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Stimulatory Effect of Procaine on the Growth of Several Microalgae and Cyanobacteria

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Abstract

Procaine has been used to stimulate plant growth and it has been noted that it also promotes growth of microorganisms. The effect of procaine hydrochloride concentration on the growth rates of several species of microalgae and cyanobacteria was studied under both photoautotropic and heterotrophic growth conditions. Procaine hydrochloride was added to cultures at concentrations over the range $0{\cdot}01-1000\,mg\,L^{-1}$.

A stimulating effect of procaine hydrochloride on photoautotrophic growth was observed for the cyanobacteria *Anabaena cylindrica* and *Anabaena variabilis*, and for the salt-tolerant green algae *Dunaliella primolecta* and *Dunaliella parva*. During active growth in batch culture an increase in growth rate (compared with control culture without procaine hydrochloride) of about 25% was observed at $0{\cdot}1\,mg\,L^{-1}$ of procaine hydrochloride for *A. cylindrica*. However, procaine hydrochloride was toxic at concentrations of $> 10\,mg\,L^{-1}$. Simultaneous administration of hydrolysis products of procaine, *p*-aminobenzoic acid and diethyl aminoethanol, in lieu of procaine hydrochloride, was as effective as procaine in stimulating growth of *A. cylindrica*. Heterotrophic growth of *Chlorella ellipsoidea* and *Prototheca zopfii* was not stimulated by procaine hydrochloride over the concentration range studied ($0{\cdot}1-10\,mg\,L^{-1}$).

The combined effects of procaine hydrochloride concentration and four other environmental factors (temperature, light intensity, CO_2 concentration in the flushing gas and NaCl concentration) on growth rate of *D. primolecta* was modelled using both a neural network approach and a response surface method. These results indicate that procaine hydrochloride exerts different effects on the growth of microalgal and cyanobacterial cells as functions of dosage, species and culture conditions.

Procaine (4-aminobenzoic acid 2-(diethylamino) ethyl ester) exhibits several physiological effects in man and animals and has been used as a local anaesthetic. It is also known to stimulate plant growth (Cachita-Cosma & Ardelean 1996). Procaine has been used in Romania to increase plant productivity and promote the development of fruits and seeds, and it has been noted that it promotes the growth of microorganisms such as bacteria, yeast, algae and protozoa (Cachita-Cosma & Ardelean 1996). Although the mechanism of action has not been fully elucidated, it seems that procaine affects the cell cycle or the rate of cell division. Interest in algae and cyanobacteria (blue-green algae) as potential sources of pharmaceutical agents has increased in recent years (Glombitza & Koch 1989; Suzuki et al 1998a). In this context, procaine might prove useful for the industrial cultivation of algae since algae are not rapidly growing organisms. Susceptibility of algae to procaine would likely depend upon the individual species and its cultivation conditions. Unfortunately, only a limited amount of information on the response of algae to procaine is available (Cachita-Cosma & Ardelean 1996).

The aim of this study was to determine the effect of procaine hydrochloride concentration on the growth of several species of microalgae and

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cyanobacteria. We also examined the effect of procaine hydrochloride concentration in conjunction with several other environmental factors known to affect the photosynthetic growth of these microorganisms.

Materials and Methods

Chemicals

Procaine hydrochloride and 2-diethylaminoethanol were products of the Tokyo Chemical Industry (Tokyo, Japan). *p*-Aminobenzoic acid was obtained from Wako (Osaka, Japan).

Microorganisms

The microorganisms used were: *Chlorella ellipsoidea* Gerneck (IAM C-87), *Dunaliella primolecta* Butcher (IAM C-525), *Dunaliella parva* Lerche (IAM C-527), *Anabaena cylindrica* Lemmermann (IAM M-1) and *Anabaena variabilis* Kützing (IAM M-3), all from the Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan; and *Prototheca zopfii* Krüger ATCC 30253.

Photoautotropic growth

Stock cultures of each algal species were incubated in a liquid medium in 100-mL cotton-stoppered oblong flat flasks. The flasks were placed in a thermostatically-controlled water bath and illuminated from one side with fluorescent tubes. The cultures were aerated continuously with or without additional CO_2 (Suzuki et al 1995). Media were prepared for each species according to its particular needs (Watanabe 1960).

