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The energy of chloroplast movements in *Lemna trisulca* L.

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INTRODUCTION

In spite of extensive researches into the movements of chloroplasts controlled by light conditions, the mechanism of these movements still remains unexplained. Some recent investigations indicate that the movements of chloroplasts are not solely caused by the physical processes associated with the absorption of light, as was assumed in older hypotheses (for a review of these hypotheses see Zurzycki 1962a), but are strictly related to the metabolism of the cell. In the conversion from chemical energy produced in the course of metabolic processes to the mechanical energy of the movements an essential part seems to be played by energy-rich phosphates, in particular by adenosine triphosphate (ATP). The significance of this compound in the conversion of energy was demonstrated for e.g. the movements of flagella in algae (Marcus and Mayer 1963), of the protoplasm in slime molds (Kamiya 1959) and in cells having cell walls (Kasantzev, Taggeva, Tairbekow 1964). There are four main sources from which a protoplast of the plant cell can obtain ATP (Fig. 1). Two of them are the processes of dissimilation, which may

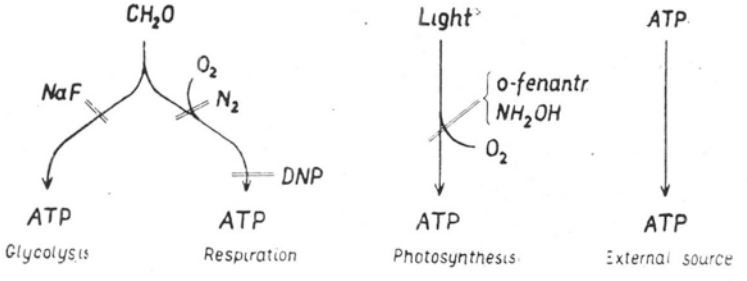

Fig. 1. Schematic diagram showing the sources of ATP in a cell

be either anaerobic (glycolysis) or aerobic (oxygen respiration). ATP is also formed in the process of photosynthetic phosphorylation under conditions in which photosynthesis is possible. Finally, the cell can utilize the energy of ATP supplied to the medium experimentally.

In this investigation an attempt was made to inhibit the processes which could be a source of ATP and then the influence of the inhibitors on the principal types of chloroplast movements was examined. Glycolitic processes were poisoned with sodium fluoride and respiration was inhibited by applying anaerobic conditions;

the inhibitors of photosynthesis were hydroxylamine and o-phenantroline; finally, experiments were made with 2,4 dinitrophenol, an inhibitor uncoupling the phosphorylation processes.

Similar experiments with the effect of inhibitors and anaerobiosis on the movements of chloroplasts in *Mugeotia* carried out by Haupt and Fetzer (1963) and Fetzer (1963) make possible the comparison of the energetic conditions involved in these processes in two species, which have been studied and differ essentially in the nature of the mechanism of chloroplast movements.

MATERIAL AND METHODS

In the experiments young leaves of *Lemna trisulca* were used. The method of choosing leaves, the system of recording the chloroplast arrangements, and the cinematographic technics applied for recording the movements of chloroplasts were described in an earlier paper (Zurzycki 1962b). The material used in the experiments carried out in the summer seasons of 1963 and 1964 came from three

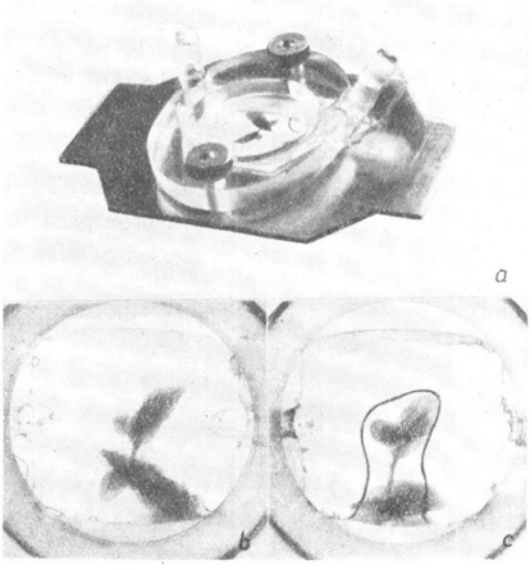

Fig. 2. *a* — Microscope chamber, *b* — Leaf in chamber in water medium, *c* — Leaf in chamber adapted to changes of gaseous media

different populations and this explains why the course of the displacements in the controls showed certain differences between the particular experimental series.

Phototactic movements were observed in blue light obtained with interference filters of maximum transmission 481 nm and half-width 8,8 nm. Light intensities were measured with a microthermo-element described elsewhere (Zurzycki 1951). The chloroplast arrangement of weak light (epistrophe) was obtained in blue light of intensity 35 to 40 erg/cm²sec. The strong light reaction (epistrophe → parastrophe) was induced by increasing the intensity of light to 1600 erg/cm²sec. Only when the

initial arrangement of chloroplasts had to be the arrangement of strong light the leaves were irradiated with white light at the intensity of 17 000 erg/cm²sec (spectral range 400—700 nm) and colour temperature about 5300°K; this was necessary because blue light of the intensity necessary to induce the strong light arrangement of chloroplasts caused after prolonged irradiation the agglomeration of chloroplasts into shapeless masses. Blue light of wave length 481 nm was also used to define how the arrangement of chloroplasts depended on the intensity of light. The displacements of chloroplasts induced by strong red light were obtained by applying an interference filter of maximum transmission 668 nm and half-width 11 nm. The intensity of red light was 80 000 erg/cm²sec. The displacements of chloroplasts in darkness (epistrophe → apostrophe) were recorded after lighting the preparation with red light only sufficiently to make the counts possible and only for the time of counting.

For the experiments the leaves were placed in a specially designed microscope chamber (Fig. 2*a*) constructed so that the medium inside could be changed very rapidly. The chamber was cut in a piece of plexiglass mounted in a brass plate placed on the microscope table. The chamber was fitted with two conduits for connecting rubber tubes carrying the medium to and from the chamber. The preparation was fixed in position inside the chamber with a drop of warm vaseline applied to the mother leaf as far away as possible from the point to be observed (Fig. 2b). The chamber was closed with a cover glass sealed with vaseline and pressed down by means of a ring of plexiglass with two screws. The inside of the chamber was entirely filled with the liquid medium. Only in experiments with anaerobiosis the leaf was placed in a small drop of water and the rest of the chamber was filled with a gaseous medium (Fig. 2*c*); though with this method the conditions for diffusion were not as favourable as when the leaf was in direct contact with the ambient gas, the irradiation of the leaf could be maintained at a strictly defined, unchanging intensity.

The medium filling the chamber was 0,02 M phosphate buffer the pH of which was the same as the pH of the applied inhibitor solutions. To change the medium about 20 ml of the new liquid were passed through the chamber in 10 sec and then the rate of flow was reduced to about 20 ml/hour. In the case of gaseous media the rate of flow through the chamber was about 2500 ml/hour.

The solutions used in the experiments were:

(i) 2,4 dinitrophenol (DNP) in phosphate buffer, pH 5,8 (in a few instances pH was 7,1),

(ii) ATP sodium salt in the form produced by British Drugs House Works or by Polfa under the trade name Myotrifos adjusted to pH 6,8 by adding NaOH, and

(iii) o-phenantroline, hydroxylamine hydrochloride or sodium fluoride in buffer at pH 6,8.

Nitrogen was obtained by chemical reaction from sodium nitrite ($NaNO_2$). The gas was then carefully washed in KOH solution of pyrogallol.

If not stated otherwise, the inhibiting solution was never supplied to the chamber before the chloroplasts had reached the arrangement characteristic of the specified light conditions. The action of the inhibitor was maintained for one hour under

unchanged light conditions and then the phototactic reaction was induced by changing the intensity of light.

