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Growth Responses of Blue-green Algae to Sodium Chloride Concentration

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Summary. General characteristics of blue-green algal halotolerance were studied by growth experiments and selected analyses. Variation in NaCl concentration was used to mimic salinity. Marine isolates were more halotolerant (8—10% NaCl) than non-marine isolates (2% NaCl). The Na^+ requirement for growth was saturated at 1 mg NaCl/l for non-marine isolates and 100 mg NaCl/l for marine isolates. Intracellular Na^+ values were affected by washing; however, bound-K^+ values for both marine and fresh-water blue-green algae were fairly constant, 1—3 µg/mg cells. A specific Na^+ function was implied by the retention after washing of $^{22}Na^+$ (0.1 µg/mg cells) by *Agmenellum quadruplicatum* (PR-6), a marine coccoid blue-green alga.

High concentrations of NaCl apparently inhibit growth more by ionic (Na^+) stress than by osmotic stress. Changes in light, temperature, pH, or composition of the basal medium failed to alleviate this stress.

In contrast to marine bacteria, cells of PR-6 grown in Medium ASP-2 + 90 g NaCl/l did not undergo lysis when suspended in distilled water. However, viability of cells grown in Medium ASP-2 + 90 g NaCl/l decreased rapidly compared to cells grown in Medium ASP-2 + 18 g NaCl/l.

Cells of PR-6 grown in ASP-2 + 90 g NaCl/l were larger than normal, formed chains (3—16 cells), and appeared bleached. Analyses of such cells revealed an overall decrease in fatty acids, hydrocarbons, and pigment levels. Electron micrographs showed that NaCl stressed cells were little altered in morphology.

The photosynthesis of PR-6 cells was immediately depressed when the cells were transferred from 18 g NaCl/l to 70 g NaCl/l medium. When held in the latter for several hours the rate recovered and approached the initial photosynthetic rate maintained before NaCl-shock. This phenomenon was never seen with non-marine isolates. The explanation may lie in the ability of the cell to adjust to sudden Na^+ increase via an ion (Na^+) pump, for example, adenosine triphosphatase (ATPase). Subsequent assays suggested more ATPase activity in a marine isolate than in a non-marine isolate. The ATPase was not, however, ouabain sensitive.

It is suggested that marine blue-green algal isolates are characteristically more halotolerant, perhaps by selection, than fresh-water forms. This difference may be due in part to inherent capacity of the cell to extrude Na^+. Alternatively, in fresh-water forms rhe Na^+ functional sites may be more Na^+ sensitive than in marine forms.

Data accumulating from the study of marine and halophilic bacteria suggest that the Na^+ requirement is specific and largely ionic in nature. Brown (1964) indicated that the roles of water activity and osmotic

pressure in ionic phenomena are probably of minor importance. General characteristics of bacterial cells which tolerate or require (halophilic bacteria) a high concentration of NaCl include: 1. in marine bacteria an overall NaCl requirement (0.2—0.3 M) for optimal growth (MacLeod and Onofrey, 1957); 2. cell wall composition changes according to NaCl concentration; e.g., mucopeptide content and diaminopimelic acid content decrease as extracellular NaCl increases (Brown, 1964; Larsen, 1962); 3. lysis upon dilution of external medium (Larsen, 1962); 4. certain enzymes of halophiles have higher salt optima than corresponding enzymes of non-halophiles (Larsen, 1962); and 5. K^+ accumulation coincident with increase in NaCl levels (Christian and Waltho, 1961).

Although like the bacteria in many respects, blue-green algae are a unique group of micro-organisms. They are procaryotic with O_2-yielding photosynthesis, widely distributed, and usually considered very resistant to environmental extremes (Fritsch, 1952). There are several field observations suggesting that blue-green algae are halotolerant or perhaps even halophilic (Elazari-Volcani, 1943; Hof and Fremy, 1933; Pillai, 1954). However, in an experimental sense, little is known of the meaning of the term "halotolerance" in blue-green algae.

The work described herein, based largely on growth experiments, attempts to define the nature of the response of certain blue-green algae to NaCl (salinity). In addition to the growth data, selected analyses were done to probe for changes in cells at the biochemical level as NaCl concentration was varied during growth.