Heterotrophic growth

The two *Anabaena* spp. used in this study can not grow heterotrophically in the dark on glucose or sorbitol as sole carbon and energy sources, so tests of the effects of procaine hydrochloride on heterotrophic growth were confined to *C. ellipsoidea* and *P. zopfii*. Modified Bristol medium (Watanabe 1960) supplemented with 1% (w/v) D(+)-glucose (Koso Chemical, Tokyo, Japan) was autoclaved, and procaine hydrochloride was added aseptically (by filtration) to the medium to give the desired final concentration. The initial cell concentration of the algal inoculum was adjusted to be the same as that for tests of photoautotrophic growth, and cells were cultured in complete darkness in reciprocally-shaken Erlenmeyer flasks. *P. zopfii* was also grown in Sabouraud Dextrose Broth (Difco, USA).

Measurement of growth

Biomass concentration was determined by measuring optical density at 635 nm (OD635) or cell number. A spectrophotometer (Shimadzu UV-1200) or haemocytometer, or both were used to assess cell concentrations. The initial cell concentrations were adjusted to $1{\cdot}52 \times 10^6$ cells mL^{-1} for *C. ellipsoidea*, $7{\cdot}00 \times 10^5$ cells mL^{-1} for *D. primolecta* and *D. parva*, $1{\cdot}42 \times 10^6$ cells mL^{-1} for *P. zopfi*, and 0·10 in OD635 for *A. cylindrica* and *A. variablis*.

Statistical analysis

Analysis of variance with Microsoft Excel was employed to determine whether there were significant differences ($P < 0{\cdot}05$) in growth rates or final cell concentrations between treated and control cells.

Results and Discussion

Effect of procaine on photoautotrophic growth

Four of the five species studied were stimulated by procaine hydrochloride: *A. cylindrica* (growth condition: temperature, 30°C; light, 2 klux; flushing gas, air), *A. variabilis* (30°C, 2 klux, air), *D. primolecta* (25°C, 2 klux, air, NaCl, 0·68 M) and *D. parva* (25°C, 2 klux, air, NaCl, 0·68 M) (Table 1). The effect of procaine hydrochloride (0·1–1000 mg L^{-1}) on algal growth was graded as follows: ++, a strong stimulation; +, weak stimulation; 0, no effect; −, weak inhibition; −−, strong inhibition. The responses of these four species to procaine hydrochloride were similar, and maximal growth rates occurred in cultures treated with 0·1 mg L^{-1} procaine hydrochloride. Procaine hydrochloride was increasingly inhibitory to algal growth as the concentration exceeded 1·0 mg L^{-1}.

For *A. cylindrica*, an increase of about 25% in the logarithmic growth rate was observed at 0·1 mg L^{-1} of procaine hydrochloride, relative to control cultures lacking procaine (Figure 1). The original growth curves were shown in a preliminary study (Suzuki et al 1998b). However, procaine hydrochloride at 0·5 or 1 mg L^{-1} showed no significant effect on cell proliferation. Procaine hydrochloride concentrations > 10 mg L^{-1} resulted in significant growth inhibition in a dose-dependent manner. The EC50 (the concentration required to inhibit growth by 50% relative to controls) value obtained from a linear regression of the log phase specific growth rate (μ) was 20 mg L^{-1} procaine hydrochloride. The ratio of EC50 to optimal procaine hydro-

Table 1. Effect of procaine hydrochloride on growth rates of some microalgae and cyanobacteria.

Species	Strain	Procaine hydrochloride concn (mg L^{-1})						
		0·01	0·1	0·5	1	10	100	1000
Photoautotrophic growth								
Anabaena cylindrica Lemmermann	IAM M-1	+	++	0	−	−−	−−	−−
Anabaena variabilis Kützing	IAM M-3		++		−	−−		
Chlorella ellipsoidea Gerneck	IAM C-87		0		0	−		
Dunaliella primolecta Butcher	IAM C-525		++		+	−		
Dunaliella parva Lerche	IAM C-527		++		0			
Heterotrophic growth								
Chlorella ellipsoidea Gerneck	IAM C-87	0	0		0	0		
Prototheca zopfii Krüger	ATCC 30253	0	0		0	0		

++: strong growth stimulatory effect; +: weak growth stimulatory effect; 0: no effect; −: weak inhibitory effect; −−: strong inhibitory effect.

