The effects of simultaneous action of the short and long wave parts of spectrum on the movements of chloroplasts

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INTRODUCTION

In the previous paper (Zurzycki 1962) it was established for *Lemna trisulca* that the phototactic displacement of chloroplasts and typical phototactic arrangements are controlled by the short wave range of the spectrum within the limits 360 — 530 m μ , but when exposed to the long wave range (550 — 700 m μ) the chloroplasts achieve an arrangement similar to that in darkness, though the speed of reaching the arrangement depends on the intensity of radiation. The influence of the long wave part of the spectrum was defined as photokinetic.

In this paper an analysis is attempted of the effects of simultaneous action of a chosen range of short and long wave parts of the spectrum.

METHOD

The investigations were carried out on the leaves of *Lemna trisulca*. The manner of preparing the material, selection of spectral ranges, measurements of light, and registration of results was similar to that

Fig. 1. Scheme of the illuminating system of the microscope

O — objective; K — condensator; m_1 — semi-transparent mirror; m_2 — mirror; I_1 — blue interference filter; I_2 — red interference filter; L — lens; d — diaphragm.

of the previous paper (Zurzycki 1962). In order to make possible the simultaneous exposure to light of the microscopic object by means of two ranges of monochromatic light a double illuminating arrange-

ment of the microscope was constructed which is shown schematically in Fig. 1. As semitransparent mirror m_1 a half-silvered glass plate reflecting 20 or 50% of light was used. Both optical systems, were set according to the Köhler principle and the diameters of diaphragms d_1 and d_2 were so chosen that the surface of light spots formed in the plane of the preparation exactly covered each other. In order to obtain short-wave radiation and interference filter Zeiss SIF with maximum transmission of $481\text{ m}\mu$, and as long-wave radiation red light ($669\text{ m}\mu$) was used.

RESULTS

A. The influence of one hour's additional exposure to red light

On Fig. 2 is shown the dependence of the arrangement of chloroplasts (% E) on the intensity of blue light. If after reaching a stable state additional exposure to red light was used the state obtained after one hour of additional exposure is shown by the remaining curves. As results from the graph, additional exposure to red light does not essentially influence the arrangement of chloroplasts when its intensity does not exceed some thousand $\text{erg/cm}^2\text{sec}$. Higher intensities, however, cause a distinct effect. In the range of intensity of blue light 100 — 1000 $\text{erg/cm}^2\text{sec}$ additional exposure to red light lowers % E. The displacement of the curves seems to indicate the lowering of the thresh-

Fig. 2. Dependence of arrangement of chloroplasts (axis Y — % of epistrophe) on the intensity of blue light (axis X) applied alone or together with various intensities of additional red radiation.

hold of the reaction. In the range of weak intensities of blue light 5 — 100 erg , i.e. in the range in which full epistrophe exists, additional strong red light causes also a certain lowering of % E so that 100% E is not maintained on any point.

B. The course of displacements

The results of statistic and film registration of the course of displacement in time is exemplarily presented for two intensities of blue light.

Weak intensity of blue light ($24.7 \text{ erg/cm}^2 \text{ sec}$) causes a full and more or less stable flat arrangement of chloroplasts (100% E) with numerical

Fig. 3. The course of displacements in blue light $24.7 \text{ erg/cm}^2 \text{ sec}$. From the moment marked by an arrow additional exposure to red light with $80,000 \text{ erg/cm}^2 \text{ sec}$ was applied. Axis X — time in hours, axis Y — % of epistrophe. Upper curve — general percentage of epistrophe, lower curves — % of epistrophe on the wall towards the light (proximal) —O— and that away from the light (distal) —●—.

advantage of chloroplasts on the wall towards the light (proximal) (Fig. 3). The application of additional strong red light ($80,000 \text{ erg/cm}^2 \text{ sec}$) causes a not very rapid but distinct decrease of % E strengthening in the course of time. Characteristic here are fluctuations of both general % E and the number of chloroplasts on the wall of the cell towards the light (proximal) and on that away from the light (distal). Similar fluctuations had been observed previously when applying strong red light. The non stability of the arrangement indicates increased agility of the chloroplasts. This is confirmed by cinematographic investigations (Fig. 4). In weak blue light chloroplasts which are in flat position perform slow displacements with characteristic irregular vibrations or a slow progressive movement with variable direction (average speed $0.133 \mu/\text{min}$). Additional exposure to red light does not basically change the character of tracks but causes — usually only after 10 — 20 min of action — a distinct increase of speed up to the average value $0.40 \mu/\text{min}$ (momentary maximum speeds $5 - 7 \mu/\text{min}$). It is possible to differentiate here periods in which the given chloroplast shifts with a slow movement, interrupted by periods of accelerated displacements. The sequence of these phases (which does not always appear very

Fig. 4. Movement of chloroplasts according to film analysis. The intensity of blue light $90 \text{ erg/cm}^2 \text{ sec}$. At the point marked by an arrow additional red light of intensity $78,000 \text{ erg/cm}^2 \text{ sec}$. was switched on. Left top — the tracks of chloroplasts; below — time distance curves; points denote the position of chloroplasts every 10 minutes.

distinctly) is irregular for each chloroplast and does not show synchronization with the movements of other chloroplasts within the given cell.

