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INTERACTION OF MINERAL NUTRITION AND TEMPERATURE ON THE GROWTH OF *EUGLENA GRACILIS*

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Introduction

Euglena gracilis Klebs (strain "Z" and var. *bacillaris* Pringsheim) is a suitable object for plastid investigation. It is usually cultured in a defined inorganic basal medium, mostly devised by Hutner et al. (1956), or by Cramer and Myers (1952). For practical reasons it is often grown in the so-called rich or complex medium which supports an average growth rate, somewhat greater than that supported by the Cramer-Myers medium, where the variation of the individual generation time is reduced (Cook and Cook 1962). It was first devised by Pringsheim and Hovasse (1948) and it contains 0.1 per cent sodium acetate + 3 H_2O, 0.2 per cent Bacto-peptone, 0,1 per cent Difco beef extract and 0.2 per cent Difco yeast extract. The same medium with slight modifications (mostly omission of beef extract) was used by a number of workers (e.g. Gibbs 1960, Rosen and Gawlik 1961, Pogo et al. 1966 etc). Some additional nutrients especially salts were added to this basal medium by some authors (e.g. Mg SO_4 + 7 H_2O, KH_2PO_4, Na_2HPO_4 and $CaCl_2$ by Kirk 1962; KH_2PO_4, K_2HPO_4, $MgSO_4$ + 7 H_2O and glucose by Mego 1964). In most cases *Euglena* is grown at room temperature under continuous light. It has also been induced to divide synchronously by means of light-dark cycles (see review by Padilla and James 1964).

The present study investigated optimum conditions for cultivation of *Euglena* in the complex medium in terms of growth (expressed as increase in cell number, chlorophyll and carotenoid content) at different temperatures with addition of various nutrients.

Euglena gracilis strain "Z" could be grown well in a chemically defined medium by Sager and Granick for *Chlamydomonas* (1954) slightly modified (Marčenko unpublished). The modification consisted

in replacing ammonium nitrate by ammonium monoH-orthophosphate because nitrates are not utilized by *Euglena* (Provasoli 1958), in omitting sodium citrate and ferric chloride, adding 0.1 mg/l vitamin B_1 (thiamine HCl) and 0.4 µg/l of vitamin B_{12} and 1,2 ml/l of the trace element solution by Wall et al (1952) with added $FeSO_4 + 7\ H_2O$ 40 mg/100 ml. The micronutrient solution used by Sager and Granick is also suitable. Various salts from this modified Sager-Granick medium were added to the complex medium in the same relative amounts as in the original medium but at higher concentrations, as shown below.

Materials and Methods

Euglena gracilis strain "Z" was grown in the modified complex Pringsheim medium as described previously (Marčenko 1970) in 5 ml solution in test tubes. One drop of the concentrated solutions of salts was added to 5 ml of the complex medium.

The concentrations of the added salts were the following (after addition):

K_2HPO_4	0.08	per cent (w/v)
KH_2PO_4	0.08	per cent
$K_2HPO_4 + KH_2PO_4$	0.16	per cent
$MgSO_4 + 7\ H_2O$	0.24	per cent
$(NH_4)_2HPO_4$	0.0546	per cent
$CaCl_2 + 2\ H_2O$	0.016	per cent

Trace elements solution contained the following concentrations of salts after addition to the basal medium:

H_3BO_3	450.0 µg	per 100 ml medium
$CoSO_4 + 7\ H_2O$	190.0 µg	"
Na_2MoO_4	360.0 µg	"
$CuSO_4 + 5\ H_2O$	15.0 µg	"
$MnCl_2 + 4\ H_2O$	1.4 µg	"
EDTA (Na salt)	4.0 µg	"
$ZnSO_4 + 7\ H_2O$	340.0 µg	"
$FeSo_4 + 7\ H_2O$	320.0 µg	"

Each test tube was inoculated with ca 1×10^4 cells/ml. They were placed either in an incubator (at temperatures 25° and 32 °C respectively) illuminated from the outside with a light intensity of ca 1500 lx, or in a waterbath (cooled with running tap-water) at a temperature of 18 °C. The cultures were illuminated for 14 hours daily or continuously.

After five days 2 ml of the culture were extracted with 80% acetone. Total chlorophyll content of the extract was determined according to the method of Arnon (1949). Dividing the amount of chlorophyll (per ml volume) by the number of cells (per ml volume) $\times 10^6$, values for chlorophyll content per 10^6 cells were obtained. The total carotenoid content was determined according to the method of Kirk and Allen (1965). The value of ΔA car 480 was introduced into the equation of Goodwin (1955 p. 297) for carotenoid estimation with an average $E^{1\%}_{1cm}$ of 2500. By dividing the amount of carotenoid per ml by the number of cells per ml $\times 10^6$, values for carotenoid content per 10^6 cells were obtained.