Measurements of the gas exchange were made with a microrespirometer (Zurzycki 1955, Starzecki 1961). The rate of photosynthesis was measured in white light at the intensity of 22 000 erg/cm²sec (spectral range 400—700 nm) and colour temperature about 2800°K corresponding approximately to 3600 lux. This light intensity was about three times higher than the intensity corresponding to the saturation point of photosynthesis. After preliminary measurements of respiration and photosynthesis the leaf was transferred for one hour to the inhibiting solution and then the gas exchange of the leaf was measured again the results being expressed as the percentage of the uptake or the output of oxygen characterizing the leaf.

The viability of cells was controlled in the plysmolysis test in 1 M KNO_3.

RESULTS

1. Nitrogen

When cells with chloroplasts in the position of weak light were submitted to anaerobic conditions while the light intensity remained unchanged, the chloroplast arrangement was not maintained. Some chloroplasts moved to the side walls this movement becoming noticeable already 6 to 10 minutes after the beginning of the nitrogen supply to the chamber and lasting for about one hour; the movements resulted in the achievement of about 70% E and then stopped (Fig. 3*b*). Similarly the strong light arrangement was not maintained under anaerobic conditions: the proportion of epistrophe chloroplasts increased gradually to stabilize at the level of about 30% E after 1 hour. This means that neither of the two main phototactic arrangements of chloroplasts could be maintained after the substitution of nitrogen for oxygen even if the light conditions had not been changed.

On the ground of the results obtained in an earlier investigation (Zurzycki 1962b) the suggestion was then advanced that the principal phototactic arrangements (epistrophe and parastrophe) were associated with some state of tension within the cell, whereas the state of equilibrium was the darkness arrangement, which in *Lemna* was typically expressed by 40 to 50% E. The question, therefore, arose, whether the stabilized arrangements of 70% E in weak light and 30% E in strong light under anaerobic conditions were the symptom of a phototactic tendency of chloroplasts or whether this stabilization was the outcome of the mechanism of the movements being damaged before the state of relaxation had been attained. In order to obtain more information about the damaging effect of anaerobiosis on the apparatus of chloroplast movements the following experiment was carried out: after keeping the leaf under anaerobic conditions for 30 minutes, 1 hour and 2 hours the chamber was washed with a stream of air and the light conditions were changed. The results of these experiments are shown in Fig. 3 *a*. As is to be seen after 30 minutes of anaerobiosis the chloroplasts fairly rapidly reacted to the new conditions and assumed an almost complete arrangement characteristic of the new

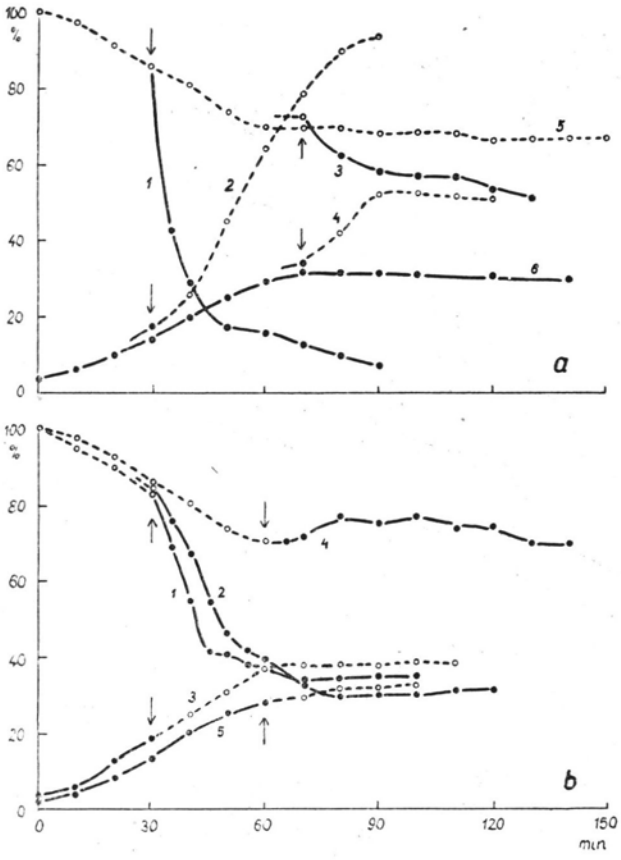

Fig. 3. The anaerobic effect. X—time in minutes, Y—%E. Nitrogen introduced at 0 time

a — Chamber washed with air and light conditions changed 30 min (curves *1*, *2*) and 70 min (curves *3*, *4*) after nitrogen had been introduced. Curves *5* and *6* — constant anaerobiosis and illumination; *b* — Light conditions changed during uninterrupted anaerobiosis 30 min (curves *1*, *2*, *3*) or 60 min (curves *4*, *5*) after nitrogen had been introduced. – – – low light intensity, ——— strong light intensity.

light conditions. This means that 30 minutes in nitrogen was not a noxious dose. After one hour of anaerobiosis the change of environment and light conditions produced much smaller displacement of chloroplasts and the final arrangement was only about 50% E regardless of the light conditions. The damage to the apparatus of chloroplast movements was, therefore, sufficient to prevent the typical phototactic arrangement. Aeration and a change of light conditions after 2 hours caused no significant changes in the arrangement of chloroplasts. These results lead to the conclusion that prolonged anaerobiosis injured the apparatus of chloroplast movements and that, therefore, the stabilized arrangement after one hour in nitrogen seems to be due to the damage in the apparatus and to the inhibition of chloroplast movements. Because of this damaging effect the time of adaptation to anaerobic conditions in the principal experiments with the movements of chloroplasts in the absence of oxygen was, besides the standard one hour period, also 30 minutes

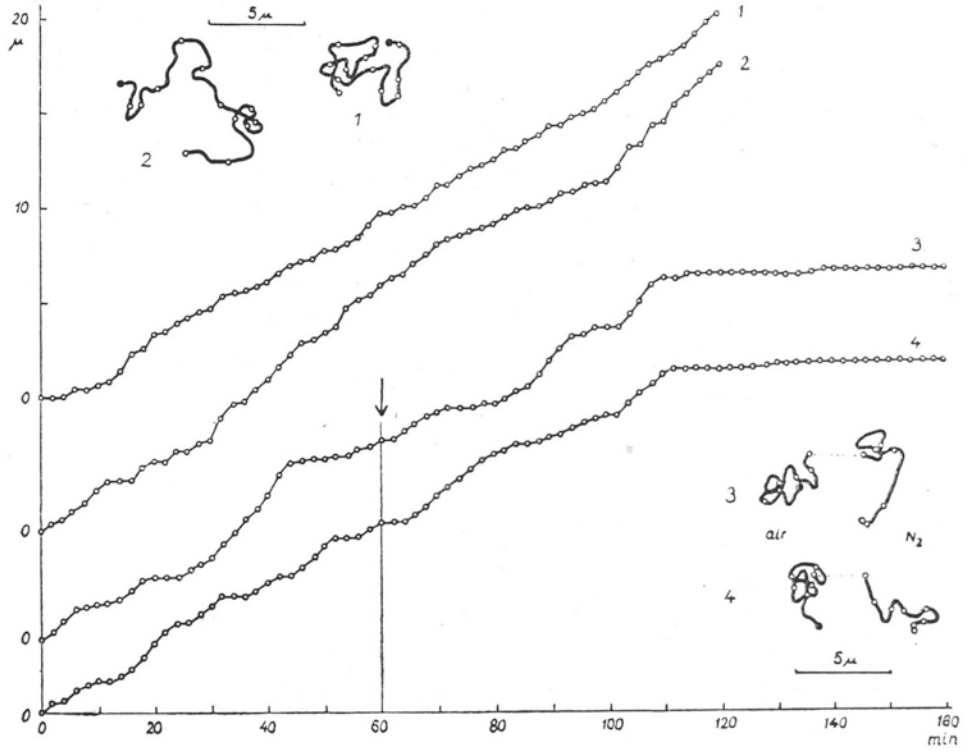

Fig. 4. Paths and speeds of chloroplasts during the movements in darkness (*1* and *2*) and in weak light (*3* and *4*). In the paths traced by chloroplasts points mark the position every 10 minutes: black point — starting position.

