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# PRELIMINARY INVESTIGATIONS ON THE EFFECT OF (2-CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE (CCC) ON THE METABOLISM OF *CHLORELLA*

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**Abstract**

*Chlorella* cultures were grown in light with low and high concentrations of CCC and the rate of growth, photosynthesis, respiration, nitrate and nitrite assimilation were studied. CCC at low concentrations up to 6.3 x $10^{-6}$M, did not have any effect on these processes whereas at higher concentrations 0.5 x $10^{-4}$ to 0.5 to $10^{-2}$M, all these processes were affected differently. CCC brings about qualitative change in the soluble pool of the amino acids in treated cells of *Chlorella.*

## Introduction

(2-chloroethyl) trimethyl ammonium chloride (CCC) is a plant growth regulator which produces different effects in a wide variety of plants. The effect of CCC on the growth and metabolic activities of higher plants have been extensively reviewed (Cathy, 1964) but the literature on the effect of CCC on algae is scanty. Cathy (1964) reported that $10^{-6}$M or lower concentration of allyl trimethyl ammonium bromide (AMAB) stimulated the growth of filamentous alga (*Ulothrix subtillisma.* Rabenh), whereas higher concentrations inhibited growth. Kim (1962) observed that CCC inhibited the growth of *Chlorella.* Supniewska (1963) studied the effect of CCC on the growth of *Lemna minor* and *Chlorella pyrenoidosa. Lemna* was found to be more sensitive to the action of growth retardant, it was killed at 0.6 x $10^{-1}$ M whereas *Chlorella* was more resistant than *Lemna.*

No attempt has so far been made to study the effect of this growth retardant on the metabolism of algae. The present investigation reports the results of the effect of CCC on the growth and metabolism of *Chlorella.*

## Meterial and Methods

### *Growth of Organism*

The alga used in this investigation was *Chlorella fusca* (a kind gift of Prof. P.J. Syrett, University College Wales, Swansea, England). Cultures were grown as described by Syrett & Morris (1963).

*General Experimental Procedure*

CCC solution after passing through the millipore filter was added to the culture medium in appropriate concentrations. An incoulum of *Chlorella* suspension giving a final concentration of 300 cells per cubic mm was added to the culture solution. During the growth period of 4 days the cells were counted after every 24 hours by Neubauer haemocytometer. They were then harvested by centrifugation at 400 x g for 5 minutes, washed once in nitrogen free medium (full growth medium minus N-source) and resuspended in the same medium. The cell density was adjusted turbidiometrically to 3 mgs dry weight cells per ml. Dry weight standard curves were prepared for each concentration of CCC. All experiments were performed at 25°C and pH 6.1.

*Gas Exchange*

Evolution and consumption of oxygen was measured by conventional Warburg manometry. Depending upon the experiment, illumination of 8500 Lux was provided at the surface of the flasks. Carbon dioxide was removed by including 20% potassium hydroxide (plus filter paper) in the centre well of Warburg flask.

*Analytical Procedures*

Assimilation of nitrate and nitrite was measured by following their disappearance from the medium. The methods were the same as described by Ahmed (1968). Amino acids were investigated qualitatively using two dimensional paper chromatography in a manner described by Fowden (1951). Chlorophyll was estimated by the method of Maelaclam & Zalik (1963).

## Results

*Effect on Growth*

In preliminary experiments (data not included in this paper), low concentrations of CCC (ranging from 6.6 x $10^{-10}$ to 6.3 x $10^{-6}$M) did not have any effect on the growth and metabolism of *Chlorella* when they were incubated for a period of 2 to 24 hours in light or dark. Higher concentrations of CCC ranging from 0.5 x $10^{-4}$M to 0.5 x $10^{-1}$M were therefore used in all the subsequent experiments. The bleaching of cells occured at 0.5 x $10^{-1}$M CCC concentration. Growth rate was studied by following cell counts and dry matter production (Fig. 1). Dry matter production was inhibited at all the three concentrations of CCC used. At low concentrations of 0.5 x $10^{-4}$M & 0.5 x $10^{-3}$M, percentage inhibition remained almost constant during the entire growth period whereas at 0.5 x $10^{-2}$M it decreased linearly with time.