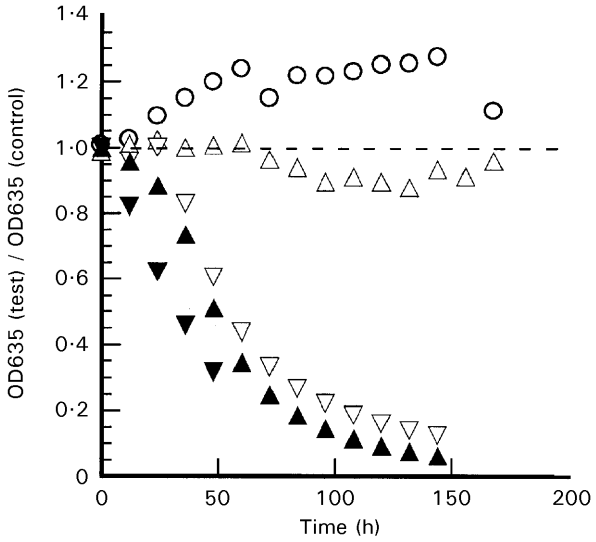

Figure 1. Effect of procaine hydrochloride (○ 0·1; △ 1; ▽ 10; ▲ 100; and ▼ 1000 mg L^{-1}) on the photoautotrophic growth of *A. cylindrica* IAM M-1 (30°C, aeration with air, 2 klux light intensity). Points are means of triplicate determinations. Growth rate changes were expressed by the ratio of optical density at 635 nm (OD635; 1 unit of optical density at 635nm = 0·351 g of dried cells per litre of culture) of test sample to that of the control. The initial cell densities were adjusted to approximately 0·1 in OD635.

chloride concentration was 200, suggesting a wide safety margin for practical use of procaine hydrochloride in photosynthetic algal cultivation. In contrast, the stimulatory effect of procaine hydrochloride on autotropic cultures of *C. ellipsoidea* (25°C, 2 klux, air) was not significant at the concentrations tested. Growth inhibition was seen at 10 mg L^{-1} of procaine hydrochoride.

Procaine-stimulated multiplication has been reported previously for the green algae *Scenedesmus acutus* and *Stichococcus bacillaris* (Cachita-Cosma & Ardelean 1996), and the optimum concentrations of procaine hydrochloride for cell growth were comparable with those found in the present study. The effects of five plant growth substances, indole-3-acetic acid, indole-3-butyric acid, indole-2 propionic acid, naphthalene acetic acid and gibberellic acid on three green algae (*Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Chlorococcum* spp.) have been reported (Prasad 1982). This study revealed that the growth stimulating effects of these compounds varied from species to species (e.g. indole-3-acetic acid had a positive effect on growth of *S. obliquus* and *Chlorococcum* spp., whereas it had a negative effect on growth of *A. falcatus*. All growth substances were highly stimulatory to the growth of *S. obliquus* in the range 1–20 ppm, and concentrations of indole-3-acetic acid and indole-3-butyric acid > 5 ppm were inhibitory to the growth of *A. falcatus*. Only indole-3-acetic acid stimulated growth of *Chlorococcum* spp. Indole-3-acetic acid and gibberellic acid prompted the formation of four-celled colonies in *S. obliquus*. This fact showed that the substances stimulated cell division rather than cellular elongation. According to our observation in the cultures of *Dunaliella*, the mean cell size of the algae treated with procaine hydrochloride was somewhat smaller than that in the control. This suggests that procaine hydrochloride mainly stimulates cell division of *Dunaliella* just like indole-3-acetic acid and gibberellic acid. However, more investigations are necessary to measure CO_2 uptake or O_2 production rates, or both, in algae to ascertain the effect of procaine hydrochloride on the rate of photorespiration. Measurement of the activity of enzymes such as peroxidase and (±)-amylase may be useful to

evaluate the mechanism of action of procaine hydrochloride. The influence of phytohormones, auxins, gibberellins, cytokinins and several substances of a hormonal nature on algae has been reviewed (Davis 1988).

