *et al.*, 1987). Little is known, however, about the mechanisms of Cs⁺ transport – in particular, Cs⁺ transport into microbial cells which play significant roles in the accumulation, cycling and transfer of heavy metals and radionuclides in the environment (Gadd, 1988, 1990; Hughes & Poole, 1989).

The high concentration of ¹³⁷Cs in radioactive wastewaters from power reactors (Watson *et al.*, 1989) indicates that aquatic ecosystems are especially susceptible to contamination with caesium. In aquatic environments, microalgae and cyanobacteria are important microbial primary producers and of these, *Chlorella* spp. have been used as a suitable test organism for toxicity and transport studies with heavy metals and radionuclides (Nakajima *et al.*, 1981; Khummongkol *et al.*, 1982; Mahan *et al.*, 1989; Bennett, 1990). Caesium transport in *Chlorella* is relevant to studies with monovalent cations as Cs⁺ is known to be transported via K⁺ transport systems in other micro-organisms, e.g. bacteria (Bossmeyer *et al.*, 1989), cyanobacteria (Avery *et al.*, 1991) and yeast (Borst-Pauwels, 1981). Two K⁺ transport systems have been shown to occur in *Chlorella* (Kannan, 1971; Solt *et al.*, 1971): a high-affinity system which permits

net K^+ influx and a low-affinity system which determines K^+/K^+ exchange (Paschinger & Vanicek, 1974). Raven (1980) concluded that K^+ influx in *Chlorella* is probably via a uniport mechanism, although some evidence has suggested that K^+/H^+ exchange may also occur (Schaedle & Jacobsen, 1965; Trombala, 1981).

The nutritional versatility displayed by several *Chlorella* species in their ability to grow both photoautotrophically and chemoheterotrophically is apparently matched by a versatility in their K^+ requirements and transport mechanisms. Under certain conditions in the natural environment, for example in densely populated algal communities, light and CO_2 are limiting factors for growth and, as a consequence, heterotrophic algae may become dominant (Droop, 1974). Eyster *et al.* (1958) observed that a tenfold higher external K^+ concentration is required for heterotrophic growth of *Chlorella* than for autotrophic growth. It is known that cyclic photophosphorylation supports a higher rate of K^+ (Rb^+) uptake than oxidative phosphorylation of endogenous substrates (Barber, 1968*a, b*; Kannan, 1971); however, this situation is reversed when glucose is supplied as an exogenous organic carbon source (Springer-Lederer & Rosenfeld, 1968).

The possibility that photoautotrophic and chemoheterotrophic growth regimes may influence the accumulation and toxicity of Cs^+ towards *Chlorella emersonii* 211 8b was investigated in this study, together with interactions of Cs^+ with cellular K^+ . In addition, the inhibition of photosynthesis and respiration following Cs^+ accumulation was examined.

Methods

Organism, media, growth conditions and preparation of cell suspensions.

Axenic cultures of *Chlorella emersonii* 211 8b were grown at 22 °C in 100 ml of BG-11 medium (initial pH ~7.6), containing nitrate as a fixed nitrogen source (Stanier *et al.*, 1971). Cultures were incubated in 250 ml Erlenmeyer flasks with rotary aeration at 150 cycles min^{-1} , with a photon fluence irradiance, incident on the surface of the flasks, of $12 \mu E m^{-2} s^{-1}$, provided by white fluorescent tubes.

For growth experiments, cells in the late exponential phase of photoautotrophic growth were inoculated to an initial cell density of approximately $2.7 \times 10^5 ml^{-1}$ ($OD_{680} \approx 0.1$) in 100 ml of BG-11 medium for autotrophic growth or 100 ml of BG-11 medium and 1% (w/v) glucose for heterotrophic growth, containing the desired amounts of Cs^+ (added as CsCl). These were incubated as above except black polythene was used to eliminate incident light in chemoheterotrophic growth.

To prepare cell suspensions for short-term Cs^+ uptake experiments, cells in the late exponential growth phase (unless otherwise specified) were collected by centrifugation (1200 g, 10 min), washed thoroughly with distilled deionized water and then suspended to a cell density of approximately $5 \times 10^6 ml^{-1}$ in 100 ml 10 mM-HEPES buffer $\pm 1\%$ (w/v) glucose, adjusted to pH 8 using solid tetramethylammonium hydroxide. Cells were allowed to equilibrate for 1 h prior to addition of Cs^+ and/or other monovalent cations. All cell suspensions were

incubated at 22 °C with rotary shaking (150 cycles min^{-1}) in the presence or absence of light as described above. All glassware was washed with 1 M-HCl and rinsed thoroughly with distilled deionized water prior to use.