Inhibition of cell division decreased with time and reached to a negligible value at 48 hours when CCC was applied at a concentrations of 0.5 x $10^{-4}$M. A marked stimulation in cell number was observed when cells were subjected to 0.5 x $10^{-4}$M CCC for a

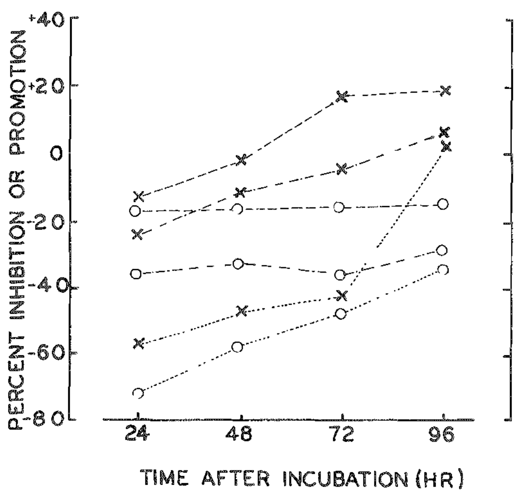


Fig. 1. CCC induced changes in cell division and dry matter production.
x——— cell division; o ——— dry matter production; ----- $0.5 \times 10^{-4}$M CCC; -.-.-.-. $0.5 \times 10^{-3}$M CCC; .......... $0.5 \times 10^{-2}$M CCC.

period of 72 hours. Increase in cell number was also observed at 96 hr. at all CCC levels and this was proportional to the concentration of CCC.

CCC treated *Chlorella* showed higher amounts of Chlorophyll "a" on dry weight basis but different concentration of CCC did not seem to have any effect on this increased amount. On cell basis the concentration of Chlorophyll "a" decreased with increasing concentration of growth retardant. Chlorophyll "b" concentration increased both on dry weight and cell basis. The ratio of Chlorophyll a/b was 1.8 in control in both the parameters and CCC treatment decreased this ratio to 1.3 at all concentrations used (Table 1). These results suggest that more chlorophyll "b" is found in CCC treated cells. It may be mentioned that Chlorophyll "b" contents also increased in *Chlorella* cultures when they were subjected to various concentration of NaCl (Chimiklis & Karlander, 1973).

*Effect on Photosynthesis and respiration*

Rate of photosynthesis increased considerably in all the concentrations of CCC when results were expressed on dry wt. basis (Table 2). Inhibition was observed at $0.5 \times 10^{-2}$M on cell basis. The enhanced rate of photosynthesis decreased with increasing concentration of CCC in both the parameters. This decrease is linear with increasing concentrations of CCC.

CCC treated cells consume more oxygen both on dry weight and cell basis (Table 3). Like photosynthesis this increased rate of respiration decreased linearly with increasing concentrations. The comparison of the photosynthetic and the respiratory capacities of treated and untreated cells reveal that the rates of both processes, were highest at the lowest concentration.