Effect of procaine on heterotrophic growth

The responses of *C. ellipsoidea* (at 25°C) and *P. zopfii* (at 25°C) to procaine hydrochloride in heterotrophic growth in the dark are shown in Table 1. The growth of neither species was stimulated in the concentration range 0·01–10 mg L^{-1}, though neither was growth inhibition observed for these algae at 10 mg L^{-1} procaine hydrochloride. For *P. zopfii*, penetration of procaine hydrochloride through the cell wall might be precluded by the strong hydrophobicity of the outermost surface of this cell (Suzuki et al 1998c).

Effect of hydrolysis products from procaine

Procaine hydrochloride is metabolised or hydrolysed in-vivo to yield *p*-aminobenzoic acid (PABA) and 2-diethylaminoethanol (DEAE), vitaminic precursors of folic acid and choline, respectively. The effects of PABA, DEAE and PABA + DEAE on growth of *A. cylindrica* (30°C, 2 klux, air) are shown in Figure 2. The doses of each chemical were equivalent to a dose of 0·1 mg L^{-1} procaine hydrochloride. The effect of a combined administration of PABA + DEAE was comparable with that of 0·1 mg L^{-1} procaine hydrochloride, whereas PABA or DEAE alone showed no significant stimulatory effect. A synergy may exist between PABA and DEAE; in any case it is not necessary to use procaine per se, a comparatively expensive compound, in order to enhance the growth of *A. cylindrica*.

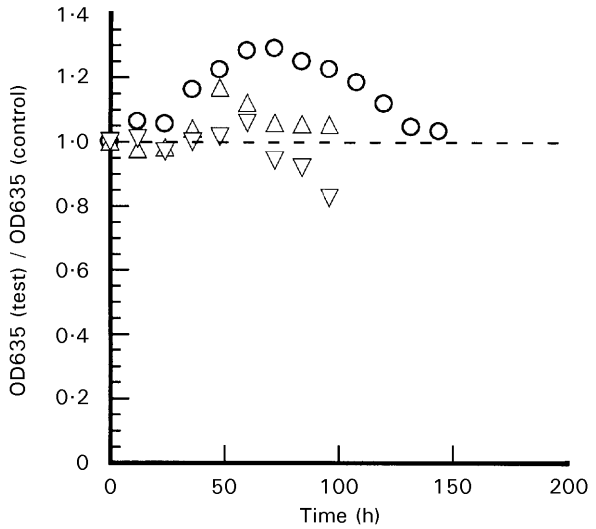

Figure 2. Effects of *p*-aminobenzoic acid (PABA, △), 2-diethylaminoethanol (DEAE, ▼) and PABA + DEAE (○) on growth rates of *Anabaena cylindrica* IAM M-1 (30°C, aeration with air, 2 klux light intensity). The doses of each chemical are equivalent to 0·1 mg L^{-1} procaine hydrochloride. Points are means of triplicate determinations.

Synergy of procaine with other environmental factors

Many factors affect growth and photosynthesis in algae. Generally, algal specific growth rates (μ) are governed by a multitude of environmental determinants such as light intensity (I), temperature (T), nutrient concentration (S) and pH, and the growth response to these determinants is species specific (eg. Goldman 1979):

$$\mu = f(I, T, S, pH, \text{species, etc.}) \qquad (1)$$

D. primolecta was chosen as a representative microorganism for further study of the combined effects of procaine hydrochloride and other environmental factors on growth. A neural network approach was used to model algal growth as a function of environmental factors. Artificial neural networks have proven useful when no rigid theoretical basis or mathematical relationship is available for describing the phenomenon to be modelled. The theory and general practice of artificial neural networks are well documented (Zupan & Gasteiger 1993).

The neural network used was three-layered (input, hidden and output) with back propagation of errors (Figure 3). There are five input neurons corresponding to procaine hydrochloride concentration, NaCl concentration, CO_2 concentration in the flushing gas, culture temperature, and light intensity; a varying number of hidden-layer neurons and only one output neuron, corresponding to μ. The five environmental factors and their range of magnitudes are shown in Table 2, where the levels of each parameter are classified into lower (−), higher (+) and basal (0) levels. The entire design of experiments are given in Table 3. Sixty experimental runs were performed. The first 26 runs were

Table 2. Range of factors selected for experiment.

Factors	Levels		
	Lower	Base	Higher
PHCl concn (mgL^{-1})	0·1	1·0	10
NaCl concn (M)	0·1	2·0	4·0
CO_2 in flushing gas (%)	1	8	15
Temperature (°C)	25	30	35
Light intensity (klux)	8	13	18

PHCl, procaine hydrochloride.