Materials and Methods

The principal algae used were *Anacystis nidulans* and *Agmenellum quadruplicatum* strain *PR-6*, hereinafter referred to as *TX20* and *PR-6* respectively. *Coccochloris elabans* strain *17A* and other blue-green algae used for comparative purposes are identified in the text. *TX20* was obtained from the laboratory of Algal Physiology, University of Texas at Austin, Texas. *PR-6* and *17A* are marine isolates of this laboratory (Van Baalen, 1962). *PR-6* and *17A* were grown in Medium ASP-2 of Provasoli *et al.* (1957), as modified by Van Baalen (1962). Low-salt medium indicates Medium ASP-2 + 18 g NaCl/l and high-salt medium indicates Medium ASP-2 + 70 or 90 g NaCl/l. *TX20* was grown in Medium Cg10, which is Medium C of Kratz and Myers (1955), containing 1.0 g glycylglycine/l in place of K_2HPO_4 as a buffer and 10 mg Na_2EDTA/l in place of sodium citrate as chelator (Van Baalen, 1967). NaCl was added directly to Medium Cg10 as needed. The pH of all media was adjusted to 8.1 with NaOH or HCl.

All *chemicals* used were reagent grade and were not further purified. EDTA treated NaCl was obtained from Mann Research Laboratories (New York, New York 10006).

PR-6, *17A*, and *TX20* were grown at 39° using the test-tube method of Myers (1950). Illumination was provided by four (two on each side) F48T12/CW-XHO fluorescent lamps 9.0 cm from the tubes. In some cases F20T12/CWX fluorescent lamps were used. Air enriched to 1% CO_2, (v/v), was continually bubbled through the culture tubes.

Growth of the liquid cultures was followed colorimetrically after the method of Kratz and Myers (1955), using a Lumetron Model 402-E Colorimeter. Linearity between O.D. and cell concentration was verified. The growth values reported are the specific growth rate constant, k, in $\log_{10}$ units/day. When $k = 0.301$, the generation time is 24 hours. Viable cell counts on *PR-6* were done after the method of Van Baalen (1965, 1967).

Osmotic pressures were measured by freezing-point depression in an Advanced Osmometer Model No. 68-31 LAS (Advanced Instruments, Inc., Newton Highlands, Mass. 02161).

Media used for enrichment cultures were ASP-2 + 110 g NaCl/1, ASP-2 + 150 g NaCl/l, and seawater concentrated to one-fourth and one-sixteenth original volume. The concentrated seawater media were supplemented with vitamin B_{12} (4 µg/l), vitamin B_1 (1 mg/l), $NaNO_3$ and KH_2PO_4 (5 mg/l). Inocula consisted of mud samples and scrapings from hypersaline environments kindly supplied by the Morton Salt Company and by Dr. Gerald M. Friedman of the Renssalaer Polytechnic Institute, Troy, New York. Culture flasks were incubated at room temperature under various light intensities.

The absorption spectra of whole cells (Shibata, 1958) were made with 1.0 cm cuvettes with scattering plates (Lucite 1/8″, No. 7328), in a Bausch and Lomb Spectronic 505 spectrophotometer (5 nm slits).

Cell volumes were measured by pipetting a known volume of a cell suspension into a calibrated (mm^3) cell-volume tube. The tube was then placed in an International Centrifuge, Universal Model U.V., and spun at 3,300 rpm for 1 hr.

Analyses of cellular lipid fractions containing fatty acids and hydrocarbons were done after the methods of Parker *et al.* (1967) and Winters *et al.* (1969). Gas chromatography was performed using a Perkin-Elmer Model 880 gas chromatograph with 1/8″ columns packed with 5% FFAP on Chromosorb G. Retention times were determined by use of fatty acid and hydrocarbon standards; quantitative data was obtained by measuring peak areas.