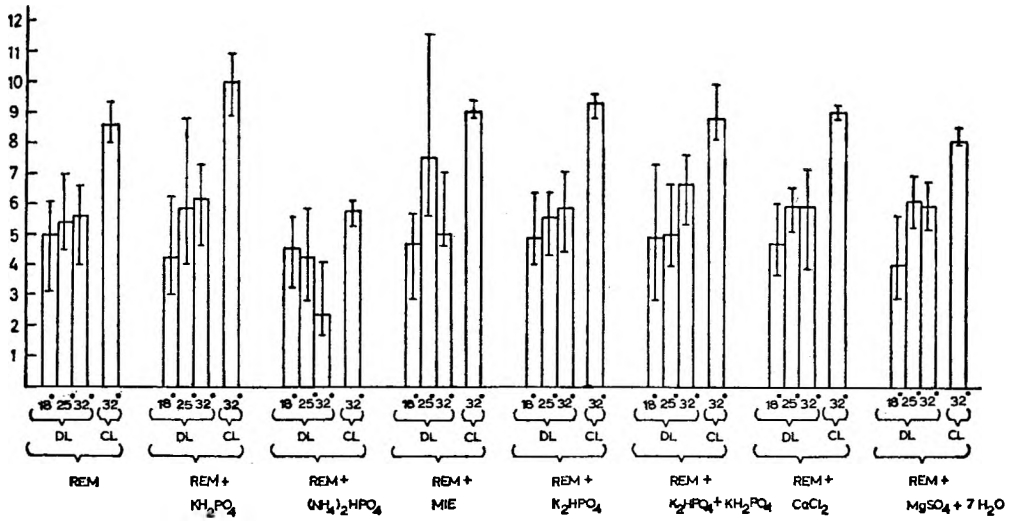

Fig. 1. Effect of temperature and additional nutrients on growth of *Euglena gracilis* strain »Z« grown on a complex medium. The height of the columns represents cell number/ml $\times 10^5$ (mean values of 2 experiments; each experiment was performed in triplicate) after five days growth.

DL = grown in dark-light cycles; *CL* = grown in continuous light; *MiE* = microelements; *REM* = rich *Euglena* medium.

The vertical line represents the interval between extreme values.

Sl. 1. Efekt temperature i dodatka soli na rast vrste *Euglena gracilis* soj »Z« uzgojene na kompleksnom mediju. Visina stupaca prikazuje broj stanica po ml $\times 10^5$ (srednja vrijednost 2 pokusa; svaki pokus napravljen je u tri primjerka) nakon pet dana rasta.

DL = uzgojene kod izmjenične tame i svjetlosti; *CL* = uzgojene kod kontinuirane svjetlosti; *MiE* = mikroelementi; *REM* = bogati *Euglena* medij.

Vertikalna linija prikazuje interval među ekstremnim vrijednostima.