Time distance curves: X — time in minutes, Y — distance travelled in μ. For the chloroplasts *3* and *4* after 60 min. air was replaced by nitrogen

(Fig. 3*b*). A change of light intensity in the absence of oxygen after one hour of anaerobic conditions caused no significant displacements of chloroplasts. Contrary to expectations, the change from weak to strong light even led to a small increase in the proportions of epistrophe chloroplasts; the same change of light intensity after 30 minutes of anaerobic adaptation caused a difference in the reactions to strong and to weak light. When strong light was applied under anaerobic condition the displacements were at first markedly accelerated and then gradually slowed down to stop after from 1 to 1,5 hour, the final arrangement being 30 to 40% E (Fig. 3*b*). On the other hand, the change from strong to weak light only slightly accelerated the displacements, though their rate as well as the final arrangement did not differ significantly from what they would be in strong light of constant intensity.

The course of displacements under anaerobic conditions as recorded by statistical technics was, at least in the first stage, similar to the displacements taking place in darkness. A more detailed analysis of both types of displacements was made by cinematographic technics (Fig. 4). In darkness the movements of chloroplasts were

analogous to what was described in an earlier report (Zurzycki 1962b). The chloroplasts strayed in various directions with periodic variations of speed, their average speed being 0.188 μ/min. The paths traced were haphazard curves with numerous loops and the chloroplasts manifested no attachements to any definite parts of the cell (Fig. 4,1). In the arrangement of weak light the chloroplasts showed minor displacements with a tendency to remain in a definite part of the cell (Fig. 4,2). When the medium was changed to nitrogen the character of the movements remained similar, but the displacements of the chloroplasts became greater and a number of the plastids hitherto remaining near the side walls now moved onto the side walls of the cells. Although the average speed of the chloroplast movements in weak light in the presence of oxygen was the same as during the first 30 to 40 minutes of anaerobiosis (0,164 and 0.166 μ/sec), the speed of the movements in the absence of oxygen varied over a wider range and there were very distinct periods of acceleration (Fig. 4,3). Gradually the speed of chloroplasts submitted to anaerobiosis decreased and after 1 to 1,5 hour all movements ceased.

A comparison of the results obtained with cinematographic technics and from the statistical analysis shows that under the experimental conditions applied in this investigation the substitution of nitrogen for air at first only produced or abolished the ability of the chloroplasts to assume the typical phototactic arrangements, but that the mechanism controlling the movements was not damaged till a later stage. Much criticism is, however, essential in any attempts at the interpretation of these results, since the structure of leaves in *Lemna* is such that it includes many intercellular spaces, which may prevent the rapid achievement of complete anaerobiosis even with a steady, uninterrupted flow of nitrogen. The acceleration of chloroplast movements in strong light (Fig. 3*b*) may be interpreted as being the result of photosynthetic phosphorylation proceeding in spite of the absence of carbon dioxide, but also in this case the observed effect may be the result of CO_2 derived from glycolysis being used for photosynthesis thus leading in turn to the production of oxygen and the establishment of oxygen respiration.

2. Sodium Fluoride

Figure 5 shows the main types of chloroplast displacements taking place under different conditions of NaF concentrations after one hour of adaptation and Fig. 6 the influence of different NaF concentrations on respiration, photosynthesis and the final arrangement of chloroplasts attained one hour after the change of light conditions. Concentrations of sodium fluoride lower than 10^{-2} M/l had no significant effect on any of the chloroplast movements. The reaction to strong light also remained normal when the concentration was 3.10^{-2} and not till the concentration had risen to 6.10^{-2} M/l that the reaction was slowed down and the final arrangement of strong light could not be attained. Finally, when the concentration was 10^{-1} M/l a certain amount of displacements took place already during the period of adaptation so that the weak light arrangement was disturbed and there were no further

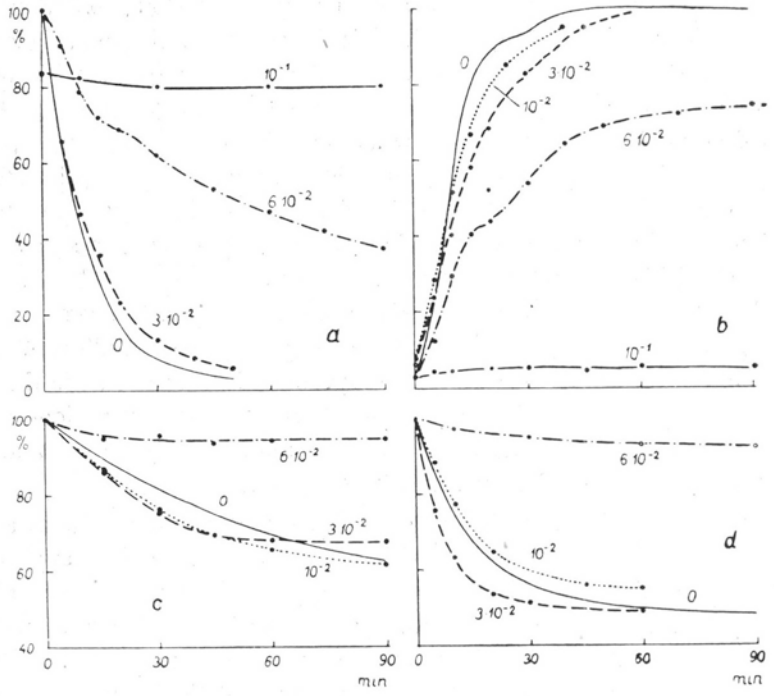

Fig. 5. Chloroplast displacements after 1 hour of adaptation in different NaF concentration. X—time in minutes, Y—% E

a — weak blue light → strong blue light; *b* — strong blue light → weak blue light; *c* — weak blue light → darkness; *d* — weak blue light → strong red light;
Figures by the curves show the concentration of the inhibitor in M/l.

chloroplast movements following the change of the light intensity (Fig. 5*a*). The chloroplasts assembled to from shapeless masses and many of them fused together loosing the individuality of their contours. The reaction strong light→weak light was more sensitive to the changing concentration. Already in sodium fluoride solutions of 10^{-2} and even more so of 3.10^{-2} M/l the rate of displacements especially in their later stage was decelerated, but the chloroplasts could attain the complete arrangement to weak light. The 10^{-1} M/l solution completely inhibited the displacements of chloroplasts (Fig. 5b). In 10^{-2} and $3 \cdot 10^{-2}$ M/l solutions the displacements taking place in darkness were somewhat accelerated as compared to the controls, but they became strongly inhibited when the concentration rose to $6 \cdot 10^{-2}$ M/l (Fig. 5*c*). Also in red light, when the concentration was 3.10^{-2} M/l, the chloroplast movements were accelerated (Fig. 5*d*).

Measurements of the gas exchange showed that photosynthesis remained unaffected by NaF in concentrations up to 3.10^{-2} M/l and as the concentration was further increased photosynthesis was inhibited and stopped alltogether when the concentration reached 10^{-1} M/l. The rate of respiration slightly increased in concentrations ranging from 10^{-3} to 3.10^{-2} M/l and then it also dropped to about 40% of the initial value when the concentration became 10^{-1} M/l (Fig. 6a).

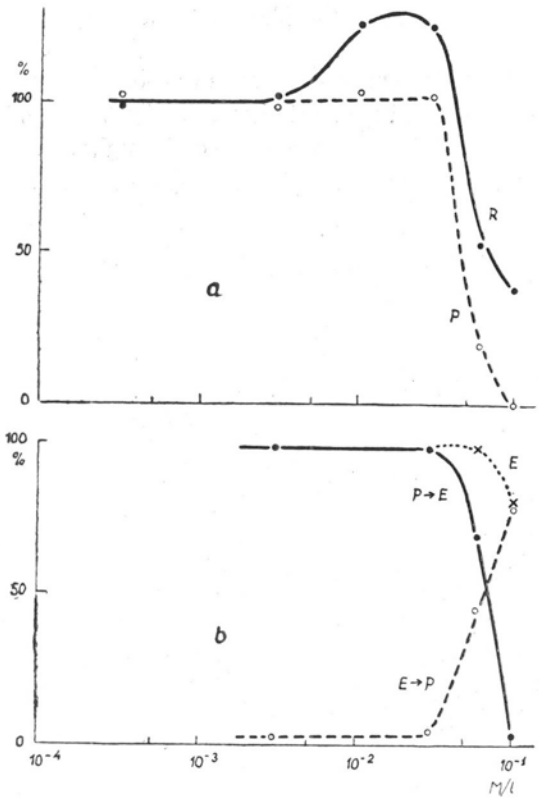

Fig. 6. *a* — Influence of NaF on the gas exchange, *b* — Influence of NaF concentraction on chloroplast arrangement attained 1 hour after the change of light conditions

a — X — concentration in M/l, Y — photosynthesis (*P*) and respiration (*R*) as % of the rate before inhibition.

b — X — concentration in M/l, Y — %E. *EP* — reaction to strong light, *PE* — reaction to weak light, *E* — constant weak light.

