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Light respiration in *Lemna trisulca*

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INTRODUCTION

Many recent investigations have shown that respiration of photosynthetic cells in light differs from that in darkness. The differences concerns not only the rate of oxygen uptake but also the way of oxydative metabolism.

The study of the effect of light on the respiration process in *Chlorella* (K o w a l l i k, 1967, 1969) showed that:

a) only the short wave range of the spectrum is active in the enhancement of the oxygen uptake,

b) the action spectrum of this process shows two maxima (by about 370 and 460 nm) similar to the absorption spectrum of flavines,

c) relatively low blue light intensities suffice to saturate the light effect.

By very many plants the response of the rearrangement of chloroplasts to light shows the same features.

The aim of this paper is to investigate the light respiration in *Lemna trisulca*, the plant which chloroplast reactions to light are well known. We shall try to answer the question if there exist any quantitative differences in oxygen uptake in light and darkness in such material, what the magnitude of these differences is in various spectral regions and radiation intensities and what a correlation could be found between effect of light on respiration and on chloroplasts arrangements.

MATERIAL AND METHODS

Leaves of *Lemna trisulca* were used as experimental material. The plants were collected from a natural pond in the vicinity of Chełmek, and kept in an aquarium. The upper part of healthy leaf blades about 4 mm long, consisting mostly of one layer of mesophyll were cut out and used for gas exchange measurements.

DCMU was used for inhibition of photosynthesis. Selection of proper concentration and time of treatment were carried out in the following experiment. After measuring the net photosynthesis in white light (inten-

sity about 9000 erg/cm²sec) the leaves are treated with various DCMU concentrations for 1,3 and 6 hours and net photosynthesis was measured again. Basing upon the results illustrated in figure 1, in the following experiments treatment with $3{:}10^{-5}$ M/1 DCMU solution for 1 hour was applied.

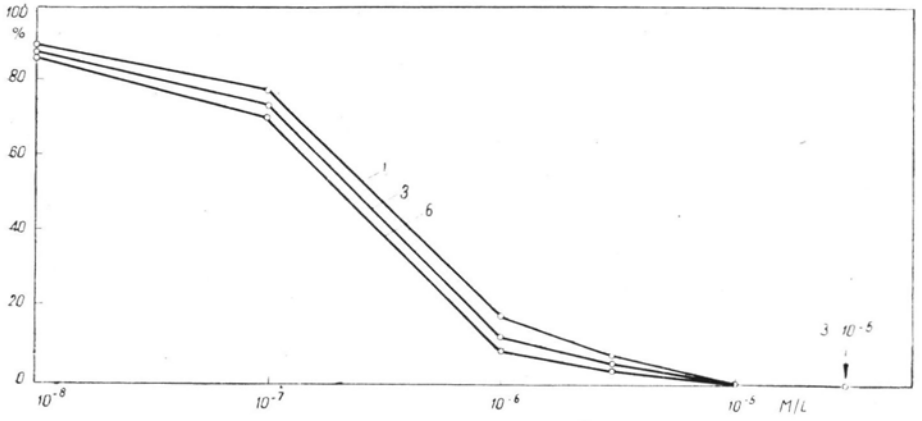

Fig. 1. Photosynthesis after 1, 3 and 6 hours treatment with DCMU solutions. X axis (DCMU concentration in M/1, Y axis — net photosynthesis in percent of that before treatment.

In some experiments the leaves were treated with DCMU solution with addition of antimycine A in 60 µg/ml concentration.

The leaf sections were pretreated in darkness for 36 hours. Than they were put into DCMU solution for 1 hour kept in darkness and mounted in a microrespirometer in a drop of the same solution. All manipulations were performed in weak red light.

For the gas exchange measurements the microrespirometric technique was used (Zurzycki, 1955; Starzecki, 1961). After measuring the dark respiration the leaves were exposed to light, where the oxygen uptake was measured during 30—50 mins. this operation being followed by another dark period. Three spectral regions of light obtained by interference filters (max 454.506 and 676 nm, half with 6, 7, and 8,5 nm respectively) were used. Technique of light intensity measurements was described elsewhere (Zurzycki, 1970).

Fig. 2. Measurements of oxygen uptake by *Lemna* leaves.