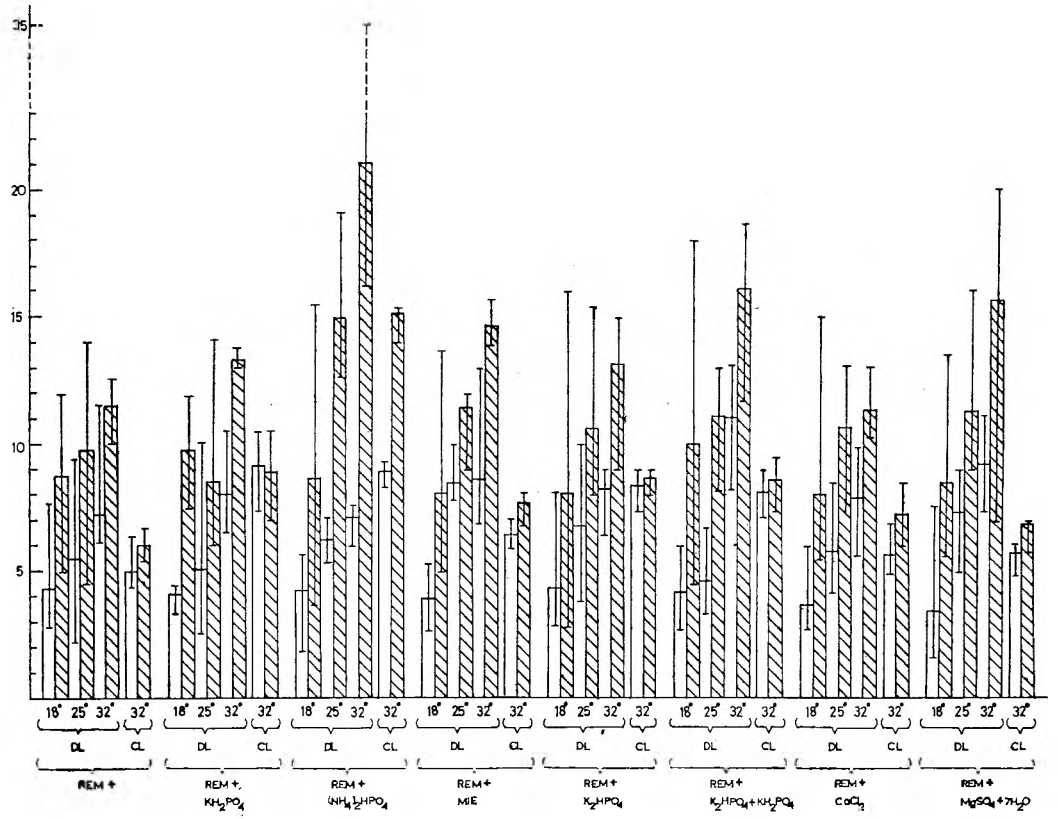

Fig. 2. Effect of temperature and additional nutrients on chlorophyll content of *Euglena gracilis* strain »Z« grown on a complex medium. The height of the columns represents total amounts of chlorophyll per ml cell suspension after 5 days of growth in μg (light columns) and amounts of chlorophyll per 10^6 cells in μg or pg per cell (hatched columns).

DL = grown in dark-light cycles; *CL* = grown in continuous light; *MiE* = microelements; *REM* = rich *Euglena* medium.

Sl. 2. Efekt temperature i dodatka soli na sadržaj klorofila kod vrste *Euglena gracilis* soj »Z« uzgojene na kompleksnom mediju. Visina stupaca prikazuje ukupnu količinu klorofila po ml suspenzije stanica nakon pet dana rasta u μg (svijetli stupci) i količinu klorofila na 10^6 stanica u μg odnosno pg po stanici (iscrtkani stupci).

DL = uzgojene kod izmjenične tame i svjetlosti; *CL* = uzgojene kod kontinuirane svjetlosti; *MiE* = mikroelementi; *REM* = bogati *Euglena*-medij.