From what was said above it results that the displacements of chloroplasts were affected only by unphysiologically high concentrations of sodium fluoride, concentrations so high that they caused substantial disturbances in photosynthesis and respiration, while lower concentrations left the chloroplast movements unaffected. Sodium fluoride being an inhibitor of glycolysis even at much lower concentrations it is, therefore, to be assumed that the inhibition of this process has no influence on the course of chloroplast movements. Of the reactions to the high NaF concentrations worth noting are the greater sensitivity in the reaction to weak light than to strong light (Fig. 5*a* and *b*) and the correlation between the inhibition of chloroplast movements and the complete stopping of photosynthesis. It seems that the accelerating influence of NaF on the movements in darkness and in red light may be explained by the rather high concentrations of Na and F ions affecting the viscosity of the protoplasm.

3. Ortho-phenantroline

The influence of the various concentrations of o-phenantroline on the movements of chloroplasts and the gas exchange are shown in Fig. 7 and 8. The initial arrangements of weak or strong light were maintained throughout the adaptation period even in the highest of the applied o-phenantroline concentrations. The reactions

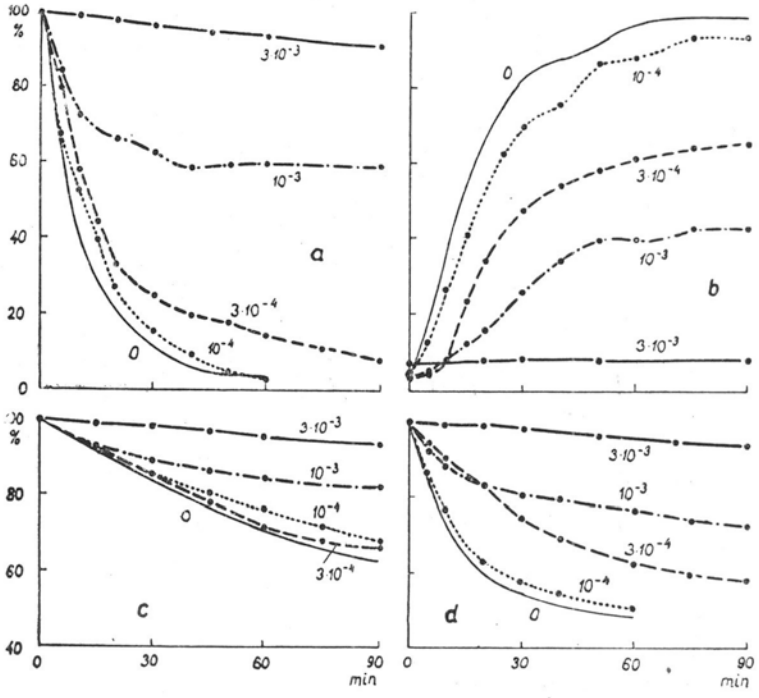

Fig. 7. Chloroplast displacements in different o-phenantroline concentration. Details as in Fig. 5.

of all types were inhibited when the concentration rose to 3.10^{-3} M/l. In concentrations lower than 10^{-4} there were no changes in the chloroplast movements as compared to the controls. The course of phototactic movements corresponding to the intermediate concentrations (Fig. 7*a* and *b*) was gradually decelerated as the concentration of the inhibitor became higher. This influence was more clearly marked in the later stages of the displacements. The reaction to weak light was clearly more sensitive than the reaction to strong light (see e.g. the curve for the concentration of 10^{-4} M/l). The displacements in darkness became distinctly retarded only when the concentration was increased to 10^{-3} M/l (Fig. 7*c*). The influence of o-phenantroline on the reaction to strong red light had some interesting aspects (Fig. 7*d*): while the 10^{-4} M/l concentration had no significant effect on the reaction, the 3.10^{-4} and 10^{-3} M/l concentrations retarded the displacements of chloroplasts making the movements similar to the movements in darkness, which means that in higher concentrations the specific effect of red light was abolished. This effect

was most clearly marked in the 3.10^{-4} M/l o-phenantroline solution, this concentration having no influence on chloroplast movements in darkness (Fig. 7*c* and *d*).

Gas exchange measurements indicate that respiration remained unchanged up to the concentration of 10^{-4} M/l, i.e. over the same range of o-phenantroline

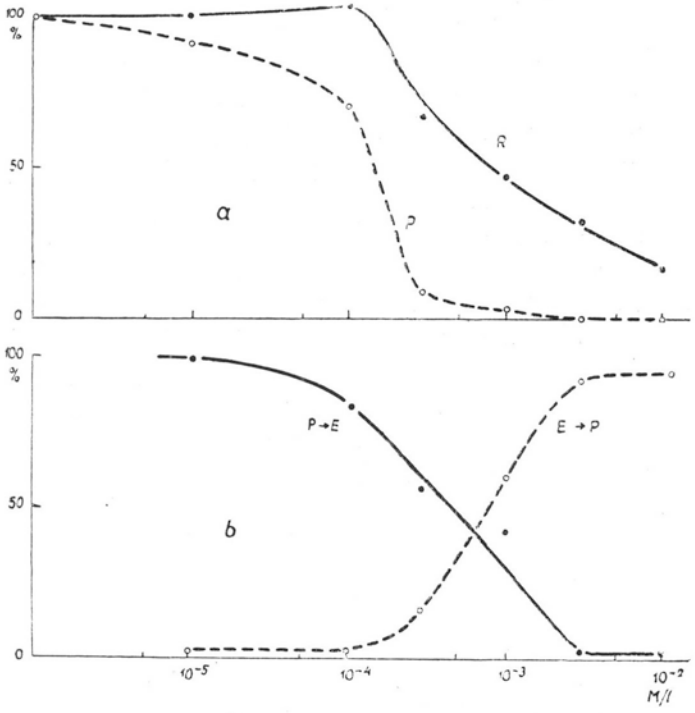

Fig. 8. Influence of o-phenantroline concentration on the gas exchange (*a*) and the chloroplast arrangement (*b*). Details as in Fig. 6

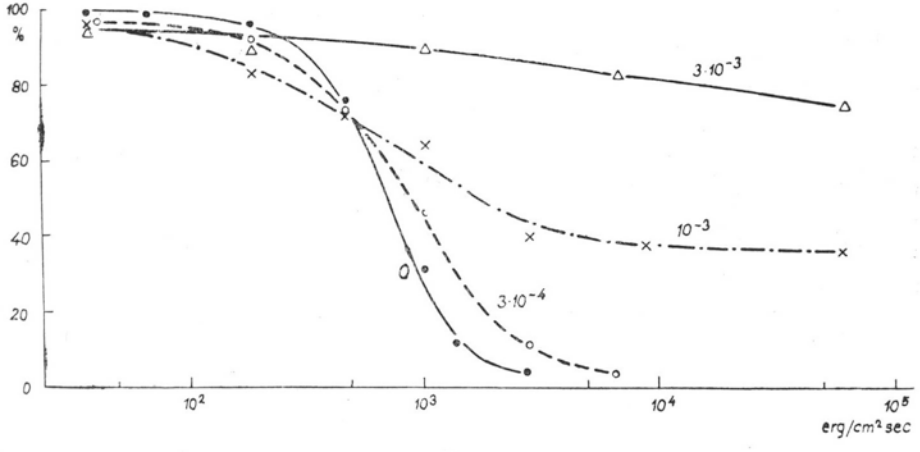

Fig. 9. Curves showing how chloroplasts arrangement (Y — %E) depend on intensity of blue light (X — erg/cm² sec). Starting position — weak light arrangement. Time of irradiation 1 hour. Figures by the curves show the concentration of o-phenantroline in M/l.

concentrations which have had no significant effects on phototactic movements. As the concentrations mounted the rate of respiration gradually dropped to from 30 to 40% of its initial value when the concentration reached 3.10^{-3} M/l (Fig. 8a). On the other hand, the inhibition of photosynthesis was much stronger: the rate of photosynthesis was depressed to about 1/3 of its initial value already in the 10^{-4} M/l solution and to residual values at concentrations ranging from 3.10^{-4} to 3.10^{-3} M/l. It is to be noted that a sharp drop in the rate of photosynthesis had no influence on the phototactic movements, whereas the complete suppression of photosynthesis was associated with the immobility of chloroplasts. On the other hand, the inhibition of photosynthesis to a small percentage of the initial rate abolished the specific effect of red light.