X axis — time in min., Y axis — 0_2 uptake. ↓ light on, ↑ light off. *a*, *b*, *d* — leaves treated 1 hour in $3 \cdot 10^{-5}$ M/1 DCMU, *c* and *e* — leaves treated 3 hours in the same concentration of DCMU + antymycine 60 g/ml.

Illumination: a — blue light 20 erg/cm² sec., b and c — blue light 12 000 erg/cm² sec., d and e — red light 12 000 erg/cm² sec.

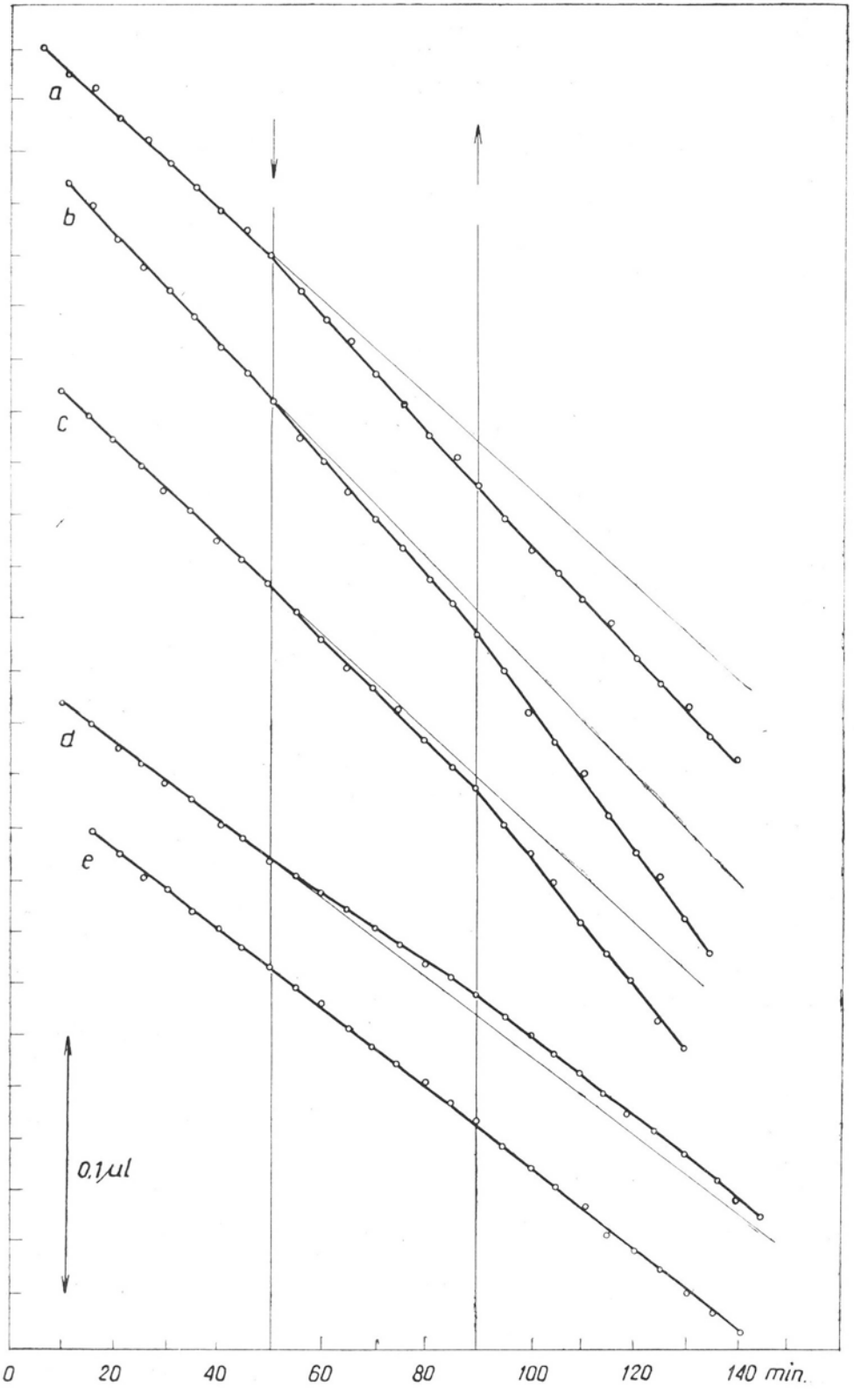

Fig. 2.