**Table 1. Effect of "CCC" on the Chlorophyll Content of Chlorella**

| Culture medium Supplemented with. | ng Chl. a /mg dry wt. | % increase (+) or decrease (−) over control. | ng Chl b /mg dry wt. | % increase (+) or decrease (−) over control. | Ratio of Chl. a/b |
|---|---|---|---|---|---|
| None | 41.8 | — | 22.7 | - | 1.8 |
| 0.5 x $10^{-4}$M CCC. | 57.2 | +36.8 | 43.4 | +91.19 | 1.3 |
| 0.5 x $10^{-3}$M CCC. | 56.5 | +35.3 | 43.2 | +90.30 | 1.3 |
| 0.5 x $10^{-2}$M CCC. | 54.8 | +31.1 | 42.2 | +85.90 | 1.3 |
| | ng Chl. a/ $10^{6}$ cells | % increase (+) or decrease (−) over control | ng Chl b./ $10^{6}$ cells. | % increase (+) or decrease (−) over control. | Ratio of Chl. a/b |
| None | 1.58 | — | 0.86 | - | 1.8 |
| 0.5 x $10^{-4}$M CCC | 1.54 | − 2.53 | 1.16 | +34.88 | 1.3 |
| 0.5 x $10^{-3}$M CCC | 1.47 | − 6.96 | 1.13 | +31.40 | 1.3 |
| 0.5 x $10^{-2}$M CCC | 1.35 | −14.57 | 1.04 | +20.93 | 1.3 |

**Table 2. Effect of Various Concentrations of "CCC" on the Photosynthesis of Chlorella.**

| Culture medium supplemented with. | n. mole $O_2$ evolved/ mg dry wt. cells. | % increase (+) or decrease (–) over control | P. mole $O_2$ evolved/ cell | % increase (+) or decrease (–) over control. |
|---|---|---|---|---|
| None | 2.679 | - | 0.101 | – |
| $0.5 \times 10^{-4}$M CCC | 5.627 | +110.04 | 0.151 | +49.51 |
| $0.5 \times 10^{-3}$M CCC | 4.317 | + 61.14 | 0.113 | +11.88 |
| $0.5 \times 10^{-2}$M CCC | 3.571 | + 33.30 | 0.088 | -12.87 |

**Table 3. Effect of "CCC" on Respiration of Chlorella**

| Culture medium supplemented with | n. mole $O_2$ consumed/ mg dry cells. | % increase (+) or decrease (-) over control. | P. mole $O_2$ consumed/ cell. | % increase (+) or decrease (-) over control. |
|---|---|---|---|---|
| None | 0.223 | – | 0.008 | - |
| $0.5 \times 10^{-4}$M CCC | 0.402 | +80.27 | 0.011 | +37.50 |
| $0.5 \times 10^{-3}$M CCC | 0.357 | +60.09 | 0.009 | +12.50 |
| $0.5 \times 10^{-2}$M CCC | 0.313 | +40.36 | 0.008 | |

*Effect on nitrogen metabolism*

Like respiration and photosynthesis the rate of nitrate assimilation also increased in treated cells The increased rates of nitrate assimilation decreased linearly with increasing concentrations of CCC in both parameters (Table 4). Rates of nitrite assimilation of CCC treated *Chlorella* cell increased insignificantly on dry weight basis but a significant decrease was observed on cell basis. (Table 5).

Effect of CCC on the soluble pool of amino acids showed that total number of amino acids increased with increasing concentrations of CCC. Number of parent amino acids, however, decreased with increasing concentrations and therefore new amino acids appeared in the soluble pool. These new amino acids were 5, 7 and 11 respectively in the increasing concentration of CCC (Table 6). No attempts has been made to identify all the amino acids but few were identified by comparing their $R_f$ values with the known amino acids. Visual observations of amino acid spots reveal an increasing level of proline in CCC treated cells.

**Discussion**

Results of this investigation have demonstrated that higher concentrations of CCC have profound effect on the growth and metabolism of *Chlorella*. Growth experiments have shown that dry matter production is inhibited at all the three concentrations of CCC used. This inhibition remained constant at low concentrations during the entire growth period whereas at higher levels it decreased linearly with time. The dependence of inhibition of cell division on the concentration and incubation period suggests that as CCC is metabolized its concentration gradually becomes low and inhibitory effect is gradually relieved. When this compound has completely been metabolized the cell division proceeds at a normal rate. It is also likely that some product of CCC metabolism or a compound produced by the action of CCC is responsible for the control of cell division. Our results confirm the earlier findings of Linser *et al* (1965) that plants treated with CCC show an increase in proline and decrease in hydroxyproline contents. Steward *et al* (1958) suggested that cell division in higher plants go with a special moiety of protein rich in hydroxyproline. Although direct relationship between cell division and hydroxyproline has not been demonstrated in algae, yet hydroxyproline containing proteins have been reported in the cell wall by a number of workers (Roberts *et al*, 1972, Gorelli & Clealand, 1968). It appears likely that inhibition of cell division in *Chlorella* with increasing CCC concentrations, may be due to an increased level of proline or decrease in hydroxyproline.