The fact that in some concentrations of o-phenantroline the characteristic arrangements of weak and strong light were never completely attained may indicate that this inhibitor changed the sensivity of chloroplasts to the intensity of light. In order to check this supposition the arrangements of chloroplasts after one hour of irradiation were examined to see how they depended on the intensity of light. As is to be seen from the results assembled in Fig. 9 the supposition just made was not confirmed. Only at the 3.10^{-4} M/l concentration there is a small shift of the curve towards the higher light intensities. At the higher o-phenantroline concentrations the complete arrangements of strong light was not attained even after exposure to light of very high intensities.

4. Hydroxylamine

As is to be seen in figs. 10 and 11 the chloroplast arrangements of weak and strong light were maintained throughout the adaptation period in all of the applied hydroxylamine concentrations. Hydroxylamine had a different influence on each of the particular types of phototactic displacements: in strong light and in darkness the inhibitor did not influence significantly the nature of the movements over the whole range of the applied concentrations, whereas in weak light and in red light the displacements of chloroplasts were distictly affected. In strong light (Fig. 10*a*) the course of the displacements in the first stage was in all of the applied hydroxylamine concentrations exactly the same as in the controls; in the later stage the movements became somewhat slower in the concentrations ranging from 10^{-2} to 10^{-1} M/l, though the final arrangement of chloroplasts was identical as in the controls. The opposite reaction (strong light → weak light) resembled the reaction in the unpoisoned controls only in the lower hydroxylamine concentrations, i.e. up to 10^{-3} M/l. In concentrations ranging from 3.10^{-3} to 3.10^{-2} M/l there was distinct deceleration of the rate of displacements and in the higher concentration within this range the complete arrangement of weak light was never attained. In the 10^{-1} M/l hydroxylamine solution the chloroplasts did not react to the change from strong to weak light. In darkness the displacements were unaffected by hydroxylamine with the exception of the highest concentration, which slightly slowed down the movements but the difference was to small that it could almost lie within the

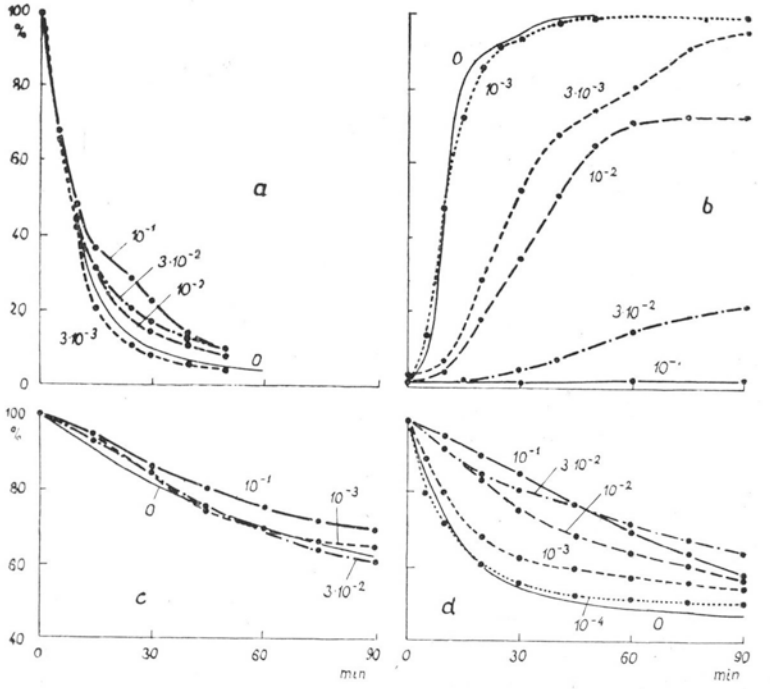

Fig. 10. Chloroplast movements in different hydroxylamine concentrations. Details as in Fig. 5

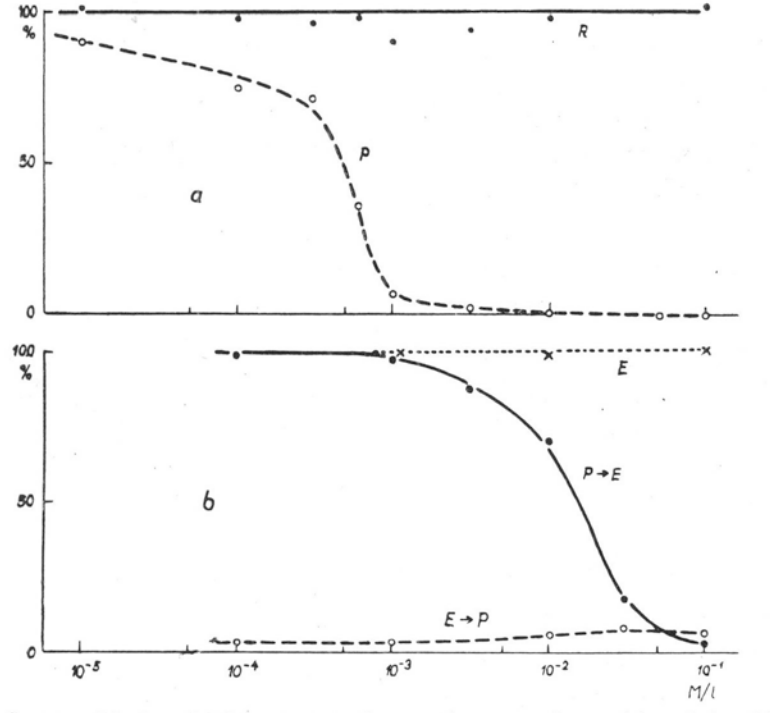

Fig. 11. Influence of hydroxylamine concentration on the gas exchange (*a*) and the chloroplast arrangement (*b*). Details as in fig. 6

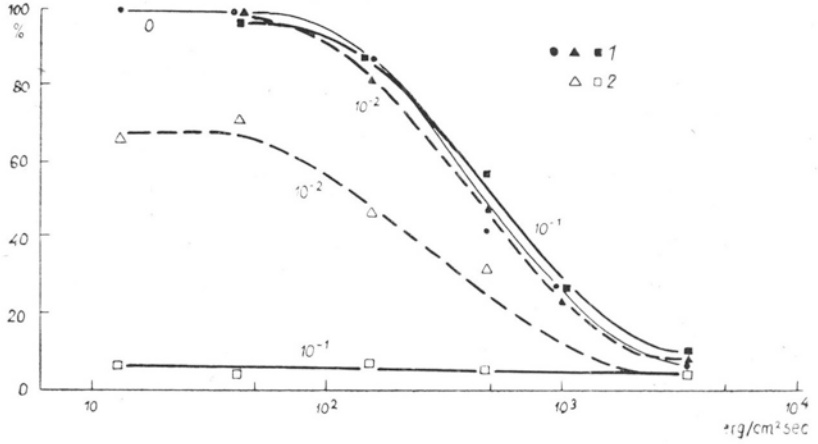

Fig. 12. Curves showing how chloroplast arrangement (Y — %E) depend on light intensity (X — erg/cm²sec). Starting position — weak light arrangement (*1*) or strong light arrangement (*2*). Time of irradiation 1 hour. Figures by the curves show the concentration of hydroxylamine in M/l.

limits of variability. Concentrations up to 10^{-4} M/l had no influence on the reaction to strong red light and in concentrations ranging from 10^{-2} to 10^{-1} M/l the chloroplasts moved with approximately the same speed as in darkness in spite of being exposed to strong red light.