Table 3. Experimental conditions and their responses or the specific growth rates of *Dunaliella primolecta*.

Run no.	Experimental conditions					Responses
	PHCl (mg L^{-1})	NaCl (M)	CO_2 (%)	Temp. (°C)	Light (klux)	μ (h^{-1})
Runs based on three-level fractional fractorial design						
1	0·1	0·10	8	30	13	0·139
2	0·1	2·00	8	30	13	0·074
3	1·0	2·00	8	30	13	0·064
4	10·0	2·00	8	30	13	0·042
5	0·1	4·00	8	30	13	0·012
6	1·0	2·00	1	30	18	0·037
7	10·0	2·00	1	30	18	0·025
8	10·0	4·00	1	30	18	0·011
9	1·0	0·10	15	30	8	0·099
10	10·0	0·10	15	30	8	0·070
11	0·1	0·10	1	30	8	0·068
12	0·1	2·00	1	30	8	0·039
13	0·1	4·00	15	35	13	0·011
14	1·0	4·00	15	35	13	0·000
15	1·0	2·00	15	35	18	0·032
16	10·0	2·00	15	35	18	0·020
17	10·0	4·00	15	35	18	0·006
18	1·0	0·10	8	35	8	0·066
19	10·0	0·10	8	35	8	0·033
20	1·0	2·00	1	25	13	0·039
21	10·0	2·00	1	25	13	0·030
22	10·0	4·00	1	25	13	0·004
23	0·1	4·00	8	25	18	0·018
24	1·0	4·00	8	25	18	0·015
25	0·1	0·10	8	25	8	0·083
26	0·1	2·00	8	25	8	0·068
Additional runs						
27	0·1	0·68	8	30	13	0·143
28	0·1	0·68	8	30	13	0·151
29	0·1	0·68	8	30	13	0·140
30	0	0·68	8	30	13	0·100
31	1·0	0·68	8	30	13	0·125
32	10·0	0·68	8	30	13	0·071
33	0	2·00	8	30	13	0·054
34	0·1	0·68	15	30	18	0·075
35	1·0	0·68	15	30	18	0·068
36	10·0	0·68	15	30	18	0·051
37	0	2·00	15	30	18	0·031
38	0·1	0·68	1	30	18	0·080
39	0·1	0·68	15	30	8	0·077
40	0	0·10	15	30	8	0·069
41	0·1	0·68	1	30	8	0·071
42	0	0·68	1	30	8	0·051
43	0·1	0·68	15	35	13	0·064
44	0	4·00	15	35	13	0·007
45	0·1	0·68	1	35	13	0·062
46	1·0	0·68	1	35	13	0·054
47	10·0	0·68	1	35	13	0·040
48	0	2·00	1	35	13	0·026
49	0·1	0·68	15	35	18	0·057
50	0·1	0·68	8	35	8	0·076
51	0	0·10	8	35	8	0·044
52	0·1	0·68	15	25	13	0·065
53	1·0	0·68	15	25	13	0·057
54	10·0	0·68	15	25	13	0·039
55	0	2·00	15	25	13	0·026
56	0·1	0·68	1	25	13	0·081
57	0·1	0·68	8	25	18	0·083
58	0	4·00	8	25	18	0·012
59	0·1	0·68	8	25	8	0·078
60	0	0·68	8	25	8	0·069

PHCl, procaine hydrochloride.

based on a three-level fractional factorial design (Carlson 1992) to achieve a great saving in the number of experimental runs which are required by a complete three-level factorial design. For the concentration of procaine hydrochloride, the logarithmic dose values were used in the analysis; therefore the concentration of $1{\cdot}0\,mg\,L^{-1}$ at basal level was just the arithmetical mean of lower and higher values of the concentration (0·1 and $10\,mg\,L^{-1}$) in log units. For procaine hydrochloride and NaCl concentrations, they were tested at additional levels, $0\,mg\,L^{-1}$ and 0·68 M (equivalent to the concentration in the basal medium), respectively, keeping other factors at three levels shown in Table 2. The resulting additional experiments were those numbered from 27 to 60 in Table 3. The initial lag phase was not observed in all experimental conditions. The μ values in the logarithmic growth phase obtained are given in Table 3.