Determination of chloroplasts position and the rate of rearrangement were carried out on a normal, not DCMU treated material according to the previously described methods (Zurzycki 1962).

RESULTS

Effects of blue light 454 nm. The results of some measurements of oxygen uptake of *Lemna* leaves treated with DCMU are given in figure 2. Illumination of the leaf with relatively low intensity of blue light induces an enhancement of oxygen consumption. In the following dark period the O_2 uptake maintains on the same high level as in the light period (fig. 2a). An increase of oxygen uptake but much lower than in the case of low blue light is also obtained by illumination with very high light intensity. On the other hand, in the dark period following strong illumination, the oxygen consumption increases to the same or even higher rate as in illumination with low light (Fig. 2b).

The percentage increase of oxygen uptake in the light period compared with dark respiration is presented in figure 3 versus light intensity. Very low light intensity of the order of 0,3 erg/cm²sec. already induces an increase of oxygen uptake. Saturation of the light effect can be found by about 5 erg/cm²sec. where the mean increase of oxygen consumption is about 35% of the dark respiration. Above 100 erg/cm²sec. a decrease of light increased oxygen uptake begins. The values obtained in light intensities above 1000 erg/cm²sec. show a greater dispersion but indicate to the tendency of a further decrease of oxygen uptake. In the whole range of the used light intensities the mean light oxygen uptake was never lower than the dark respiration.

The extremely high sensitivity of the respiratory mechanism in *Lemna trisulca* to blue light allows to suppose that similar effects could be found in normal, not DCMU treated leaves, because the oxygen production in photosynthesis is insignificant in the region of some ergs/cm²sec. This is really the case. In fig. 3 the lower curve shows the light enhanced oxygen uptake for normal 36 hours dark pretreated leaves. The increase of oxygen uptake in light as compared to dark respiration is visible in the range of 1—100 ergs intensities. In higher intensities the oxygen uptake rapidly decreases because of the interference of increasing photosynthetic activity.

High rate of O_2 consumption following illumination takes place for a longer period of time before it drops to the normal rate of dark respiration. This period of time depends on the length of time of light exposure and the applied light intensity. Fig. 4 shows that after 40 min. illumination with a very low blue light intensity the rate of respiration reaches the normal dark rate in about 1 hour; for higher intensities of