Rate of photosynthesis, respiration and nitrate assimilation are increased by the action of CCC. The enhanced rates of these processes decrease with increasing concentration of CCC. The increase in photosynthesis may be related to an overall increase in chlorophylls and chloroplast proteins. CCC treatment show higher levels of total chlorophylls and specially Chl b. The increased rate of photosynthesis under saline condition has been attributed to increased levels of Chl b, which improves the efficiency of photosynthatic apparatus (Chimiklis & Karlander 1973). Stoddart (1964) studied the effect

Table 4. **Effect of "CCC" on Nitrate Assimilation of Chlorella.**

| Culture medium supplemented with | n. mole $NO_3'$-N assimilated/mg dry cells. | % increase (+) or decrease ( ) over control. | P. Mole $NO_3'$-N assimilated/ cell. | % increase (+) or decrease (–) over control |
|---|---|---|---|---|
| $NO_3'$-N + CCC | 0.079 | | 0.003 | |
| $NO_3'$-N + $0.5 \times 10^{-4}$M CCC | 0.302 | +282.28 | 0.008 | +166.66 |
| $NO_3'$-N + $0.5 \times 10^{-3}$M CCC | 0.241 | +205.06 | 0.006 | +100.00 |
| $NO_3'$-N + $0.5 \times 10^{-2}$M CCC | 0.142 | + 79.75 | 0.004 | + 33.33 |

**Table 5. Effect of "CCC" on Nitrite Assimilation of Chlorella.**

| Culture medium supplemented with. | n. mole. $NO_2'$-N assimilated/mg dry cells. | % increase (+) or decrease (–) over control. | P. mole. $NO_2'$N assimilated/ cell. | % increase (+) or decrease (–) over control. |
|---|---|---|---|---|
| $NO_2'$-N – CCC | 0.31 | – | 0.012 | – |
| $NO_2'$-N + 0.5 x $10^{-4}$M CCC | 0.33 | +6.46 | 0.009 | –25.00 |
| $NO_2'$-N + 0.5 X $10^{-3}$M CCC | 0.32 | +3.23 | 0.008 | –33.33 |
| $NO_2'$-N + 0.5 x $10^{-2}$M CCC | 0.32 | +3.23 | 0.008 | –33.33 |

**Table 6. Effect of "CCC" on the Soluble Fraction of Nitrogen of Chlorella.**

| Culture medium supplemented with. | Total No. of amino acids present | Number of amino acids whose Rf. value correspond with the Rf. values of amino acids present in control. | Number of new amino acids appeared in treated cells. |
|---|---|---|---|
| None | 12 | – | – |
| 0.5 x $10^{-4}$M CCC | 15 | 10 | 5 |
| 0.5 x $10^{-3}$M CCC | 14 | 7 | 7 |
| 0.5 x $10^{-3}$M CCC | 16 | 5 | 11 |

of CCC on *Lolium temulentum* and observed that free amino acids which are utilized for the formation of structural proteins are diverted towards the synthesis of chloroplast proteins. Specific contribution of these factors in the enhancement of photosynthesis was not evaluated. We assume that increase in photosynthesis is due to the cumulative effect of increased levels of chloroplast protein and chlorophylls. Increased rates of respiration, nitrate and nitrite assimilation is indirectly related to photosynthesis which supply the substrate and reduced pyridine nucleotide for these processes.

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