Except for procaine hydrochloride concentration, all input and output data were transformed to normalized values x' between 0·05 and 0·95 (Suzuki & Ishida 1995), using the following formula:

$$x' = 0 \cdot 9(x_i - x_{min})/(x_{max} - x_{min}) + 0 \cdot 05 \quad (2)$$

where x_i is the value of the ith input or output value, x_{min} and x_{max} are its minimum and maximum data over the data set. For the procaine hydrochloride concentrations, 0, 0·1, 1·0 and $10\,mg\,L^{-1}$, were assigned to 0·05, 0·35, 0·65 and 0·95, respectively, to give proper values for the inputs.

The sigmoid function has been selected as the transfer function for each neuron. Weight adjustment has been derived by the back propagation algorithm employing the generalized delta rule to minimize the mean square error between observed and calculated μ data. For the parameters of the model, the learning rate and momentum are initially set to 0·5 and 0·6, respectively, and gradually decreased. The number of hidden neurons were varied from two to five, and the networks were optimized (Suzuki et al 1997). The optimum network model with four neurons was selected from the 5000 iteration cycles. With this architecture, the model contains a total of 29 adjustable parameters. Thus, the number of available data points (60) is about two times greater than the number of model parameters and this ratio is included in a guideline of determining the number of hidden neurons being employed (Andrea & Kalayeh 1991). This model consists of three individual submodels according to three different starting configurations (Table 4), and the neural network model output is calculated as the average of the output values of these three individual models. The neural network model gave the squared correlation coefficient of 0·903 and the root mean squares error of 0·008 as the quality of the fitting. For the predictive ability of the model, the 'leave-one-out' cross-validation procedure was carried out. The cross-validated squared correlation coefficient of 0·619 and the root mean squares error of 0·022 showed the reliability of the model.

The relative dependence of each factor in affecting the μ values in the neural network model was assessed. The variations of simulated μ values of the neural network model were monitored by changing the value of one factor while the other four factors were constant at 25, 50 and 75% of their corresponding ranges in the data set. On the basis of the resulting plots shown in Figures 4a–e, one can see that there is a parabolic dependence of the procaine hydrochloride concentration. In view of the individual contributions of the environmental factors to the algal growth, procaine hydrochloride is useful for promoting production of microalgal

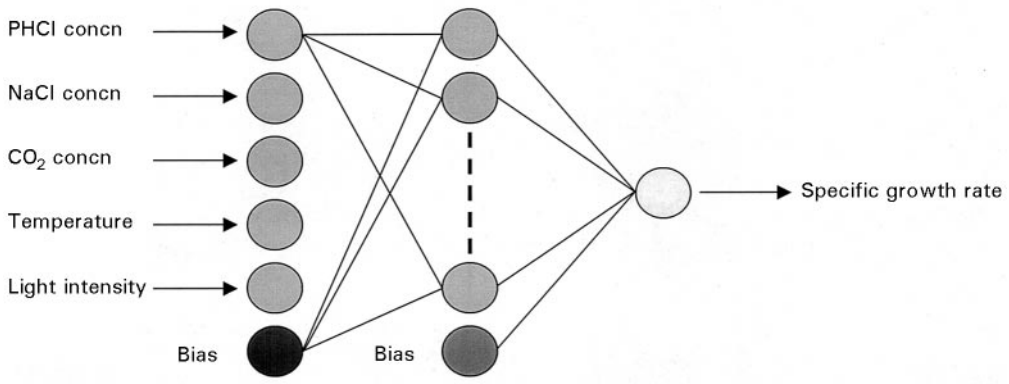

Figure 3. Three-layer neural network model for simulating the combined effects of procaine hydrochloride and other four environmental factors on the specific growth rate (μ) of *Dunaliella primolecta*. PHCl: procaine hydrochloride.

Table 4. Optimum parameters (weights) of the neural network model with four hidden-layer neurons.