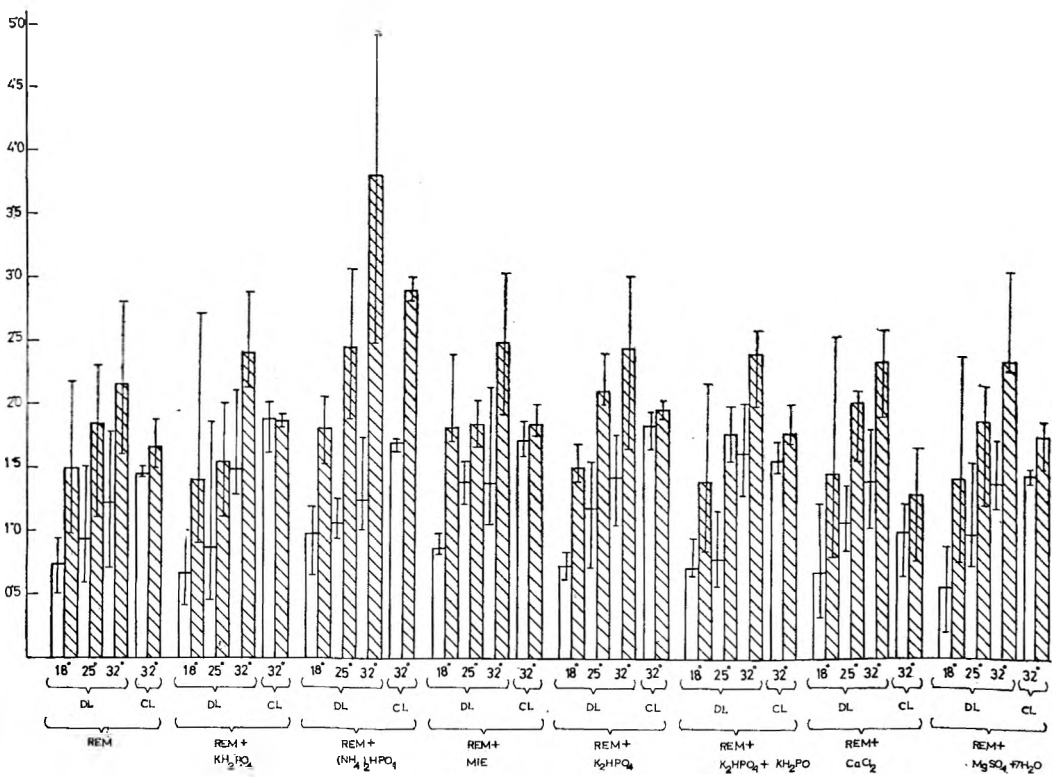

Fig. 3. Effect of temperature and additional nutrients on carotenoid content of *Euglena gracilis* strain »Z« grown on a complex medium. The height of the columns represents total amounts of carotenoid per ml cell suspension after five days of growth in μg (light columns) and amounts of carotenoid per 10^6 cells in μg or pg per cell (hatched columns).
DL = grown in dark-light cycles; *CL* = grown in continuous light; *MiE* = microelements; *REM* = rich *Euglena* medium.

Sl. 3. Efekt temperature i dodataka soli na sadržaj karotenoida kod vrste *Euglena gracilis* soj »Z« uzgojene na kompleksnom mediju. Visina stupaca prikazuje ukupnu količinu karotenoida po ml suspenzije stanica nakon pet dana rasta u μg (svijetli stupci) i količinu kartenoida na 10^6 stanica μg odnosno pg po stanici (iscrtkani stupci).
DL = uzgojene kod izmjenične tame i svjetlosti; *CL* = uzgojene kod kontinuirane svjetlosti; *MiE* = mikroelementi; *REM* = bogati *Euglena* medij.

Cell counts were made with a haemacytometer after fixation with a drop of formaldehyde.

All experiments were performed twice in triplicate except those in the continuous light, which were carried out only once and should be considered as preliminary observation.

Results

Fig. 1 shows the growth of *Euglena gracilis* strain "Z" expressed as an increase in cell number at temperatures of 10 °C, 25 °C and 32 °C in light-dark cycles (14 hours of light: 10 hours of darkness) and in continuous light at 32 °C in the complex medium and in the complex medium with various additional nutrients.

The highest rate of growth was observed in continuous light at 32 °C and the lowest at 18 °C.

In discontinuous light at 18 °C (the lowest temperature used) there was no increase in the growth rate of *Euglena* cultivated in the solution with additional nutrients in comparison with the control growth. Somewhat better growth (the increase was rather low) was achieved at 25°C and especially at 32 °C with addition of phosphates (KH_2PO_4 and K_2PHO_4, or both) magnesium sulphate and a mixture of microelements. Ammonium phosphate on the contrary has been found to inhibit growth: at higher temperatures more than at lower ones. In continuous light better growth was obtained with additional phosphates. In comparison with the control the difference is rather small, perhaps because in that case the cell density approached its saturation point and the values had to be read after a shorter lapse of time.

There was an even more pronunced increase in the total amount of chlorophyll and carotenoids with the rise in temperature in all cases (Fig. 2 and 3). Although growth (i.e. cell division) seemed to be more rapid at continuous illumination, chlorophyll synthesis proceeded at a similar or even lower rate than in cells grown in the light-dark cycle, and the amount of chlorophyll per cell was lower (Fig. 2).

Ammonium phosphate at the concentration used had an inhibitory effect on cell division but not on chlorophyll or carotenoid synthesis so that cells with very high chlorophyll content were formed.

Discussion

Various strains of *Euglena* have different optimum temperatures of growth (P r i n g s h e i m and P r i n g s h e i m 1951). It has been shown that the optimum growth temperature for a streptomycin bleached strain of var. *bacillaris* lies between 25 and 30 °C (B u e t o w 1962) in terms of generation time while protoplasmic growth is better at lower temperatures. The »Z« strain belongs to the »high-temperature« strains with pronounced thermophily. It has a large capacity for utilizing exogenous nutrients, which is supposed to be the result of a higher osmotic tolerance and heightened permeability (B a k e r et al. 1955).