Measurement of photosynthesis and respiration. Photosynthesis and respiration by *C. emersonii* cell suspensions were measured at 25 °C, either in the light at a photon fluence irradiance of $250 \mu E m^{-2} s^{-1}$ for photosynthesis or in the dark with 1% (w/v) glucose for respiration, using a Rank oxygen electrode linked to a Servoscribe potentiometric recorder. The chamber volume used was 3 ml and calibration was by means of air-saturated distilled water; oxygen solubility values were obtained from Golterman (1969).

Metal analyses. Cells were harvested by centrifugation (1200 g, 10 min) and washed twice with distilled deionized water. Cell pellets were digested for 1 h in 0.5 ml 6 M- HNO_3 at 100 °C and, after subsequent addition of 2.5 ml distilled deionized water and mixing, cell extracts were centrifuged (1200 g, 10 min) to remove debris. Caesium, potassium and sodium concentrations in the supernatants were determined using a PYE Unicam SP9 atomic absorption spectrophotometer with reference to appropriate standards.

Results

*Effect of Cs^+ on photoautotrophic and chemoheterotrophic growth of *C. emersonii* and on cellular K^+ and Na^+*

Addition of CsCl to a final concentration of 1 mM in BG-11 medium resulted in inhibition of photoautotrophic growth of *C. emersonii*. The cell doubling time during the exponential phase of growth was increased by approximately 34% from 1.60 d to 2.15 d and the final cell yield after incubation for 35 d was reduced by approximately 83% (Fig. 1*a*). Chemoheterotrophic growth of *C. emersonii* was considerably faster than photoautotrophic growth and was exponential over the first 6 d, with a doubling time of 0.9 d, reaching stationary phase after 10 d (Fig. 2*a*). The final cell yield after 35 d incubation was approximately 5×10^7 cells ml^{-1} . In comparison to growth under photoautotrophic conditions, addition to the medium of CsCl to a final concentration of 1 mM had no apparent effect on either the doubling time or final cell yield during chemoheterotrophic growth (Fig. 2*a*).

Cells were analysed for intracellular Cs^+ , K^+ and Na^+ throughout growth under the two nutritional regimes. Accumulation of Cs^+ during photoautotrophic growth of *C. emersonii* proceeded in three phases (Fig. 1*b*). During the first 6 d, Cs^+ accumulated to approximately 16 nmol (10^6 cells) $^{-1}$; this was followed by a slower phase of accumulation to a maximum level of approximately 23 nmol Cs^+ (10^6 cells) $^{-1}$ after 20 d, which was then followed by loss of the ion. The final Cs^+ level present in cells after incubation for 35 d was approximately 18 nmol (10^6 cells) $^{-1}$. The initial accumulation of Cs^+ paralleled a rapid accumulation of K^+ in cells incubated in the absence of Cs^+ (Fig. 1*c*). However, internal K^+ levels in cells incubated in the presence of Cs^+ remained

Fig. 1

Fig. 2

Fig. 1. Effect of caesium accumulation on photoautotrophic growth of *Chlorella emersonii* and levels of cellular monovalent cations. Cells were grown in the absence (open symbols) or presence (filled symbols) of 1 mM- $CsCl$ in the light. (a) $\log_{10}$ cell number ($\circ$, $\bullet$). (b) Intracellular Cs^+ ($\times$) and external K^+ (∇ , $\blacktriangledown$). (c) Intracellular K^+ ($\square$, $\blacksquare$). (d) Intracellular Na^+ ($\triangle$, $\blacktriangle$). Mean values from three replicate determinations are shown $\pm$ SEM values where these exceed the dimensions of the symbols.