	Hidden-layer neuron				
	1	2	3	4	Bias
			First starting configuration		
Input layer					
1	−8·5029	4·8296	0·5589	0·3333	
2	5·8529	3·2564	−3·4581	−3·8327	
3	5·1583	3·3829	−2·0487	−13·9345	
4	0·1373	0·1764	−0·4980	0·1814	
5	−0·9640	1·0318	0·6026	−4·6750	
Bias	−2·5622	−6·7910	1·1008	7·9219	
Output layer					
1	−2·1007	−3·1383	2·0301	−2·8971	1·5146
			Second starting configuration		
Input layer					
1	0·1944	−5·3760	3·5839	−0·6467	
2	−1·4745	0·9499	1·0506	−2·5502	
3	0·9458	−4·6523	3·6534	−0·8105	
4	−1·9602	11·9342	−8·9534	0·4736	
5	−2·1624	−21·6795	14·4775	4·3925	
Bias	1·8174	8·0431	−6·5894	−2·6925	
Output layer					
1	2·6875	−6·8412	−7·7914	3·5248	4·5941
			Third starting configuration		
Input layer					
1	0·8956	5·4481	1·2820	−6·1797	
2	3·1682	−5·4008	3·8569	−0·2704	
3	−11·3340	−8·2085	0·2360	−2·7718	
4	0·2526	0·0683	0·4625	0·0200	
5	6·7004	−3·3545	1·3986	−0·8599	
Bias	3·3857	1·5823	−3·1065	2·3602	
Output layer					
1	2·4760	−2·0307	−5·8846	−3·1398	1·5193

and cyanobacterial mass and can be used to compensate for limiting environmental factors such as light intensity; light differs from the other environmental factors in that it is instantaneous, and is supplied only to the surface of the culture. The simulation of the effects of procaine hydrochloride at various light intensities is shown in Figure 4f. In this figure, the remaining three parameters are held at their suboptimal values (NaCl = 0·1 M, CO_2, 7·0%; temperature, 32°C). The suboptimal concentration of procaine hydrochloride can be found at approximately 0·1 mg L^{-1}.

The relationships and interrelationships of the variables in the above system can also be assessed by the response surface model including linear, interaction and second-order terms:

$$\mu = b_0 + \sum_{I=1}^{5} b_i X_i + \sum_{I<j}^{5} b_{ij} X_i X_j + \sum_{j=1}^{5} b_i j X_j{}^2 \quad (3)$$

where μ is the response or algal specific growth rates, X_i and X_j are the factors, and b parameters are regression coefficients calculated from the experimental data by multiple linear regression. For this modelling, the scaled independent variables used in the neural network modelling were employed. The following statistically significant model with the squared correlation coefficient of 0·801 and the root mean squares error of 0·016 was obtained after the significance test at 95% confidence level:

$$\mu = 0{\cdot}072 + 0{\cdot}0819X_1 - 0{\cdot}0787X_2 + 0{\cdot}126X_3 - 0{\cdot}0688X_4X_5 - 0{\cdot}0967X_1{}^2 - 0{\cdot}124X_3{}^2 - 0{\cdot}0501X_4{}^2 - 0{\cdot}0246X_5{}^2 \quad (4)$$

where X_1 is the procaine hydrochloride concentration, X_2 is the NaCl concentration, X_3 is the CO_2 concentration in the flushing gas, X_4 is the culture temperature and X_5 is the light intensity.

Although the response surface model containing three linear, four quadratic and one interaction terms gave somewhat inferior fit compared with the neural network model, it was interesting to note that the result was consistent with some of the findings from the neural network model. Both models agreed that the biological response has a parabolic dependence on procaine and CO_2 concentrations, respectively, and a linear dependence on NaCl concentration. For the interaction of factors, only the X_4X_5 term was significant. The interrelation between temperature (X_4) and light