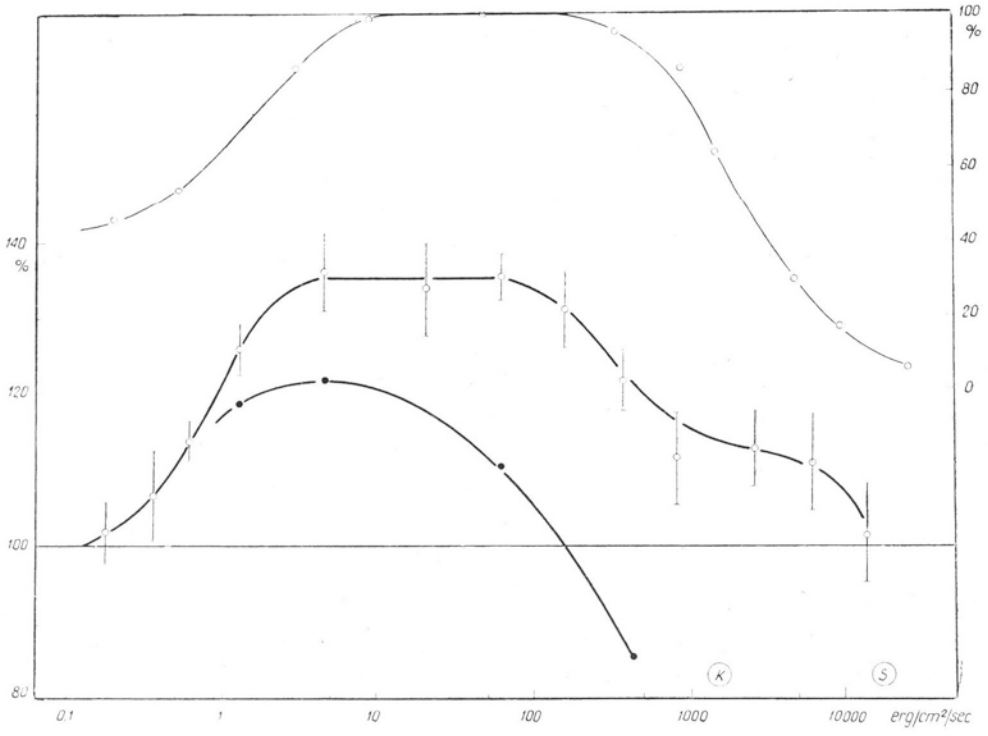

Fig. 3. Effects of blue light 454 nm

Oxygen uptake in light (lower curves) in percent of the dark respiration (Y axis, left) after ilumination with various light intensities (X axis). —O— material treated with DCMU, —●— normal DCMU untreated leaves. Vertical lines mark the mean error. Upper curve — position of chloroplasts (Y axis right) as measured for untreated material. k — compensation, S — saturation points of photosynthesis.

blue light this time may be prolonged well above 6 hours. The curves permit to suppose that the used 36 hours time of pretreatment in darkness is sufficient to eliminate the later effects of the former illumination.

Effects of green light 506 nm. The effects induced by illumination with green light are principially the same as for the blue one; the sensitivity of leaves only for this spectral region being much lower (Fig. 5). The lowest intensity inducing enhancement of oxygen uptake is about 1 erg/cm²sec., whereas its saturation takes place by about 50 erg. In high intensity a decrease of the enhancement effect can be stated. Using leaves not treated with DCMU the effects of increased O_2 uptake can be also found in low light but these are not so explicit as in blue light because of an earlier interference of photosynthesis.

Effects of red light 676 nm. Red light illumination of dark adapted DCMU treated leaves induces a reverse effect as obtained by using the short wave part of the spectrum. The O_2 uptake becomes lower. This effect is reversible and in the following it dark period the

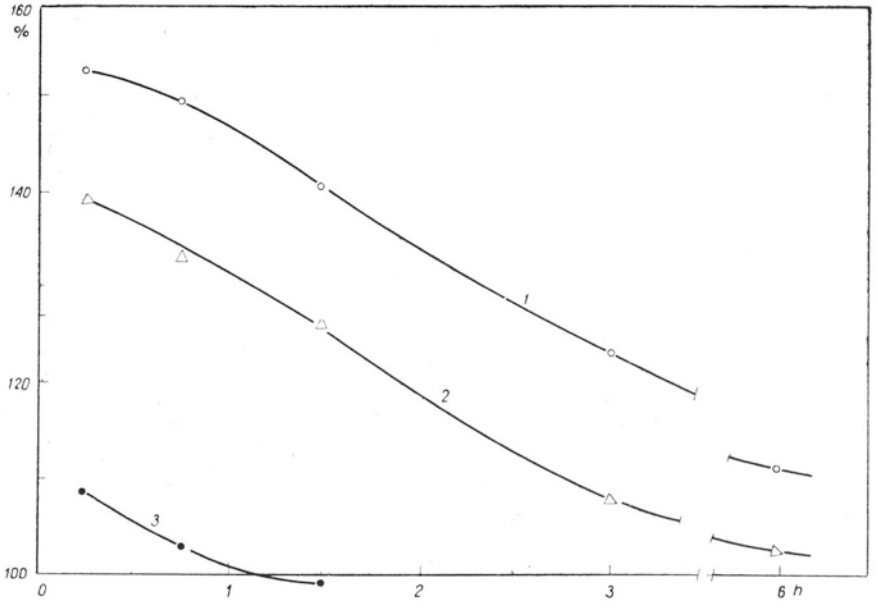

Fig.4. Recovery to normal dark respiration rate after 40 min. illumination with blue light of the intensity 12 000, 70 and 0,4 erg/cm²sec. (curves 1, 2, 3 respectively) X axis — time in hours, Y axis — oxygen uptake in percent of the former dark respiration.