The curves (Fig. 12) illustrating how the arrangements of chloroplasts after one hour of irradiation depended on the intensity of light indicate that, as long as the initial position was the arrangement of strong light, hydroxylamine had no influence on the sensivity to the intensity of light. However, the same concentration of hydroxylamine caused a complete inability to react when the change was from strong light to weak light.

Table 1
Influence of hydroxylamine on output and uptake of oxygen

NH_2OH concentration M/l	Darkness			Light 3600 lx		
	before hydroxylamine was used Arbitrary units	1 hour after hydroxylamine was used Arbitrary units	respiration %	before hydroxylamine was used Arbitrary units	1 hour after hydroxylamine was used Arbitrary units	true photosynthesis %
10^{-1}	- 4.8 - 3.6	- 5.0 - 3.6	104 100	+ 21.1 + 27.6	- 7.6 - 6.5	- -
5.10^{-2}	- 4.25 - 3.7	- 4.6 - 3.85	108 107	+ 26.0 + 18.6	- 6.2 - 5.8	- -
10^{-2}	- 5.1 - 4.3	- 4.6 - 4.65	90 108	+ 17.9 + 14.4	- 5.6 - 4.65	- 0
3.10^{-3}	- 3.8 - 5.4	- 4.0 - 4.7	105 97	+ 21.8 + 21.1	- 3.5 - 4.1	1.95 2.25
10^{-3}	- 4.3 - 3.5	- 3.75 - 3.4	87 97	+ 20.5 + 16.0	- 3.45 - 1.5	1.2 9.8

The specific effect of hydroxylamine seems to be associated with its action on cell metabolism (Fig. 11*a*). Even the highest concentrations of hydroxylamine caused no changes in the rate of respiration, but hydroxylamine inhibited photosynthesis; the rate of this process was slowed down slightly at first in concentrations ranging from 10^{-5} to 10^{-4} M/l and then, as the concentrations increased, the rate of photosynthesis dropped very sharply to a residual value in concentrations ranging from 10^{-3} to 10^{-2} M/l. The exact concentration at which all photosynthetic activities, stopped altogether was difficult to define. The curve in Fig. 11*a* was calculated, similarly as the analogous curves for the other inhibitors, on the

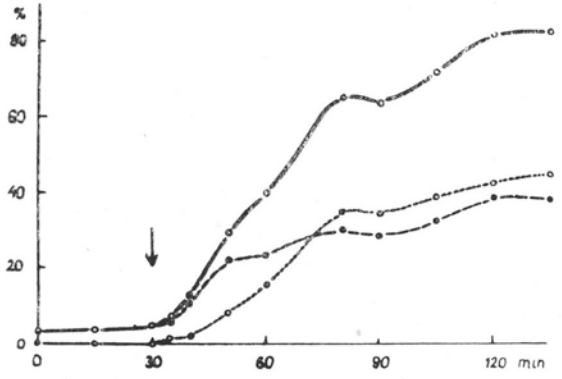

Fig. 13. Chloroplast displacements in 10^{-1}M/l hydroxylamine solution. At 0 time weak light was switched on after the 1 hour period of adaptation in strong light. After 30 minutes irradiation with red light (intensity 30000 erg/cm²sec) was additionally provided. Solide line — total per cent chloroplasts in flat position, dashed line — per cent of chloroplasts on distal side, dotted line — per cent of chloroplasts on proximal side of the cell

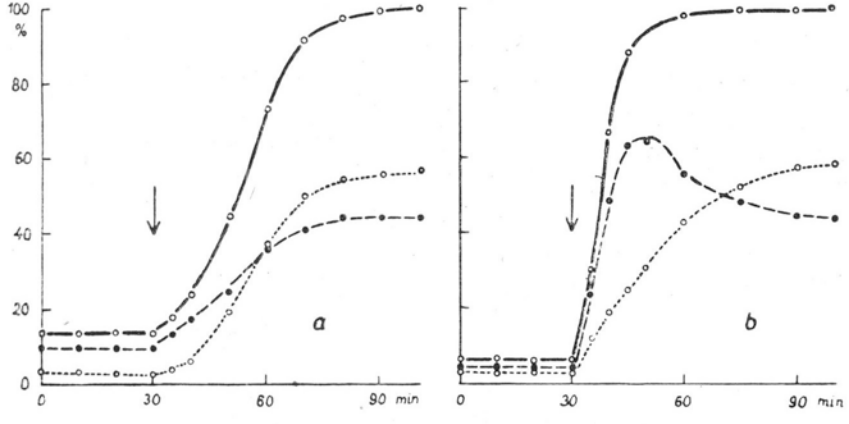

Fig. 14. *a* — Chloroplast displacements in 10^{-1} M/l hydroxylamine solution. Starting position— strong light arrangement after 1 hour adaptation in hydroxylamine solution. At 0 time weak light was switched on and after 30 minutes hydroxylamine was replaced by the phosphate buffer. *b*—Control. Chloroplasts displacements in strong light → weak light reaction. Details as in fig. 13.

assumption that the rate of respiration in light equals the rate of respiration in darkness. If this assumption was correct it would mean that photosynthesis dropped to zero when the concentration of hydroxylamine was 6.10^{-3} M/l. The results of the measurements of gas exchange assembled in Table 1 indicate that the uptake of oxygen in light was distinctly higher than the uptake of oxygen during respiration in darkness. If it be assumed that this effect was not caused by the specific action of hydroxylamine on respiration, but was the result of the normal increase of the rate of respiration in light, which had become discernible owing to the complete or near complete inhibition of photosynthesis, then the conclusion would have to be that residual photosynthesis was still taking place at the concentration of 10^{-1} M/l. This problem does not appear in the case of the other inhibitors, because their concentrations sufficiently high to block photosynthesis were also always sufficient to inhibit respiration.

The suppression in the high hydroxylamine concentrations of the reaction normally induced by the change from strong to weak light may be explained by the absence of even traces of photosynthesis in weak light and a residual photosynthetic process still taking place in strong light. This explanation is based on the assumption that at least a trace of photosynthesis was necessary for normal phototactic movements. This assumption was checked by adding red light to the normal irradiation of the strong light → weak light reaction red light being phototactically inactive but active in photosynthesis The apparatus used for the additional irradiation with red light was described in an earlier report (Zurzycki 1964). The results of this experiment (Fig. 13) indicate that the addition of red light stimulated phototactic displacements even when the concentration of hydroxylamine was 10^{-1} M/l, i.e. when under the other conditions there were no displacements in the strong light → weak light reaction, but that the final chloroplast arrangement was not the complete weak light arrangement and only amounted from 60 to 80% E.

The blocking of the weak light reaction by hydroxylamine was reversible (Fig. 14): when the leaf was washed with a buffer while the light conditions remained unchanged, the chloroplasts rapidly moved to the position characteristic of weak light. It is remarkable that while the chloroplast movements were being prevented by hydroxylamine the prolonged exposure to weak light nevertheless exerted an influence on the movements of the plastids after the inhibitor had been washed away: contrary to the normal course of the reaction to the change from strong to weak light, as soon as hydroxylamine was washed away from a leaf exposed to weak light all the chloroplasts move almost simultaneously to the proximal and distal cell walls (see Fig. 14*a* and *b*).

