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# **The effect of high temperature and salt on the metabolic heat rate of** *Chlorella* **cells 1**

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#### **Abstract**

The metabolic heat rate of plants is an integral indicator of the state of the organism. The metabolic heat rate reflects changes in all the anabolic and catabolic processes in plant cells between optimal and extreme conditions. The present studies determined the effects of extreme temperatures (45°C) and high NaC1 concentrations (450 mM) on the rate of heat evolution by *Chlorella* cells. At first, the rate of heat production increases as compared with the control; thereafter it decreases to a steady-state value which is lower than that of the the control. The decrease in metabolic rate may be connected with changes in metabolic processes associated with adaptation of the organism to stress condition. © 1998 Elsevier Science B.V.

*Keywords:* Adaptation; *Chlorella;* Heat rate; Stress

#### **1. Introduction**

The effects of stress on plant growth and development [1], photosynthesis, the ultrastructure of chloroplasts [2,3], respiration [4], ion and metabolite transport [5] have been thoroughly studied. However, there is little information on the effects of stress on energetic indexes such as metabolic heat rate.

According to Refs. [6,7], the rate of heat production is the most integral indicator of total metabolism, because it indicates the efficiency of energy use. These measurements of metabolic heat rate can contribute to an understanding of the response mechanisms of plants under different environmental stresses.

Some aspects of the effects of high temperature and salt on the energetics of *Chlorella* cells, i.e. energy losses (dark metabolic heat rate) and the rate of energy storage, have been studied. The basic aim of this work is to determine the adaptive changes in energetics of plant cells under stress.

## **2. Experimental**

The single-cell algae, *Chlorella,* is the object of this investigation. *Chlorella* was grown in the Tamiya medium [8] at 30°C, illuminated at  $1 \times 10^4$  lx with a photoperiod of light/dark of 12/12 h. Cell suspensions were bubbled with  $0.3\%$  CO<sub>2</sub> in air. The optical density was maintained constant at  $100-150 \times$  $10<sup>6</sup>$  cells/ml. NaCl and ATP were added to the culture vessels.

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The dark heat production rate of *Chlorella* suspensions were measured in an LKB-2277 microcalorimeter [9]. The thermal equilibrating time of the ampuls is 20-30 min, so a heat rate measurement takes ca. 40-50 min. The heat production rate is determined from the equation  $\sum_{n=1}^{n} Q = q \times t/m$ , where q is the metabolic heat in Joules,  $t$  the time in hours and  $m$  the dry weight in milligrams. The rate of lightenergy storage was determined by photomicrocalorimetry [ 10].

#### **3. Results**

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At high temperatures and salt concentrations, a twophase response of *Chlorella* is observed. Fig. 1 presents the data on the dark metabolic heat rate as a function of time at the extreme temperature  $(45^{\circ}C)$ and high concentration of NaCI (450 mM). Extreme temperature was maintained in a microcalorimeter for a period of 6 h. NaC1 was added to *Chlorella* suspension just before the experiment. At the beginning, the heat rate increased compared with the control. Thereafter, the rate of heat production decreased to a lower rate of energy loss.

In the following experiments (Fig. 2), the dark metabolic heat rate was determined at 30°C after exposure to 45°C for a period of 1 h. For the first 1.5 h after exposure ended, the rate of heat production was below that of control. Beyond 1.5 h, the thermograms of the control and the sample under stress are

Fig. 1. Dark metabolic heat rate of *Chlorella* cells at 45°C and salt at 30°C throughout the experiment.

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Fig. 3. The influence of exogenous ATP on the rate of light energy storage by *Chlorella* cells at optimal and high temperature. A control;  $\overline{B}$  – ATP 10<sup>-5</sup> M; C – ATP 10<sup>-3</sup> M (30°C); D – 45°C 1 h; E –  $45^{\circ}$ C+ATP- $10^{-5}$  M; and F –  $45^{\circ}$ C+ATP- $10^{-3}$  M.

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Fig. 2. The rate of heat evolution by *Chlorella* cells at 30°C after exposure to high temperature for l h.

very similar. Apparently, the disturbance of the metabolic rate was reversible.

The rate of energy stored was determined by photomicrocalorimetry [10,12]. The rate of light-energy storage by stress compared to the control is decreased. Both, the 1 h exposure to  $45^{\circ}$ C and the exposure to 450 mM NaC1 decreased the rate of storage of light energy by ca. 20%. One reason for the rather high rates of the energy storage under stress may be activation of cyclic photophosphorylation. According to the literature [13], cyclic photophosphorylation is more resistant to the unfavorable environmental conditions than non-cyclic photophosphorylation.

The results given in Fig. 3 support the assumption, namely that plants under stress require an additional expenditure of metabolic energy. Addition of ATP to the *Chlorella* suspension, after an exposure for a period of 1 h at the high temperature, restored or increased the rate of energy storage compared to that of the control, depending on the concentration. Addition of exogenous ATP to the suspension inhibits the rate of energy storage at the control conditions, probably, because the cells do not require the additional energy under optimal conditions.

## **4. Discussion**

Plants must respond to constantly changing environmental conditions. Optimization of energy metabolism contributes to functioning of plant cells under stressful conditions. Because adaptation is connected with energy expenditure, stress resistance through altered cellular metabolism is possible. Metabolic heat rate is an integral indicator of the metabolic response to stress. In this study, the initial response to stress was an increase in the metabolic heat rate. At a prolonged interval, the metabolic heat rate was reduced by the stress. The rate of storage of light energy was reduced by stress, except in the presence of exogenous ATP. These results indicate that, under stress, plants need additional energy for the organism to adapt to the new conditions. The initial increase in metabolic rate, seen after a stress is applied, probably is the initial stage of adaptation. As noted in Ref. [11], an active intensification of the organism's adaptation potentialities does occur, but no passive expenditure of the initial store of "adaptation energy" may take place. After the initial response, the metabolic rate falls to a new steady state, corresponding to adaptation of the plant cells to the new condition. Briefly, plants adapt energy metabolism to resist unfavorable conditions. Maintenance of the energy supply under extreme conditions is important for conservation of the structure and functions of

the organism and helps the plant to survive under unfavorable conditions.

### **5. Conclusion**

Plants adapt energy metabolism to unfavourable conditions. The establishment of a new steady-state level of the energetic exchange is an indicator of plant adaptation. The decrease in metabolic heat rate is accompanied with a certain inhibition of metabolism, which leads a system to the more stable state. The maintenance of the energy supply under the action of extreme factors at a rather high level is an important condition for the conservation of structural-functional integrity of the organism and the possibility for adaptational changes, which help the plant to survive under new unfavourable conditions.

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