For analyses of intracellular Na^+ and K^+ cells of *PR-6* or *TX20* were harvested during exponential growth. After centrifugation, cells were washed with distilled water or $MgSO_4 \cdot 7H_2O$ (5 gm/l for PR-6, 0.25 gm/l for TX20). With unwashed cell pellets the centrifuge tubes were inverted and carefully rinsed with distilled water to the level of the pellet. Cell digestion with 1×10^{-2} M HCl or 1×10^{-2} M HNO_3 gave slightly lower values than digestion with 1×10^{-2} M trichloracetic acid (TCA); therefore, all values represent cells digested by TCA. Following digestion, samples were diluted and analyzed in a Beckman Model 105 Flame Photometer. Values for unwashed pellets were calculated by subtracting the computed Na^+, K^+ content of intercellular volume (40%) as determined with Blue Dextran 2000 (Pharmacia Fine Chemicals, Uppsala, Sweden).

Photosynthesis was measured with a Gilson Medical Electronics Clark-type electrode (OX700) and water-jacketed cell (OX705). Changes in electrode current were amplified on a Keithley 150B Microvolt-Ammeter and recorded on a 1 mv Brown Recorder Model Y153X18-(V)-II-III-(118)-(V). Carbon dioxide was supplied in a 5-min gassing period as 1% CO_2-in-nitrogen prior to closing the electrode chamber. Illumination was provided by a Standard 500 Junior Projector; intensity was controlled by voltage or calibrated screens.

Radioactive sodium, $^{22}NaCl$ in 0.5 N HCl (0.1 mc/ml, carrier free) was obtained from New England Nuclear Corp. One-tenth-ml samples of cells or supernatant containing $^{22}Na^+$ were placed on aluminum planchets and dried under a heat lamp. The samples were counted with a Nuclear-Chicago D-34 tube and Model 181A scaler.

Larger samples (up to 100 mg dry weight cells) were counted with a Nuclear-Chicago Model DS5 scintillation detector, Model 1810 radiation analyzer and Model 181A scaler.

Results and Discussion

The analyses of Clarke (1924) and others (Sverdrup *et al.*, 1947; Usiglio, 1849) showed that NaCl accounts for about four-fifths of the total salts in seawater. Thus it was felt that for the work described herein, the control of NaCl concentration in artificial media would effectively mimic salinity.

Fig. 1 illustrates the two basic growth responses of certain blue-green algae to NaCl concentration. Clearly, the marine coccoid forms *PR-6* and *17A* were considerably more tolerant to high levels of NaCl than the fresh-water coccoid *TX20*. Additional growth experiments showed that growth-rate depression was not simply due to trace contaminants (I^- or Br^-, heavy metals) in the NaCl. For *PR-6* similar growth rates were also seen when the total salts of ASP-2 were varied instead of just NaCl.

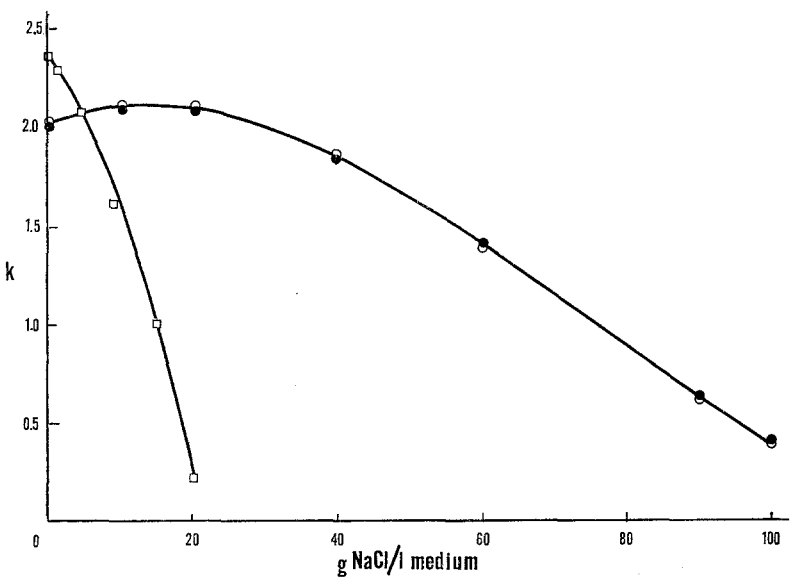

Fig. 1. Growth rates (*k* values of *PR-6*, *17A*, and *TX20* as a function of NaCl concentration in the medium. Base media, ASP-2 for *PR-6* and *17A*, Cg10 for *TX20*, contained no added NaCl. Organisms grown at 39°, with 1% CO_2-in-air, F48T12/CW-XHO fluorescent lamps (650 ft-c). The points indicated by solid circles represent a typical growth rate of *PR-6* at a given NaCl concentration; open circles represent *17A*, open squares represent *TX20*. At any given NaCl concentration the growth rate was stable