Fig. 2. Effect of caesium accumulation on chemoheterotrophic growth of *Chlorella emersonii* and levels of cellular monovalent cations. Cells were grown in the absence (open symbols) or presence (filled symbols) of 1 mM- $CsCl$ in the dark with 1% (w/v) glucose. Symbols as in Fig. 1.

approximately constant at 20 nmol $(10^6 \text{ cells})^{-1}$ during this period and the resulting difference in intracellular K^+ in cells incubated in the absence and presence of Cs^+ was maintained for the remainder of the growth cycle (Fig. 1c). After incubation for 6 d, release of K^+ occurred from all cells. The loss of K^+ from cells incubated in the absence of Cs^+ correlated with K^+ depletion of the medium as the growth cycle progressed (Fig. 1b, c). No decrease in extracellular K^+ concentrations resulted from growth in the presence of Cs^+ (Fig. 1b), although the observed loss of internal K^+ from these cells continued up to 20 d which coincided with the maximal level of intracellular Cs^+ (Fig. 1b, c). Internal Na^+ levels remained low throughout photoautotrophic growth of *C. emersonii* and no detectable loss of Na^+ resulted from Cs^+ accumulation (Fig. 1d). However, some increase in Na^+

levels was observed after 20 d incubation of cells with and without Cs^+ .

Accumulation of Cs^+ during chemoheterotrophic growth also proceeded in three phases, although these differed quantitatively from those described for photoautotrophically growing cells. An initial phase of uptake decreased in rate as the exponential phase of growth progressed, but after incubation for 6 d, which corresponded with the onset of stationary phase, a marked increase in Cs^+ accumulation occurred which continued until day 20 to a maximal level of approximately 16 nmol $(10^6 \text{ cells})^{-1}$ (Fig. 2b). Subsequently, some loss of intracellular Cs^+ was observed and the final level attained after growth for 35 d was approximately 10 nmol Cs^+ $(10^6 \text{ cells})^{-1}$. Intracellular K^+ fluctuated considerably over the first 10 d of growth, although the

Fig. 3. Effect of increased levels of caesium on chemoheterotrophic growth of *Chlorella emersonii*. (a) Growth of *C. emersonii* in the dark with 1% (w/v) glucose in external CsCl concentrations (mM) of: 1 (○); 5 (●); 10 (□); 20 (■); and 50 (△). (b, c) Intracellular Cs^+ (●) and K^+ (○) levels after growth for 2 d (b) or 6 d (c). Mean values from three replicate determinations are shown $\pm$ SEM values where these exceed the dimensions of the symbols.

large accumulation of K^+ initially observed during photoautotrophic growth of *C. emersonii* (Fig. 1c) was not evident (Fig. 2c). In contrast, a rapid decline in extracellular K^+ gave rise to an equally rapid decline in intracellular K^+ , which occurred between 4 and 10 d in dividing cells incubated in both the absence and presence of Cs^+ , (Fig. 2b) and coincided with the onset of stationary phase and the concurrent stimulation of Cs^+ accumulation in cells incubated in the presence of Cs^+ . The increased accumulation of Cs^+ between 6 and 20 d resulted in both a further K^+ loss from cells growing in the presence of Cs^+ (Fig. 2c) and a sharp rise in the external K^+ concentration (Fig. 2b); neither phenomenon was evident in cells growing in the absence of Cs^+ . As with photoautotrophically growing cells of *C. emersonii*, the excess amount of K^+ lost from cells growing heterotrophically in the presence of Cs^+ was generally slightly less than the amount of Cs^+ accumulated, although here Cs^+ accumulation resulted in an approximate 35% decrease in intracellular Na^+ , which was maintained throughout growth (Fig. 2d).

To determine concentrations of Cs^+ that were inhibitory to chemoheterotrophic cultures of *C. emersonii*, cells were grown in the presence of 1, 5, 10, 20 and 50 mM- Cs^+ (Fig. 3a). Doubling times were similar (~ 0.9 d) at all Cs^+ concentrations during the first 2 d growth. However, the onset of stationary phase was premature, at 5 and 2 d, for cells grown in the presence of 20 and 50 mM- Cs^+ respectively. Final cell yields were reduced at all the concentrations tested; by 26% at 5 mM- Cs^+ , 39% at 10 mM, 90% at 20 mM and by 97% at 50 mM- Cs^+ . Intracellular levels of Cs^+ and K^+ after growth for 2 and 6 d in the presence of these elevated Cs^+ concentrations are shown in Fig. 3(b, c). The onset of stationary phase for cells incubated in the presence of 50 mM- Cs^+ coincided with a marked reduction in internal K^+ , to approximately $2 \text{ nmol (10}^6 \text{ cells)}^{-1}$, as compared with