5. 2,4 Dinitrophenol

The influence of DNP on the main types of phototactic movements and on the gas exchange is illustrated in Figs. 15 and 16. In DNP solutions of concentrations below 10^{-5} and above 10^{-4} M/l the complete weak light arrangement was maintained unchanged throughout the one hour adaptation period, whereas within the

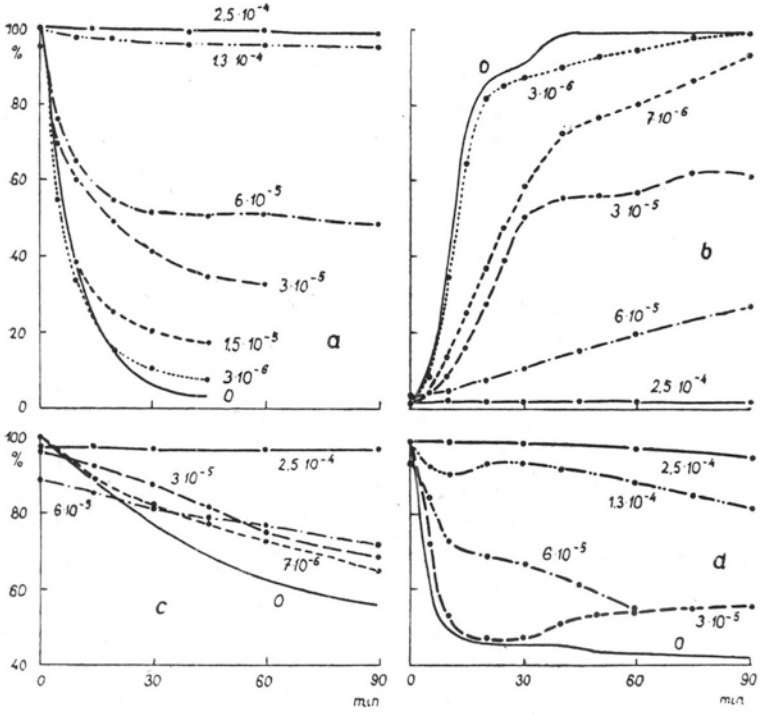

Fig. 15. Chloroplast movements in different 2,4 dinitrophenol concentration. Details as in Fig. 5.

range of concentrations between these two values there was a small drop in the proportion of epistrophe chloroplasts (Fig. 16). Concentrations above 10^{-4} M/l made all chloroplast displacements impossible and froze the arrangement existing at the time the inhibitor was introduced. In the case of chloroplasts in the position of weak light the only noticeable reaction was contraction, the loosening of the mosaic arrangement, and the adoption of a somewhat less regular distribution while there were no movements whatever to the side cell walls. In lower concentrations the displacements of the strong light reaction at first proceeded at a similar rate as in the controls, but soon they were inhibited and the proportion of epistrophe chloroplasts was established at a level depending on the concentration (Fig. 15*a* and 16). In 10^{-5} M/l DNP solution the phototactic movements were practically unchanged as compared to the controls. The sensivity of the reaction to weak light was much greater: the rate of displacements slowed down and full epistrophe became impossible already in the 5.10^{-6} M/l DNP solution. Very often in the reaction to weak light almost all the chloroplasts moved to the distal wall of the cell, i.e. to the wall further away from the source of light, while hardly any remained on the proximal wall (Fig. 17). A similar, though less clearly marked effect of DNP was sometimes observed in the reaction to strong light: the movement of the chloroplasts away from the flat position was then more rapid on the distal side of the cell

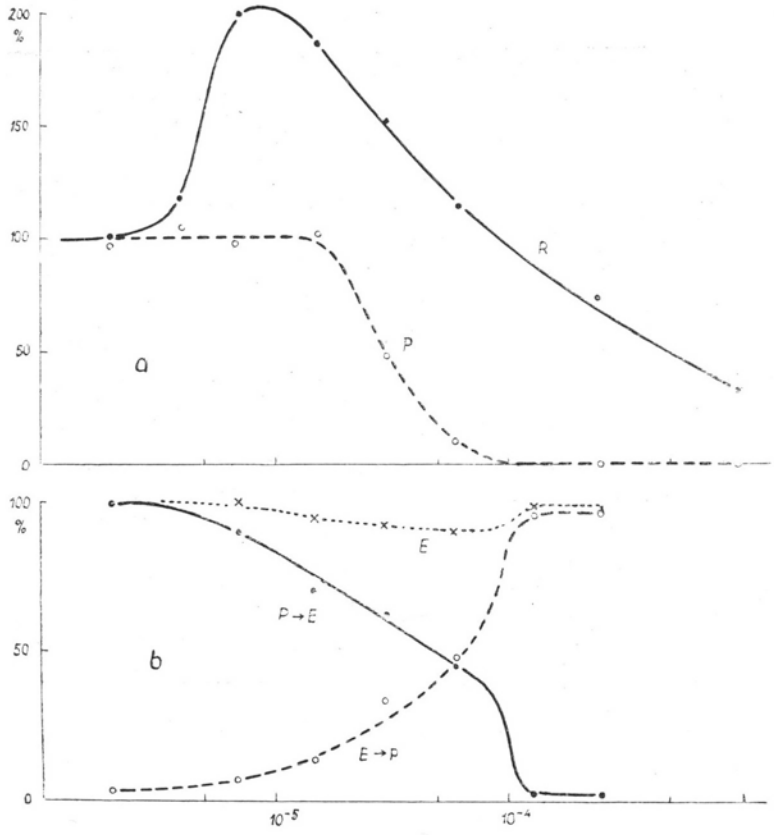

Fig. 16. Influence of DNP concentration on the gas exchange (*a*) and on the chloroplast arrangements (b). Details as in Fig. 6

than on the proximal side, this being the opposite to what takes place in cells untreated with DPN.

The gas exchange was not disturbed in DNP concentrations lower than 10^{-5} M/l. With a rise of concentration the uptake of oxygen in respiration process rapidly increased to the maximum at $1{,}5.10^{-5}$ M/l and then dropped again. In the 10^{-4} M/l DNP solution the uptake of oxygen was approximately the same as it had been before the inhibitor was applied and dropped to about 80% of the initial value with the rise of the concentration to $2{,}5.10^{-4}$ M/l. Photosynthesis was increasingly inhibited in concentrations from 3.10^{-5} M/l up till it dropped to zero in the DNP solution of about 10^{-4} M/l.

In the case of DNP the inhibition of phototactic movements was associated with the complete blocking of photosynthesis, though the cells were still alive. The plasmolysis test showed that cells kept in $2{,}5.10^{-4}$ M/l DNP solution for as long as 24 hours still retained the ability to plasmolyse. What is more, the ability to plasmolyse was not abolished in cells kept in 10^{-3} M/l DNP solution for 6 hours.