Table 1. *Growth response of blue-green algae to NaCl*

Temp.	Medium	Organism	g NaCl/l								
			0	1	5	10	20	40	60	80	100
39°	ASP-2	*Oscillatoria williamsii*[1,a]	NT	+	NT	+++	+++	++	+	+	—
39°	ASP-2	*Microcoleus chthonoplastes*[1,a]	++	++	NT	++	++	+	+	—	—
39°	ASP-2	*Fisherella ambigua*[1,b]	NT	+++	NT	+	+	—	—	—	—
39°	Cg10	*Fisherella ambigua*	++	+	+	+	—	—	—	NT	NT
30°	Cg10	*Fisherella ambigua*	++	+	+	+	—	—	—	NT	NT
39°	ASP-2	*Anabaena variabilis*[2,b]	NT	++	+	+	—	—	—	—	—
39°	Cg10	*Anabaena variabilis*	++	+	+	—	—	—	—	NT	NT
39°	Cg10	*Chlorogloea fritchii*[3,b]	++	NT	++	++	+	±	NT	NT	NT
39°	Cg10	*Tolypothrix tenuis*[2,b]	+++	NT	++	+	+	—	NT	NT	NT
32°	Cg10	*Eucapsis* sp.[1,b]	+++	NT	+	—	—	NT	NT	NT	NT

NT not tested, — no growth observed, + light growth, ++ moderate growth, +++ heavy growth. In addition to medium and temperature indicated, incubation conditions also included F48T12/CW-XHO fluorescent lamps, 1% CO_2-in-air.

[1] Axenic isolate of this laboratory.

[2] Obtained from Laboratory of Algal Physiology, University of Texas, Austin.

[3] Obtained from Dr. D. S. Hoare, Microbiology Dept., University of Texas, Austin.

[a] Isolated from marine environment.

[b] Isolated from fresh-water environment.

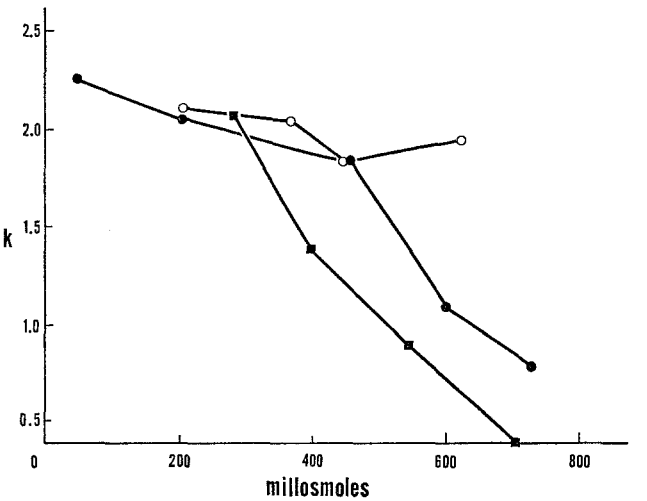

Fig. 2. Growth rate of *TX 20* as a function of osmotic pressure. Cells grown in Medium Cg10 at 39°, F20T12/CWX fluorescent lamps, 1% CO_2-in-air. Solid circles represent growth rates in Medium Cg10 plus NaCl (0, 5, 10, 15, and 20 g/l), open circles represent NaCl replaced by glycerol (15, 30, 40, and 55 g/l), solid squares represent NaCl replaced by Na_2SO_4 (10, 20, 30, and 40 g/l)

To further substantiate the pattern seen in Fig. 1, seven other blue-green algae were tested for initial NaCl tolerance when transferred directly from Cg10 or ASP-2 + 18 g NaCl/l. The results, Table 1, again suggest two general categories of growth response: 1. marine isolates which initially tolerated up to 8—10% NaCl, and 2. fresh-water isolates which only infrequently showed good growth beyond 2% NaCl. It is of interest to note that *Fisherella ambigua*, grown at two different temperatures and in two different media, maintained a consistent growth response. *Anabaena variabilis* (Hecker strain) also showed the same response to NaCl in two different media. This indicated that for these two organisms growth was in fact affected by NaCl levels and not by variation in other medium components.