cells incubated in 10 and 20 mM- Cs^+ [16 and $14 \text{ nmol K}^+ \text{ (10}^6 \text{ cells)}^{-1}$ respectively] (Fig. 3b). Although intracellular Cs^+ levels after 2 d were slightly higher for cells incubated in 50 mM- Cs^+ [$23 \text{ nmol Cs}^+ \text{ (10}^6 \text{ cells)}^{-1}$] than for those incubated in 10 mM- Cs^+ [$20 \text{ nmol Cs}^+ \text{ (10}^6 \text{ cells)}^{-1}$] they were lower than for those incubated in 20 mM- Cs^+ [$31 \text{ nmol Cs}^+ \text{ (10}^6 \text{ cells)}^{-1}$]. After 6 d growth, no change had occurred in the intracellular level of Cs^+ for cells incubated in the presence of 20 mM- Cs^+ although the onset of growth inhibition at this stage coincided with a marked decline in internal K^+ to approximately $2 \text{ nmol (10}^6 \text{ cells)}^{-1}$ (Fig. 3c). This value was the same as that found for cells incubated in 50 mM- Cs^+ after 2 d when inhibition of growth was also apparent, but lower than that in cells incubated in concentrations of Cs^+ below 20 mM, where substantially reduced growth inhibitory effects were evident.

Effect of culture age on the ability of photoautotrophically and chemoheterotrophically grown cell suspensions of C. emersonii to accumulate Cs⁺

Intracellular K^+ in photoautotrophically growing cultures of *C. emersonii* varied throughout growth (Fig. 4a). These fluctuations, however, had little effect on the ability of cells to accumulate Cs^+ . The amount of Cs^+ accumulated by cells of varying culture age, after incubation in buffer for 12 h in the presence of 1 mM- Cs^+ , showed only a slight increase from approximately $12 \text{ nmol (10}^6 \text{ cells)}^{-1}$ in cells grown for 5 d, to approximately $17 \text{ nmol (10}^6 \text{ cells)}^{-1}$ in cells grown for 30 d. A decline in intracellular levels of K^+ occurred in chemoheterotrophically growing cultures of *C. emersonii*, from approximately $19 \text{ nmol (10}^6 \text{ cells)}^{-1}$ after 5 d to approximately $9 \text{ nmol (10}^6 \text{ cells)}^{-1}$ after 30 d (Fig. 4b). However, as with photoautotrophically growing cells the age of these cells had little effect on their ability to accumulate

Fig. 4. Effect of culture age on the ability of *Chlorella emersonii* to accumulate Cs^+ . Cells of the specified culture age, growing either photoautotrophically (a) or chemoheterotrophically (b) were suspended to 5×10^6 ml $^{-1}$ in 10 mM-HEPES buffer, pH 8, with 1 mM- $CsCl$ either in the light (a) or in the dark with 1% (w/v) glucose (b). The graph shows intracellular K^+ prior to the addition of $CsCl$ ($\square$) and intracellular Cs^+ after 12 h incubation in the presence of $CsCl$ ($\bullet$). Mean values from three replicate determinations are shown $\pm$ SEM values where these exceed the dimensions of the symbols.

Cs^+ . The amount of Cs^+ accumulated, in the dark with glucose, by chemoheterotrophically grown cells of varying culture age remained approximately constant (Fig. 4b).

*Inhibition of Cs^+ accumulation by K^+ and Na^+ in photosynthesizing and respiring cells of *C. emersonii**

When K^+ was supplied to photosynthesizing cells of *C. emersonii* at an equimolar (1 mM) concentration to Cs^+ , accumulation of Cs^+ after 12 h was reduced by approximately 37% (Table 1). When the external concentration of K^+ was increased to 10 mM, and Cs^+ maintained at 1 mM, a 75% reduction in Cs^+ accumulation resulted, as compared to cells incubated in the absence of K^+ . In contrast, when respiring cells of *C. emersonii* were subjected to similar increases in external K^+ concentrations, approximate reductions in Cs^+ accumulation were only 30% (1 mM- K^+ ; 1 mM- Cs^+) and 50% (10 mM- K^+ ; 1 mM- Cs^+).