The effect of various nutrients, such as phosphates, iron, sulfur and acetate on the growth of *Euglena* has been studied in detail in defined

inorganic media (Price and Carell 1964, see also review by Kirk 1967, Buetow and Schuit 1968). High concentrations of phosphates, sulfur, acetate and some metals were found to be inhibitory to growth and chlorophyll synthesis (see also Rosen and Gawlik 1961). Most of these experiments were performed at 25 °C. It is highly probable that at an increased temperature (30 — 32 °C) these nutrients should be requiered in larger amounts. In fact there was no inhibition of growth except in the case of added ammonium phosphate, although high concentrations of salts were used. The reason for the inhibitory effect of ammonium phosphate is not known since it was used in the inorganic medium by Cramer and Myers (1952) as a sole nitrogen source in a concentration of 1.0 g/l.

The content of phosphorus in the medium after addition to the basal medium was in the range of optimum values as estimated by Buetow and Schuit (1968) in an inorganic medium (see Marčenko 1970). Phosphorus content of the medium itself without additional phosphates seems to be sufficient at lower temperatures (18°). At higher temperatures however additional phosphates stimulate growth, chlorophyll and carotenoid synthesis.

Addition of calcium at the concentration used (which may be too high) seems to have no prononunced effect on the growth of *Euglena*.

In the mixture of micronutrients iron is very probably the most important metal. The concentration of added iron was 10^{-5} M, which corresponds to the optimum value for chlorophyll synthesis (at 25 °C) according to Price and Carell (1964); even lower concentrations were found to be sufficient (10^{-6} M) for growth.

In the experiments described only one concentration of one nutrient was performed at a time. Additions of combination of salts (phosphates, micronutrients and magnesium sulphate) should be studied in future.

Orientational experiments comparing cell division and biosynthetic rates of cells grown in continuous illumination and in light-dark cycles indicate that, while cell division is more rapid in continuously illuminated cultures, chlorophyll synthesis seems to proceed at a lower rate on a per cell basis.

Growth of cultures in light-dark cycles should be more natural with the respect to the history of cell evolution (Warburg 1958) and more studies should be geared in this direction. The degree of synchrony of cell division and cell biosynthesis was not studied in our experiments but it is possible that a partial synchrony took place. As shown by Cook (1961) in an autrophic culture of Euglena on a 16 hour light cycle all divisions occured during the dark period and most of biosynthesis during the light period.

Although it has been shown that chlorophyll synthesis of etiolated *Euglena* proceeds at the same rate with or without nutrients (Stern et al. 1964), several nitrogen sources as well as some metals have been found to stimulate it or shorten the lag phase but not over 10—15 per cent (Grenson 1964, Kirk 1967). It has been established however, that chlorophyll synthesis is dependent on protein synthesis (Kirk and Allen 1965). In growing cells dependence on the exogenous nutrients may be more pronounced as the reserve substances of the cells are used up in growth.

Summary

In *Euglena gracilis* strain »Z« grown in the complex medium (on a repetitive light-dark cycle) the need for exogenous nutrients rises with temperature. At 18 °C no need in added nutrients has been observed.

The rate of growth as expressed in increase in cell number, amount of chlorophyll and carotenoids rises at higher temperatures with additional phosphates (K_2HPO_4 and KH_2PO_4), mixture of microelements and magnesium sulphate.

Addition of ammonium mono-H orthophosphate at the concentration used inhibits cell division but does not affect chlorophyll synthesis, and cells with very high chlorophyll concentration are obtained.

The study indicates that the illumination regimen induces marked differences in the relative rates of cellular biosynthesis.

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SADRŽAJ

MEĐUSOBNI ODNOS MINERALNE ISHRANE I TEMPERATURE NA RAST VRSTE *EUGLENA GRACILIS*

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Potreba za dodatkom pojedinih soli kompleksnom mediju za uzgoj vrste *Euglena gracilis* soj »Z« raste s povišenjem temperature. Kod 18° nema potrebe u dodatnim hranidbenim tvarima.

Dodatkom amonijevog fosfata (K_2HPO_4 i KH_2PO_4), mješavine mikroelemenata i magnezijevog sulfata, s porastom temperature povećava se brzina rasta izražena porastom broja stanica, porastom količine klorofila i karotenoida.

Dodatak amonijevog fosfata pri upotrebljenoj koncentraciji djeluje inhibitorno na diobu stanica, ali ne i na sintezu klorofila, tako da se dobivaju stanice s vrlo visokom koncentracijom klorofila.

Istraživanja pokazuju da režim osvjetljavanja izaziva znatne razlike u relativnim odnosima biosintetskih funkcija stanice.

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