TX 20 would not grow when transferred from Cg10 + 20 g NaCl/l to Cg10 with higher concentrations of NaCl. With *PR-6, 17A* or *Oscillatoria williamsii*, adaptation to 115 and 140 g NaCl/l, respectively, was seen. Slow growth at these high NaCl levels persisted upon successive transfers.

Fig. 2 shows the growth rates of *TX 20* as a function of osmotic pressure. The *k* values achieved in Medium Cg10 adjusted to similar osmotic strengths with NaCl or glycerol suggested that the growth drop for *TX 20* (Figs. 1 and 2) was largely an ionic effect with a possible small osmotic pressure effect. For *PR-6*, Na_2SO_4 or glycerol could not be sub-

stituted for NaCl, since they were toxic at the concentrations required. Wetherell (1963) similarly concluded that the inhibition of growth of *Scenedesmus* in saline media was not entirely due to osmotic stress.

Back-transfers of both *PR-6* and *17A* from the 100 g NaCl/l cultures directly to ASP-2 + 18 g NaCl/l resulted in k values of 1.78 and 1.84 respectively with only a slight lag period (1 hr). In view of these back-transfer data and general observations from quantitative plating experiments (single cell counts), it is inferred that cells of *PR-6* are tolerant to high-salt conditions by acclimatization rather than by mutation. A similar adaptability by *PR-6* to rapid salt changes was also seen in short-time photosynthesis measurements (see *Effect of NaCl on Photosynthesis*). Evidence supporting this view in bacteria has been reported by Doudoroff (1940), Forsyth and Kushner (1969).

Although blue-green algae have been observed in many hypersaline environments (Baas-Becking, 1931; Elazari-Volcani, 1943; Hof and Fremy, 1933; Pillai, 1954), none has been demonstrated to be truly halophilic in that large amounts of NaCl are required for growth. Efforts were made to obtain halophilic or highly halotolerant blue-green algae by inoculating high-NaCl ASP-2 or enriched ($NaNO_3$, KH_2PO_4, vitamins B_{12} and B_1) concentrated seawater media with mud or water samples from hypersaline environments and solar-evaporation ponds. During incubation under high and low light samples were examined microscopically for blue-green algae. None was detected in salt concentrations beyond the range (0—140 g NaCl/l) already found with the laboratory cultures. Taken together, the growth results with pure cultures and from enrichment cultures suggest that the explanation of the existence of blue-green algae in hypersaline environments is, as documented here, their wide halotolerance.

Halotolerance as Affected by Chemical and Physical Changes in Growth Conditions

Extensive growth experiments with *PR*-6 and *TX20* showed that NaCl stress could not be alleviated by changes in basal medium composition or physical conditions (light-temperature, pH) of incubation. The Ca^{++} relief of salinity stress reported in bean plants (LaHaye and Epstein, 1969) was not observed, nor was Mg^{++} uptake affected by NaCl as seen in bacteria by Tempest and Meers (1968). Additions of vitamins, different nitrogen sources, purines, pyrimidines, and complex organic materials also failed to relieve NaCl inhibition of growth. It is also noteworthy that since iron and manganese solubilities are affected by high salinities (Carpelan, 1957; Pillai, 1955) analyses by atomic absorption of cells of *PR*-6 grown at 0 or 70 g NaCl/l showed no cellular deficiencies of either element: 450 μg Fe/g dry wt, 21 μg Mn/g dry wt.

Attempts to replace NaCl with other solutes (NaI, NaBr, RbCl, LiCl, KCl, Na_2SO_4, sucrose, mannitol) were not successful because of toxicities encountered at the concentrations used.

Growth experiments with *PR-6* at different NaCl/KCl ratios suggested that NaCl-depressed growth rates were reflections of cationic effects more than Cl-effects. Growth was completely repressed in conditions of high K^+ (0.8 M), low Na^+ (0.01 M). K^+ antagonism was relieved by slightly increasing NaCl, indicating a possible specific Na^+ site or function in the cell.