The effect of externally supplied Na^+ on Cs^+ accumulation differed from that of K^+ . When Na^+ was supplied

at equimolar (1 mM) concentrations to Cs^+ a slight stimulation of Cs^+ accumulation was evident, and only when a 50-fold higher Na^+ concentration was present did significant inhibition of Cs^+ accumulation occur in both photosynthesizing (27% reduction) and respiring (20% reduction) cells. Cs^+ accumulation was virtually eliminated in both cases in the presence of 0.5 M- Na^+ (Table 1).

*Effect of Cs^+ accumulation and K^+ loss on rates of photosynthesis and respiration in *C. emersonii**

The photosynthetic and respiratory rates of cells of *C. emersonii* in the exponential phase of photoautotrophic and chemoheterotrophic growth were measured prior to, and after, incubation for 12 h in the absence and presence of 10 mM- Cs^+ (Table 2); this high Cs^+ concentration was employed to produce a rapid detectable change in oxygen evolution/uptake rates. Incubation of cells for 12 h in the presence of Cs^+ resulted in rates of photosynthesis and respiration similar to those observed with cells incubated in the absence of Cs^+ . However, the rates of photosynthesis decreased by

Table 1. Effect of K^+ and Na^+ on Cs^+ accumulation in *Chlorella emersonii* during photosynthesis and respiration

Photoautotrophically grown cells were suspended to 5×10^6 ml $^{-1}$ in 10 mM-HEPES buffer, pH 8, supplemented with 1 mM- $CsCl$ and the appropriate amount of K^+ or Na^+ . Cells were harvested after 12 h incubation either in the light or in the dark with 1% (w/v) glucose. Mean values from three replicate determinations are shown $\pm$ SEM.

Metal cation added to external medium	Concn (mM)	Intracellular Cs^+ in photosynthesizing cells [nmol (10^6 cells) $^{-1}$]	Intracellular Cs^+ in respiring cells [nmol (10^6 cells) $^{-1}$]
None	—	12.4 $\pm$ 0.2	6.0 $\pm$ 0.2
K^+	1	7.8 $\pm$ 0.0	4.2 $\pm$ 0.0
	10	3.1 $\pm$ 0.2	3.0 $\pm$ 0.3
Na^+	1	13.6 $\pm$ 0.2	8.5 $\pm$ 0.3
	10	10.2 $\pm$ 0.6	7.9 $\pm$ 0.0
	50	9.1 $\pm$ 0.2	4.8 $\pm$ 0.0
	500	0.7 $\pm$ 0.3	0.3 $\pm$ 0.0

Table 2. Effect of Cs⁺ accumulation and K⁺ loss on rates of photosynthesis and respiration in *Chlorella emersonii*

Cells were harvested from the late exponential phase of either photoautotrophic or chemoheterotrophic growth and suspended to 5×10^6 ml⁻¹ in 10 mM-HEPES buffer, pH 8, $\pm$ 10 mM-CsCl. Cells were harvested after 0 and 12 h incubation in the light (for photosynthesis) or in the dark with 1% (w/v) glucose (for respiration). Mean values from three replicate determinations are shown $\pm$ SEM.

Treatment	Time of incubation (h)	Cs ⁺ concn (mM)	Intracellular K ⁺ [nmol (10 ⁶ cells) ⁻¹]	Intracellular Cs ⁺ [nmol (10 ⁶ cells) ⁻¹]	Rate of photosynthesis/respiration [nmol O ₂ min ⁻¹ (10 ⁶ cells) ⁻¹]
Light	0	0	46.6 $\pm$ 3.5	0	2.88 $\pm$ 0.10
	12	0	26.6 $\pm$ 1.4	0	0.93 $\pm$ 0.18
	12	10	10.9 $\pm$ 0.7	36.5 $\pm$ 1.0	1.05 $\pm$ 0.10
Dark + glucose	0	0	27.2 $\pm$ 1.0	0	0.85 $\pm$ 0.01
	12	0	25.0 $\pm$ 0.8	0	0.85 $\pm$ 0.03
	12	10	7.9 $\pm$ 0.8	22.8 $\pm$ 2.7	0.80 $\pm$ 0.02

approximately 66% over the incubation period, whilst those of respiration remained approximately constant. Analysis of intracellular K⁺ levels at 0 and 12 h revealed that K⁺ loss occurred from both photosynthesizing and respiring cells incubated in the presence of Cs⁺; however, a reduction in internal K⁺ was also evident in photosynthesizing cells incubated in the absence of Cs⁺ that did not occur in respiring cells. Thus an approximate 71% Cs⁺-induced decrease in intracellular K⁺ had no effect on the rate of respiration whereas decreases of both 44% and 77% in intracellular K⁺ from photosynthesizing cells, incubated in the absence and presence of Cs⁺ respectively, correlated with an approximate 66% reduction in the rate of photosynthesis. Cs⁺ accumulation occurred in both photosynthesizing and respiring cells and was slightly greater than the concurrent K⁺ release in both cases.