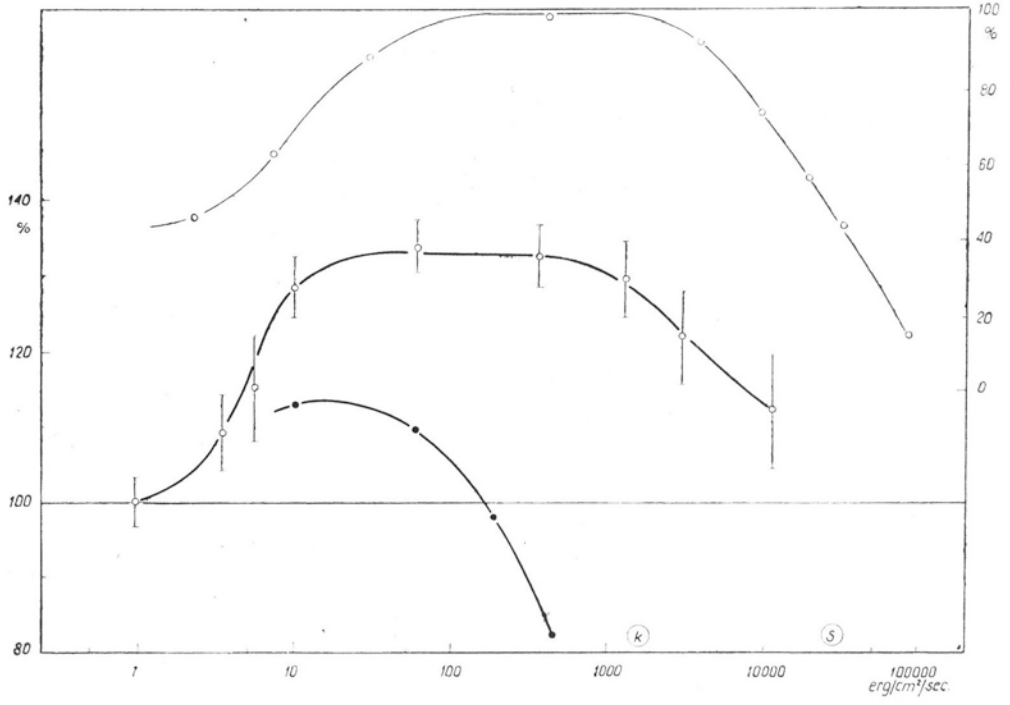

Fig. 5. Oxygen uptake and chloroplasts position after illumination with green light of 506 nm. Explanation as in fig. 3.