A Na^+ requirement for growth of blue-green algae has been reported by several investigators (Allen and Arnon, 1955; Brownell and Nicholas, 1967; Fogg, 1956; Kratz and Myers, 1955). As measured here, growth saturation for *PR-6* was approached at 100 mg NaCl/l; for *TX20* saturation value was approached at 1 mg NaCl/l. These values are in general agreement with the blue-green algal requirements (10—100 mg NaCl/l) reported in the literature (Brownell and Nicholas, 1967; Fogg, 1956). It is noteworthy that although *PR-6* demonstrated a greater requirement than *TX20*, the requirement was still very low considering its high NaCl tolerance. In addition, *PR-6* is not, in its NaCl requirement, similar to marine bacteria in that its optimum rate does not require 0.2—0.3 M NaCl (MacLeod and Onofrey, 1957; Pratt and Austin, 1963).

Cellular Response in High NaCl Conditions

Microscopic comparisons were made of both *PR-6* and *17A* grown in ASP-2 + 100 g NaCl/l. In each case high-salt grown cells were somewhat lower in pigmentation and slightly larger in size when compared to cells grown at 18 g NaCl/l ASP-2. Also conspicuous was a tendency for cells to occur in chains 3—16 cells long.

The tendency of these high-salt grown cells of *PR-6* or *17A* to form chains points to a possible inhibition of cell division by high NaCl concentrations, which may be reflected by the concomitant growth rate depression. Similar effects of high-salt have been reported for *Scenedesmus* (Wetherell, 1963), *Escherichia coli* (Doudoroff, 1940), and certain halophilic bacteria (Forsyth and Kushner, 1969). However, neither *PR-6* nor *TX20* showed any gross alteration in ultrastructure, particularly in the wall area, in response to NaCl.

Wet weight to dry weight comparisons of high-salt grown cells indicated no difference in water content compared to normal cell values. This indicates that if osmotic effects are present, they are of minor importance.

High-salt grown cells of *PR-6* washed and suspended in distilled water did not change in optical density over a period of 16 hr. Microscopic examination revealed no cell lysis as seen with marine bacteria (Brown,

1962; Pratt and Waddell, 1959; Tyler *et al.*, 1960). The viability of cells treated in such a manner was examined. Cells grown in ASP-2 minus NaCl, or with 18 or 60 g NaCl/l were harvested, washed once with distilled water and then resuspended in distilled water. Controls were resuspended in fresh growth media with respective NaCl concentrations. At intervals quantitative viable cell counts were performed after the method of Van Baalen (1965, 1967). Results showed that the duration of cell viability in distilled water increased as the NaCl concentration of original growth medium decreased.

The loss in viability associated with suspension in distilled water was prevented by single additions of KCl, $MgSO_4 \cdot 7H_2O$, $NaNO_3$, NaCl, or Tris buffer pH 8.0, all at the same concentration as in Medium ASP-2. A similar effect of single salts in preventing leakage of cell components has been seen in *Salmonella* (Christian, 1958). These data further indicate that *PR-6* is not responding osmotically to the ionic composition of the medium.

Analyses of NaCl-stressed Cells

The combined results of several attempts to establish cellular Na^+ and K^+ contents of *PR-6* and *TX 20* are shown in Table 2 and 3.

Although great difficulty was encountered in obtaining reproducible values for Na^+ content, values for K^+ repeatedly fell in the range of 1—3 μg K^+/mg cells, regardless of medium NaCl concentration. On the

Table 2. *Sodium and potassium content of* PR-6 *grown at different NaCl concentrations*

	μg Na^+/mg dry weight cells		
g NaCl/l ASP-2:	0	18	60
[a]	1.8	23.4	28.5
[b]	2.2	4.5	2.0
[c]	1.4	3.6	3.8
	μg K^+/mg dry weight cells		
g NaCl/l ASP-2:	0	18	60
[a]	1.4	1.7	2.8
[b]	1.1	1.4	0.7
[c]	1.3	1.0	0.8

Values shown are the most reproducible values obtained by flame photometric measurements.