In Fig. 5 the results of statistic registration of the arrangement of chloroplasts are presented when additional exposure to red light was

Fig. 5. The course of displacement in blue light $305 \text{ erg/cm}^2 \text{ sec}$. The remainder as in Fig. 3.

applied after reaching the stable state of partial epistrophe obtained in blue light $305 \text{ erg/cm}^2 \text{ sec}$. In this case the additional exposure increases the agility of the chloroplasts, this being expressed by fluctuations of $\% E$, the general percentage of epistrophe being distinctly lowered. Cinematographic analysis of this type of reaction (Fig. 6) shows that, similarly as in the case discussed previously, there occurs a distinct increase in the speed of movement with the average speed of $0.48 \mu/\text{min}$

Fig. 6. Movements of chloroplasts according to film analysis. The intensity of blue light $460 \text{ erg/cm}^2 \text{ sec}$. Explanations as for Fig. 4.

up to $2.13 \mu/\text{min}$, when the momentary speeds may reach the value of $6-7 \mu/\text{min}$, i.e. maximum values determined for the displacements of chloroplasts in *Lemna trisulca*.

When the chloroplasts kept in weak blue light are exposed to stronger blue light their displacement occurs up to the achievement of partial or complete parastrophe. If simultaneously with increasing the intensity of blue light an additional

Fig. 7. The course of displacements under the influence of blue light with intensity $460 \text{ erg/cm}^2 \text{ sec}$ (on the left), and blue light $460 \text{ erg/cm}^2 \text{ sec}$ and red light with intensity $80,000 \text{ erg/cm}^2 \text{ sec}$ (on the right). The starting position — epistrophe, the remainder as in Fig. 3.

strong red light is switched on, the displacements take place in an exactly similar way (Figs. 7a and b). The differences are possible to determine only in the last phase of displacements

and lead to the achievement of a lower % of epistrophe when additional red light is applied. Cinematographic analysis also shows no essential differences in the speed of movement of the two investigated cases.

Similarly in a number of other types of movements caused by the change in intensity of blue radiation (epistrophe $\rightarrow$ complete parastrophe, parastrophe $\rightarrow$ epistrophe) no essential influence of the additional red light switched on simultaneously with the changes of intensity of blue light is determined at least in the first phase of displacements.

C. The effects of various spectral ranges of long-wave radiation

In the last series of the discussed experiments a constant blue light still maintaining full epistrophe ($113 \text{ erg/cm}^2 \text{ sec}$) was applied as well as the additional exposure to light of various wave lengths and constant intensity of $60,000 \text{ erg/cm}^2 \text{ sec}$. The results obtained after one hour of additional exposure are presented in Fig. 8. As can be seen from the graph, additional radiation with a wave length longer than $700 \text{ m}\mu$ does

Fig. 8. The arrangement of chloroplasts (axis Y — % of epistrophe) in blue light with intensity $113 \text{ erg/cm}^2 \text{ sec}$ after one hour's exposure to additional radiation with intensity $60,000 \text{ erg/cm}^2 \text{ sec}$ and various wave length (axis X). Average values were obtained from 8 to 15 measurements. Dash — extreme fluctuation; rectangle — average error.

not cause essential changes of the arrangement. In the range $550 - 700 \text{ m}\mu$ additional radiation causes a decrease of percentage of epistrophe, the greatest action being shown by red light i.e. $660 - 680 \text{ m}\mu$, and the smallest by green light i.e. about $560 \text{ m}\mu$. In the shorter wave part of the spectrum a violent decrease of % E occurs (for $523 \text{ m}\mu$ it amounts to only 7.5%) caused by transition to the range acting photo-tactically.

SUMMARY

Long-wave light applied additionally together with exposure to blue light causes an effect only if it exceeds an intensity of several thousand ergs and if it is applied when the chloroplasts reach a certain arrangement regulated by blue light. In the course of the phototactic displacements caused by blue light the effect of additional red light is completely disguised. The activity of particular spectral ranges shown an agreement with the previously determined photokinetic action (Zurzycki 1962) and seems to be connected with absorption in photo-synthetic pigments.

Additional exposure to long-wave radiation causes an increase in the agility of the chloroplasts expressing itself in an increase of the speed movement, the appearance of fluctuations in the percentage of epistrophe, and a decrease in the percentage of epistrophe as compared with the value corresponding to the action of blue light itself.

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*Wpływ jednoczesnego działania krótko- i długofalowej części widma
na ruchy chloroplastów*

Streszczenie

Światło długofalowe aplikowane dodatkowo równocześnie z naświetlaniem światłem niebieskim wywiera wpływ na ruchy chloroplastów tylko wówczas, gdy jego natężenie przekracza kilka tysięcy ergów/cm² sek i o ile jest zastosowane wówczas, gdy chloroplasty osiągną pewien układ statyczny, regulowany światłem niebieskim.

W trakcie pomieszczeń fototaktycznych wywołanych światłem niebieskim efekt dodatkowego światła czerwonego zostaje zupełnie zamaskowany.

Aktywność poszczególnych zakresów spektralnych wykazuje zgodność ze stwierdzonym uprzednio działaniem fotokinetycznym.

Dodatkowe oświetlenie promieniami długofalowymi wywołuje zwiększenie ruchliwości chloroplastów wyrażające się zwiększeniem szybkości ruchu, wystąpieniem wahań procentu epistrofii oraz obniżeniem procentu epistrofii w stosunku do wartości odpowiadającej działaniu samego światła niebieskiego.