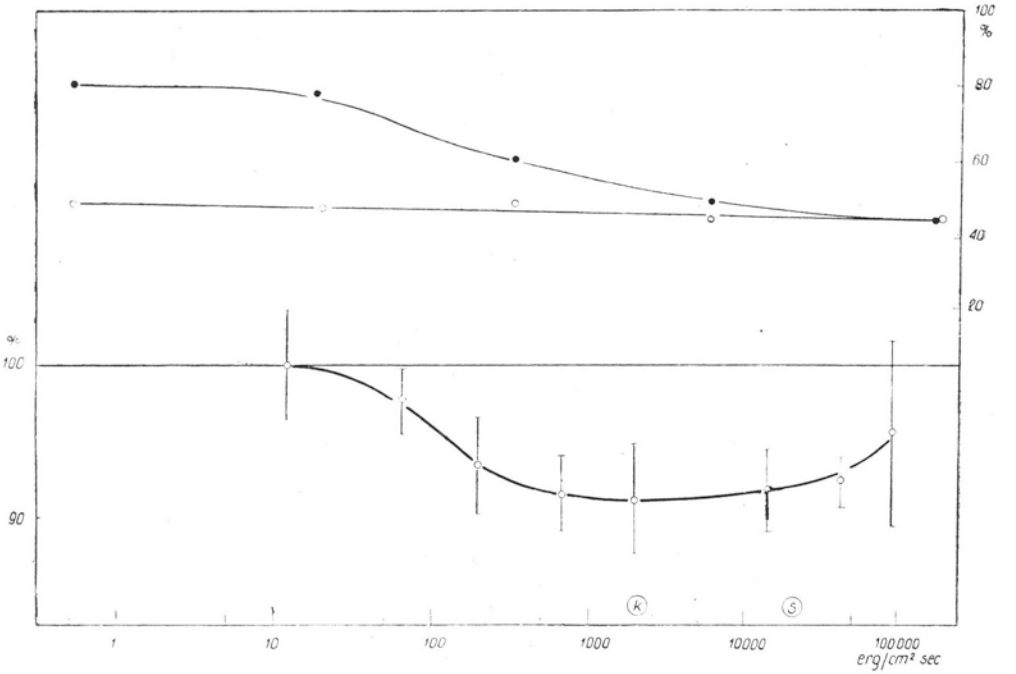

Fig. 6. Oxygen uptake (Y axis left) after illumination with red light in percent of dark respiration. Leaves treated 1 hour with DCMU.

Upper curves — position of chloroplasts (Y axis right) for untreated material as recorded after 1 hour —●— and 4 hours —O— of illumination; X axis — 1 light intensity.

rate of oxygen consumption is the same as in the former dark period (Fig. 2d). In contrast to the effects of the short wave spectral range relatively higher light intensities are necessary to induce measurable effects. The effect of red can be stated at about 100 erg/cm²sec. and the saturation of the decrease of oxygen uptake in red light takes place at about 1000 ergs (Fig. 6). In very high intensities of red the oxygen uptake decreases less but 0_2 consumption is always lower than in darkness. Very similar effects of red light on the DCMU treated *Chlorella* cells have been found by K o w a l l i k and K o w a l l i k (1969) and supposed to be connected with the cyclic photophoshorylation. To check this suggestion an inhibitor of cyclic phosphorylation — antimycin A was used to our material. After 1 hour long treatment in a solution of DCMU + antimycine the decrease of oxygen consumption in red light was only partly suppressed. A 3 hours long treatment in the same solution in red light resulted in a nearly complete suppression of the decrease of oxygen uptake (Table 1).

The suppression of the decrease of O_2 uptake in red light by antimycin strongly suggests that this effect is connected with cyclic photophosphorylation. One can suppose that the reason of the decrease of enhancement of O_2 uptake found in strong blue or green light is the same.

But this supposition does not seem to be right. Strong blue light exerts the same effects on the leaves treated for 3 hours with DCMU and antimycin A as on those theated with DCMU only (Fig. 2c). The effects induced by strong blue light result probably from other reasons.

Table 1

Oxygen uptake in red light in percent of the dark respiration. Light intensity 12 000 erg/cm² sec. DCMU in concentration 3×10^{-5} M/l, antimycin concentration 60 µg/ml

Treatment	Oxygen uptake
Dark respiration	100%
DCMU — 1 hour	91%
DCMU+Ant. — 1 h.	96%
DCMU+Ant. — 3 h.	99%

Chloroplasts position. In *Lemna trisulca* the short wave range of light only can induce low and high light arrangements of chloroplasts (Zurzycki, 1962). The curves of chloroplast position depending on light intensity as shown in figs 3 and 5 were determined for DCMU untreated leaves. They show some correlation with the response of respiration mechanism to light. Especially the intensity range in which the chloroplasts move from the dark position to the low light intensity position coincides with the enhancement of oxygen uptake.

In red light the position of chloroplasts is the same as in darkness independent on the intensity of illumination. But red light exerts an photokinetic effect i.e. it acts on the rate of movements. In strong red light the time necessary to take the dark position of chloroplasts is shorter. In fig. 6 the positions of chloroplasts are shorter. In fig. 6 the positions of chloroplasts are recorded after 1 and 4 hours of illumination. The accelerating effect of red light can be found in this intensity region in wich red light causes a decrease of the oxygen uptake.

DISCUSSION

During the last few years a lot of evidence has been collected showing that light exerts a significant influence on the oxidative processes in green cells. In contrast to dark respiration oxidative processes taking place in light, called photorespiration (Forrester, Krotkov, Nelson, 1966) are closely connected with the glycolic pathway (Zelitch, 1958). Recent investigation of *Chlorella* (Kowallik, 1969, b) strongly suggest quite a similar biochemical background of light effects in this algae.