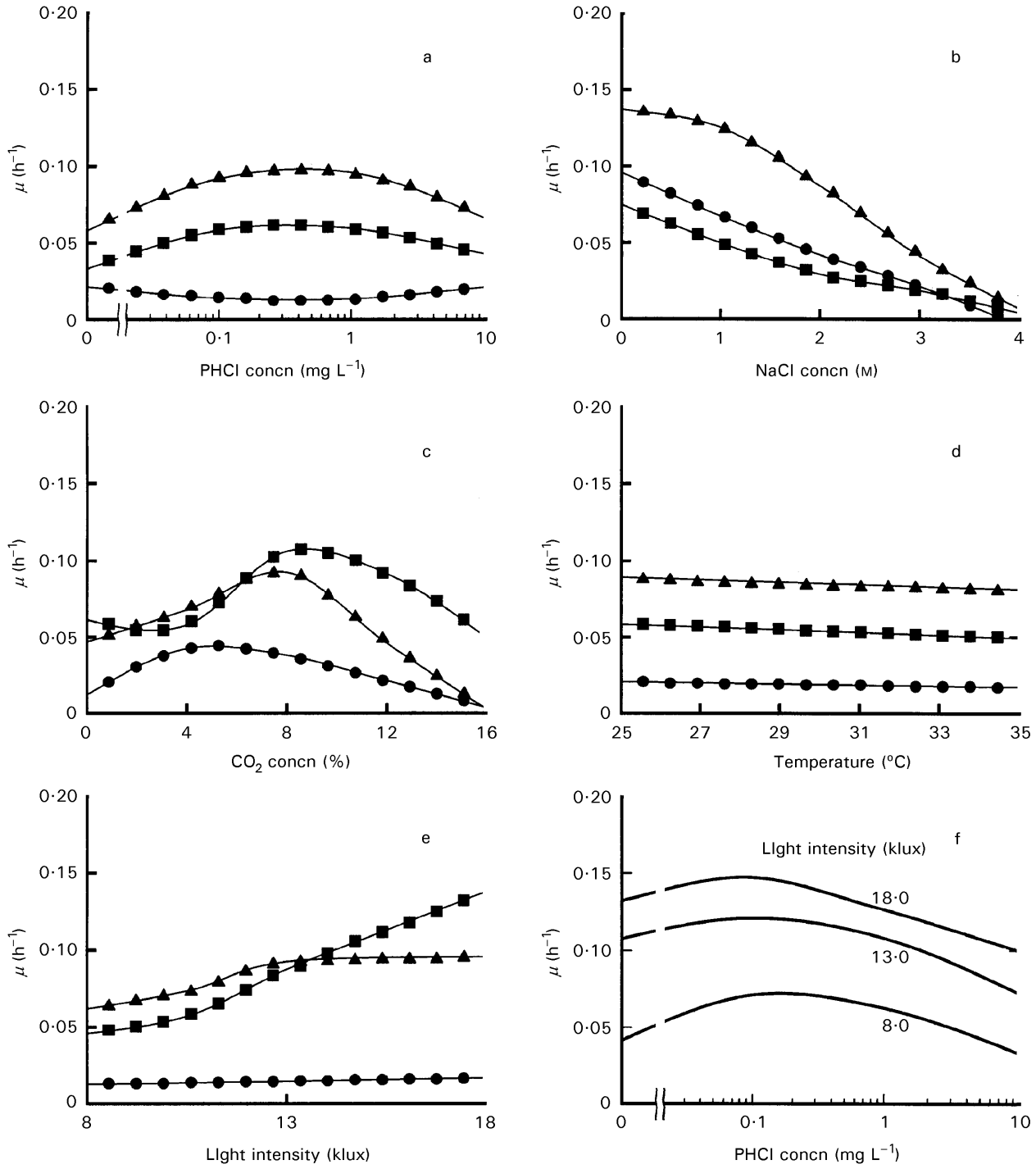

Figure 4. Neural network calculated specific growth rates of *Dunaliella primolecta* as a function of individual growth factors. The remaining four factors are held constant at 25% (■), 50%(▲) and 75% (●) of their corresponding ranges in the dataset (a–e). f. The effect of procaine hydrochloride (PHCl) concentrations at three levels of light intensity where the remaining three factors are held at their suboptimal values (NaCl, 0·1 M; CO_2, 7·0%; temperature, 32°C).

(X_5) on algal growth rates is well known (eg. Goldman 1979). The inclusion of other interaction terms into the model did not show substantial improvement of a description of the response.

Conclusions

Procaine hydrochloride stimulated photoautotropic growth of the cyanobacteria *A. cylindrica* and *A. variabilis* and of the green algae *D. primolecta* and

D. parva. The suboptimal growth promoting concentrations of procaine hydrochloride were approximately 0·1 mg L^{-1}. Procaine hydrochloride could be replaced by equimolar quantities of its hydrolysis products PABA and DEAE. The effects of procaine hydrochloride appear to be similar to those of phytohormones, although further studies are necessary to elucidate its exact mechanism of action.

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