[a] Cells not washed but corrected for Na^+, K^+ in interstitial space.

[b] Cells washed with $MgSO_4 \cdot 7H_2O$, 5 g/l.

[c] Cells washed with distilled water.

Table 3. *Sodium and potassium content of* TX20 *grown at different NaCl concentrations*

	μg Na^+/mg dry weight cells			
g NaCl/l Cg10:	0	5	10	15
[a]	5.8	—	—	—
[b]	1.7	0.9	0.7	1.0
[c]	0.8	1.5	—	3.8
	μg K^+/mg dry weight cells			
g NaCl/l Cg10:	0	5	10	15
[a]	2.6	—	—	—
[b]	0.9	0.6	0.9	0.9
[c]	1.1	0.5	—	0.4

Values shown are the most reproducible values obtained by flame photometric measurements.

[a] Cells not washed but corrected for Na^+, K^+ in interstitial space.

[b] Cells washed with $MgSO_4 \cdot 7H_2O$, 0.25 g/l.

[c] Cells washed with distilled water.

other hand, Na^+ values differed according to treatment of cells prior to analysis.

Distilled water washings were considered undesirable in view of the above effect of distilled water on cell viability. Also, it has been demonstrated (Christian, 1958; Matula and MacLeod, 1969; Rouf, 1964) that intracellular Na^+, K^+ values are affected by different washing treatments.

The data do not show the positive correlation of K^+ content with salt tolerance seen by Christian and Waltho (1961). Both *PR-6* and *TX20* showed similar K^+ contents regardless of growth medium salinity. Less confidence is felt in the accuracy of the Na^+ values. However, the values for normally grown cells are not beyond the range of other reported values for cellular Na^+ content in yeast (Norkrans, 1968) and bacteria (Christian and Waltho, 1961).

The possible biochemical role of Na^+ in a blue-green algal cell remains unknown; a Na^+ role in nitrogen metabolism has been suggested (Brownell and Nicholas, 1967).

Cells of *PR-6* were washed twice with $MgSO_4 \cdot 7H_2O$ 5 gm/l, twice with distilled water, then incubated with $^{22}Na^+$ for 6 hrs in darkness. Following 6 washings with distilled water 0.1 μg Na^+/mg cells was retained in the cells. This value agrees with the 0.35 μg Na^+/mg cells that Eagon (1968) found associated with the cell walls of *Pseudomonas aeruginosa*. Both the Na^+ requirement for growth and the notion of Na^+ binding sites in the cell are potentially important points with respect to halotolerance.

Table 4. *Effect of NaCl on fatty acids of* PR-6

Concentration NaCl in Medium ASP-2	μg fatty acid per mg dry weight			
	C16:0	C16:1	C18:1	C18:2
18 g/l	9.9	1.6	1.5	1.2
90 g/l	1.7	0.2	0.4	0.3
		Ratios		
		16:0 / 16:1	18:1 / 18:2	
18 g/l		6.2	1.3	
90 g/l		8.5	1.3	

Effect of NaCl on hydrocarbons of PR-6

Concentration NaCl in Medium ASP-2	μg hydrocarbon/mg dry weight	
	C19:1	C19:2
18 g/l	0.61	0.11
90 g/l	0.21	0.10
	Ratio	
	19:1 / 19:2	
18 g/l	5.5	
90 g/l	2.1	

Conditions. Cells grown at 39°, F48T12/CW-XHO fluorescent lamps, 1% CO_2-in-air. Values shown corrected for trace contaminants in blank.

A comparison of the pigment spectra of normally grown and high-salt grown *PR-6* showed the expected overall drop in pigment levels of high-salt grown cells. However, pigment ratios remained similar, indicating no obvious selective System I or System II effects (Myers, 1963).

The effects of NaCl concentration on the total cellular fatty acid and hydrocarbon content of *PR-6* are shown in Table 4. Compared to normally grown cells, high-salt grown cells were lower in overall content of the fatty acids; however, there were no qualitative differences. Ratios of C18:1/C18:2 were unchanged; the C16:0/C16:1 ratio increased. The drop in total fatty acids may reflect chemical change in unit membranes (photosynthetic lamellae) during NaCl stress.