Discussion

During both photoautotrophic and chemoheterotrophic growth of algae certain cell components are required to metabolize sugars. However, the diversion of a large fraction of such components to the catalysis of photoautotrophy during photoautotrophic growth results in an intrinsically slower growth rate, which, according to Raven (1988), may be as great as 2-fold. This is in agreement with the results presented here for *C. emersonii*, where an approximate 1.8-fold increase in maximal growth rate occurred when cells were grown chemoheterotrophically as opposed to photoautotrophically. These differences in growth under the two nutritional regimes coincided with differential sensitivities to Cs⁺. The extent of inhibition of photoautotrophic growth of *C. emersonii* in the presence of 1 mM-Cs⁺ was similar to that found for the cyanobacterium *Synechocystis* PCC 6803 under the same conditions (Avery *et al.*, 1991), although 1 mM-Cs⁺ had no effect on chemohetero-

trophic growth of *C. emersonii*. Avery *et al.* (1991) suggested that Cs⁺ accumulation resulted in suppression of growth through replacement of cellular K⁺. If this was the case here, a simple explanation can be proposed for the present observations according to the stage of growth at which Cs⁺-induced K⁺ loss from cells occurred during chemoheterotrophy and photoautotrophy. The decrease in intracellular K⁺, associated with initial rapid cell division and exhaustion of external K⁺, during chemoheterotrophic growth of *C. emersonii*, could not be distinguished from K⁺ loss due to Cs⁺ accumulation until day 10 when cell division had ceased. In contrast, Cs⁺ accumulation resulted in decreased internal K⁺ during the exponential phase of photoautotrophic growth and therefore had a pronounced effect on initial cell doubling times.

When *C. emersonii* was grown chemoheterotrophically in the presence of increased levels of Cs⁺, only at 20 mM-Cs⁺ was the final cell yield reduced by an amount approximating to that found for cells grown photoautotrophically in the presence of 1 mM-Cs⁺. The internal Cs⁺ and K⁺ levels in cells grown chemoheterotrophically at 20 mM-Cs⁺, both prior to (2 d), and at the point of, inhibition of cell division (6 d), are revealing. Over this period cellular Cs⁺ remained constant whereas cellular K⁺ dropped to a level presumably below that needed to support the requirements for cell division. These results clearly demonstrate that it was not the presence of Cs⁺ in the cell that was inhibitory to chemoheterotrophic growth, but rather the resultant loss of cellular K⁺, as only the intracellular concentration of the latter ion changed between 2 and 6 d. Furthermore, it can be inferred that the threshold level of K⁺, below which chemoheterotrophic cell division ceased, lies between 2 and 7 nmol K⁺ (10⁶ cells)⁻¹ and at all levels higher than this the rate of cell division remained approximately constant. This is in contrast to photoautotrophic growth where the rate of cell division after 4 d was reduced by

approximately 35% when cells, grown in the presence of 1 mM- Cs^+ , contained 19 nmol K^+ (10^6 cells) $^{-1}$, in comparison to control cells containing 42 nmol K^+ (10^6 cells) $^{-1}$. It must be stressed that possible differences in the volume of cells growing under the two nutritional regimes were not discernible through microscopic examination. The results therefore contradict those of Eyster *et al.* (1958), who reported that a 10-fold higher K^+ concentration was required for chemoheterotrophic growth of *Chlorella pyrenoidosa* than for photoautotrophic growth, although the authors admitted that their results showed 'questionable variation'.