The problem of spectral action of light on photorespiration has not been solved as yet for leaves of higher plants Only Poskuta (1968) by

use of a wide spectral ranges of light was able to show that in spruce shoots CO_2 production is higher in blue and much lower in red light than in dark respiration. In contrast to leaves of higher plants many papers concerning the action of various wave lengths on light respiration of *Chlorella* have been published. K o w a l l i k (1967, 1969) and K o w a l l i k and K o w a l l i k (1969) found that the action spectrum of oxygen uptake enhancement is limited to the short wave range only and shows two peaks of activity by about 370 and 460 nm. The results of this paper are in good agreement with those findings. The enhancement of oxygen uptake was found only in short wave radiation. Although the action spectrum was not determined it is worth to stress that the relative activity of 454 to 506 nm is about 10:1 which is near the ratio for *Chlorella* action spectra. Saturation of enhancement effects was found by much lower light intensities than in *Chlorella,* which may reflect the ecological adaptation of *Lemna* to low intensities of light.

In *Lemna* red light induces a decrease of oxygen uptake. Similar effects were stated for *Chlorella.* K o w a l l i k and K o w a l l i k (1969) made a supposition that this effect may be explained by interaction of respiratory processes with ATP produced in cyclic photophosphorylation. This supposition has been checked in our experiments. Inhibition of cyclic phosphorylation by a specyfic inhibitor reduced the effect of red light. The intensity range in which the red effect can be stated shows also that in this case a much greater energy of light is necessary.

The decrease of oxygen uptake in very high light intensities has no parallelism in *Chlorella* reactions. Only in the first minutes after illumination a decrease of oxygen uptake in higher light intensities was reported (H o c h, O w e n s, K o k, 1963; K o w a l l i k and K o w a l l i k, 1969). The observed inhibition cannot be explained by the interference of cyclic phosphorylation because the inhibitor of this process does not reduce it.

It was found in earlier studies that the rearrangement of chloroplasts in *Lemna trisulca* depends on the energy derived from oxygen respiration. The photokinetic effects of red light only can be explained by photosynthetic ATP production (Z u r z y c k i, 1965). The results of this paper strongly suggest that there is some dependence between light respiration and arrangement of chloroplast. Not only are the action spectra of both processes very similar but also the intensity range inducing enhancement of oxygen uptake coincides with the effect of light on chloroplasts position.

SUMMARY

The effect of light on oxygen uptake in DCMU treated leaves of *Lemna trisulca* was studied.

The enhancement or oxygen uptake was found only in the short wave length range. The leaves show very high sensitivity to light. In 454 nm of 0,3 erg/cm^{2}sec.

intensity enhancement effects can be found and in 5 erg/cm^{2}sec. saturation takes place. The activity of 506 nm is about ten times lower. In high intensity of short wave range a decrease of enhancement was observed.

The effects of light on the O_2 uptake show a close correlation with the effect of light on the arrangement of chloroplasts. As well action spectra as intensity range are very similar for both processes.

In red light a decrease of O_2 uptake was established. This effect is reduced by antimycin A.

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Oddychanie świetlne u Lemna trisulca

Streszczenie

Badano wpływ światła na pobieranie tlenu przez liście *Lemna trisulca*, w których fotosynteza została zahamowana przez DCMU.

Wzmożenie pobierania tlenu w stosunku do oddychania w ciemności stwierdzono tylko w zakresie krótkofalowym. Materiał wykazuje bardzo dużą wrażliwość na światło i tak dla promieniowania 454 nm efekty są już mierzalne przy 0,3 erg/cm^{2}sec., a wysycenie następuje przy ok. 5 erg/cm^{2}sec. Aktywność promieniowania 506 nm jest ok. 10 x mniejsza. W wysokich intensywnościach światła krótkofalowego obserwuje się zmniejszenie efektów.

Działanie światła na pobieranie tlenu wskazuje ścisłą korelację z wpływem światła na układ chloroplastów. Zarówno widma działania, jak i zakres intensywności działania w obu procesach jest bardzo zbliżony.

W świetle czerwonym stwierdzono zmniejszenie pobierania O_2 w stosunku do ciemności. Zjawisko to może być zahamowane przez antymycynę A.

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