The hydrocarbon level also dropped, and C19:1 of high-salt grown cells was one-third that of normal cells, whereas C19:2 remained unchanged. The results of fatty acid analyses suggest quantitative changes in membrane structure. A biochemical role of hydrocarbons in blue-green algae is at the present time unknown. However, it is tempting to consider

that they are also membrane constituents and their diminution correlates with the fatty acid changes.

Effects of NaCl on Photosynthesis

The lower growth rates and pigment levels of high-salt grown *PR-6* were, as anticipated, reflected by a lower photosynthetic rate as measured by O_2 evolution. Blinks (1951) and others (McLachlan, 1961; Vosjan and Siezen, 1968) have also found that photosynthesis is sensitive to salinity effects. It should be pointed out that although both Na^+ and K^+ requirements have been established for photosynthesis in blue-green algae (Emerson and Lewis, 1942; Kratz and Myers, 1955) the Na^+/K^+ ratio was not found to be critical.

The photosynthetic rate for control cells of *PR-6* in ASP-2 + 18 g NaCl/l was 50 µl O_2/mm³ cells/hr; for *PR-6* cells grown in ASP-2 + 60 g NaCl/l, 27 µl O_2/mm³ cells/hr. Cells of *PR-6* when subjected to NaCl stress by rapid suspension in ASP-2 + 70 g NaCl/l showed an immediate drop (6 min) to 1—10 µl O_2/mm³ cells/hr. Subsequent measurements of the NaCl-shocked cells showed recovery to a rate of 20—30 µl O_2/mm³ cells/hr after 3—6 hr incubation in darkness. This recovery phenomenon varied considerably in repeated experiments and occasionally recovery could not be clearly demonstrated. Measurements of photosynthetic rates in *17A* were similar to *PR-6*.

Normal *TX20* photosynthetic rates (ca. 50 µl O_2/mm³ cells/hr) were more difficult to establish, due for unknown reasons, to a slow decay of photosynthetic rate while the cells were in the electrode chamber. However, a depression in rate to ca. 7 µl O_2/mm³ cells/hr was demonstrated upon rapid transfer to Cg10 + 20 g NaCl/l. In contrast to *PR-6* and *17A* no recovery of photosynthetic rate in *TX20* was observed up to 10 hr.

With *PR-6* grown at various NaCl levels there was no significant change in C^{13}/C^{12} ratios of CO_2 derived from total combustion of cells, indicating that NaCl inhibition does not interfer with the isotope effect in transport or initial fixation of CO_2 (Park and Epstein, 1961).

Much evidence has been presented (Gill and Solomon, 1959; Middleton, 1970; Opit and Charnock, 1965; Wiley, 1969) that a (Na^+, K^+)-activated, ouabain-sensitive, ATPase is associated with the active transport of Na^+ and K^+ across cellular membranes in eucaryotic cells. Norkrans and Kylin (1969) have suggested that halotolerance in yeasts may be partly due to the cell's ability to extrude Na^+ and take up K^+. Such an explanation of halotolerance also apply to blue-green algae. In this connection the data of several assays using acetone powders and lysozyme-EDTA treated cells suggested that a higher level of (Na^+, K^+)-activated ATPase activity exists in *PR-6* and *17A* than in *TX20*. However, exten-

sive growth experiments and experiments patterned after $^{22}Na^+$ exchange kinetics in red blood cells (Gill and Solomon, 1959) failed to reveal a ouabain-sensitive ATPase system (Na^+ pump) in *PR-6*.

Studies of marine bacteria (Drapeau and MacLeod, 1963; Thompson *et al.*, 1969) have shown that there is no bacterial ATPase specifically inhibited by ouabain. Hence, it is with caution that halotolerance in blue-green algae is correlated with the presence of a Na^+ pump (e.g. [Na^+, K^+]-activated ATPase) in the algal cell. Perhaps the Na^+ requirement of blue-green algae is a reflection of an involvement in an ATPase reaction. Na^+ ions promote the synthesis of a phosphorylated intermediate in the Na^+/K^+ antiporter ATPase reaction (Mitchell, 1970). On the other hand, a Na^+ pump in procaryotic cells may have different properties, and its activity may not be revealed by assays such as those performed here.

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