In short-term experiments, incubation of *C. emersonii* in the dark with glucose supported a lower accumulation of Cs^+ than incubation in the light. The reverse of this situation was found for Rb^+ influx in *C. pyrenoidosa* (Springer-Lederer & Rosenfeld, 1968) although these workers conducted uptake experiments at a lower pH value of 6.5. It has since been shown that a switch from K^+/K^+ to K^+/H^+ exchange, and hence an increased net K^+ uptake, is necessary to maintain high internal pH levels when glucose is provided as an organic carbon source for *Chlorella fusca* (Tromballa, 1981). The requirement for such a mechanism to maintain a constant ΔpH would probably not be so great at lower external pH values suggesting that a reduced K^+ (or Rb^+) accumulation would result. It is more likely that the observed discrepancy may result from a K^+/H^+ exchange system operating during respiration being more selective for K^+ (or Rb^+) and against Cs^+ than a K^+/K^+ exchange operating during photosynthesis. The K^+/H^+ exchange observed in *Chlorella* cells grown on low-salt media (Schaedle & Jacobsen, 1965) has been described as occurring via a higher-affinity K^+ -transport system than that involved with K^+/K^+ exchange (Raven, 1980) and one which would presumably select more strongly against Cs^+ .

Despite the observed large variations in internal K^+ through both chemoheterotrophic and photoautotrophic growth of *C. emersonii* in the absence of Cs^+ , the effect of growth stage and internal K^+ levels on the ability of cells to accumulate Cs^+ was negligible. It is likely that the observed changes in Cs^+ accumulation through growth in batch culture were primarily due to fluctuations in external K^+ concentration, although pH changes of the medium may also play a role (Avery *et al.*, 1991). Williams (1960) described an increased Cs^+ uptake in cells of *Euglena intermedia* in the stationary phase of growth and it is probable that this may have been due to a depletion of K^+ from the medium. The inhibition of Cs^+ uptake by external K^+ has been reported previously for *Chlorella* spp. (Williams & Swanson, 1958; Williams, 1960; Plato & Denovan, 1974). The results presented here imply that this inhibition is greater for cells

metabolizing photoautotrophically than chemoheterotrophically. The evidence discussed above precludes the possibility that the K^+/H^+ exchange that operates during metabolism of glucose in *Chlorella* (Tromballa, 1981) has a lower selectivity than the K^+/K^+ exchange that operates during photosynthesis and consequently the reason for this differential sensitivity to K^+ is unknown. External Na^+ had a much lower effect on Cs^+ accumulation than K^+ and in fact a slight stimulation of Cs^+ uptake was evident at the lower concentrations of Na^+ studied. Stimulation of monovalent cation uptake by Na^+ has previously been described in yeast (Borst-Pauwels *et al.*, 1973). The observed depression of Cs^+ accumulation at elevated Na^+ concentrations is in agreement with the results of Williams (1960) with *Euglena intermedia* who attributed the observation to an alteration of plasma membrane permeability by Na^+ rather than by its antagonistic effect in the metabolic pool.

The observed strong inhibition of O_2 evolution from photosynthesizing *C. emersonii* was concurrent with K^+ loss from cells incubated in buffer in both the absence and presence of Cs^+ . Loss of K^+ under similar conditions in the absence of Cs^+ has been reported previously for cyanobacteria (Avery *et al.*, 1991) and yeast (Norris *et al.*, 1976). In marked contrast to photosynthesis, no depression of respiration rate resulted from loss of K^+ , and this was the case even when the decrease in cellular K^+ under the latter conditions was greater than that which resulted in inhibition of photosynthesis. It is significant that the lower value of K^+ in respiring cells observed here [7.9 nmol (10^6 cells) $^{-1}$] was slightly higher than that estimated earlier as being the threshold below which inhibition of chemoheterotrophic cell division results. These results are generally in agreement with those found for chemoheterotrophic and photoautotrophic growth of *C. emersonii* and imply that although the presence of Cs^+ itself in the cell may not be inhibitory, the resultant loss of K^+ is. Photosynthesis and photoautotrophic growth are substantially more sensitive to loss of K^+ , and hence Cs^+ accumulation, than respiration and chemoheterotrophic growth. Whilst it is clear that different K^+ (Cs^+) uptake systems may operate according to the mode of metabolism of *C. emersonii*, it remains unclear why there is a greater requirement for K^+ in photosynthesizing cells. K^+ is primarily required by microbial cells as an osmotic regulator, a regulator of internal pH and as an enzyme activator (Walderhaug *et al.*, 1987). Several enzymes have been identified as having a requirement for K^+ (Suelter, 1970) and it is possible that the additional enzymes required for the catalysis of phototrophy, that are not necessary during chemoheterotrophy, may have a greater requirement for K^+ than those which catalyse the breakdown of sugars.

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