The curves showing the relationship between the chloroplast arrangement and

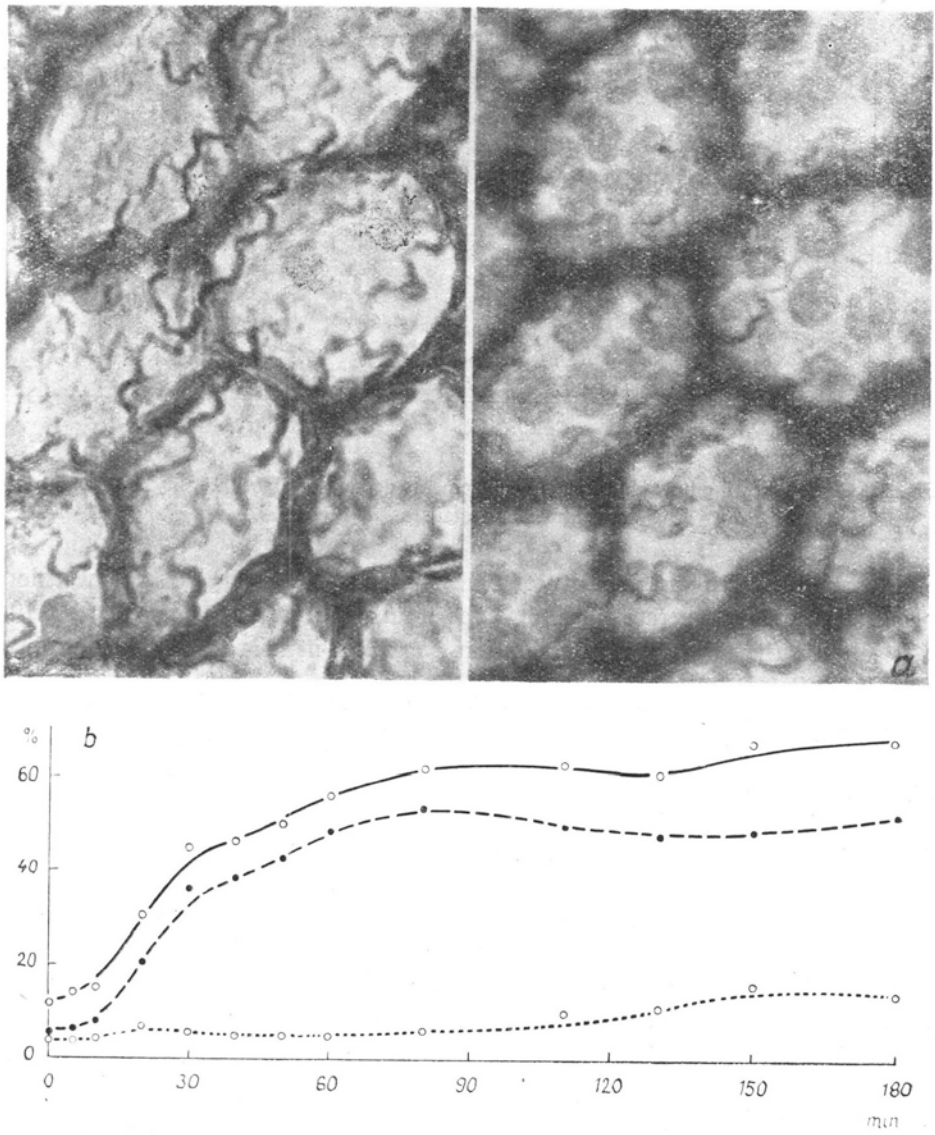

Fig. 17. Strong light → weak light reaction in 3.10^{-5} M/l DNP solution. Above (*a*) — chloroplast arrangement on the proximal (left) and distal (right) walls after 1 hour. Below (*b*) — Course of displacements. Details as in Fig. 13.

the intensity of light (Fig. 16) indicate a small drop of the sensivity to light already at $1{,}5.10^{-5}$ M/l concentration. This drop was even more distinct at the 6.10^{-5} M/l concentration when the complete chloroplast arrangement of strong light could not be attained even in light of very high intensity. Worth noting is the circumstance that, though in blue light of intensities higher than 5000 erg/cm^2 the chloroplasts agglomerated after 30 to 60 minutes of irradiation, the agglomeration of chloroplasts in DNP solution was partly or completely inhibited: e.g. in $1{,}5.10^{-5}$ M/l

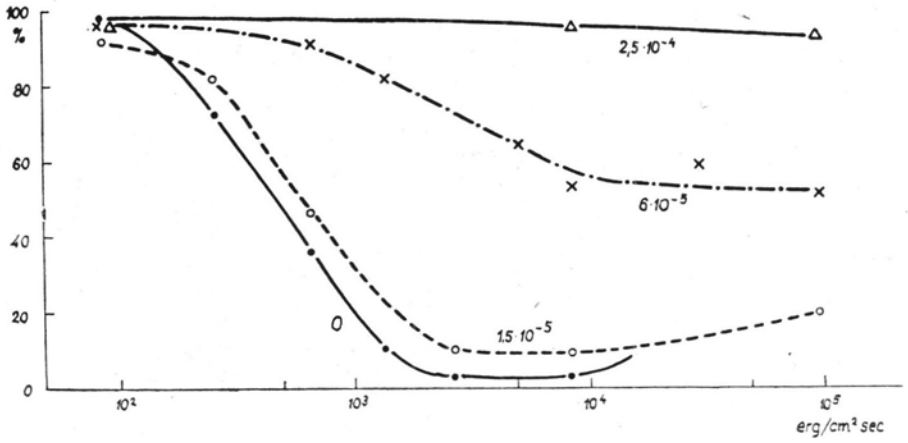

Fig. 18. Curves showing how chloroplast arrangements depend on light intensity in different DNP concentration. Details as in Fig. 9.

DNP solution when the intensity of blue light was 10 000 erg/cm²sec the chloroplasts did not agglomerate till after some hours of irradiation and in the 6.10^{-5} M/l solution there was no agglomeration even after irradiation with the strongest light intensities

In the experiments with DNP solutions of different concentrations the pH of solutions was 5,8. The effect of DNP very clearly depended on the pH. In analogous experiments with the pH set at 7,1 the effect of DNP was 10 to 20 times weaker: e.g. the effect of the $2{,}5.10^{-4}$ M/l concentration at pH 7,1 was the same as of the $1{,}5.10^{-5}$ M/l concentration at pH 5,8.

6. Adenosine Triphosphate

The influence of ATP of different concentrations on the main types of chloroplast movements and on the gas exchange is shown in figs. 19 and 20. During the one hour period of adaptation in the 10^{-3} M/l ATP solution and in lower concentrations the full arrangement of weak light remained unchanged, but when the ATP concentration was increased the proportion of epistrophe chloroplasts dropped during the first 10 minutes to a lower level, which was different in each of the different concentrations. Irradiation with strong light after the adaptation period induced the typical reaction to strong light and the speed of the chloroplast displacements in concentrations up to 3.10^{-3} M/l was the same as in the control. It was not till the concentration had been increased to 6.10^{-3} M/l and more that the displacements caused by strong light became very small. Immediately after the ATP solution of a high concentration, i.e. ranging from 3.10^{-3} to 6.10^{-3} M/l, had been introduced the chloroplasts assumed irregular arrangements, empty spaces appeared between the chloroplasts hitherto distributed in a regular mosaic, and the chloroplasts formed local agglomerations: however, 10 to 20 minutes later the chloroplasts were again dispersed and some of them moved to the side cell walls.

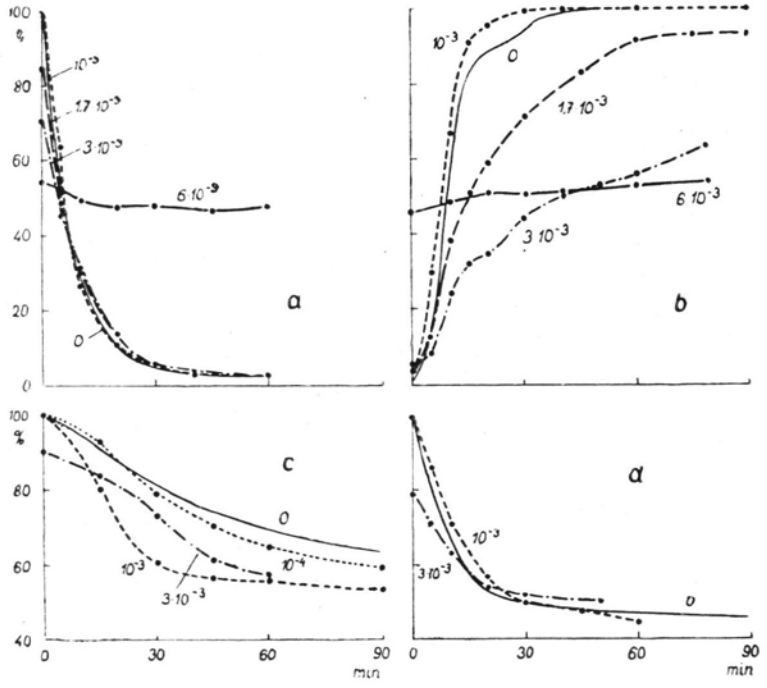

Fig. 19. Chloroplast movements in different ATP concentrations. Details as in Fig. 5

The reaction to weak light was more sensitive to ATP than the reaction to strong light. The phototactic displacements, in particular their later phase, were slowed down already by the $1{,}5.10^{-3}$ M/l ATP solution and even more so when the concentration rose to 3.10^{-3} M/l. In the case of the reaction to darkness the ATP had a specific influence most clearly marked over the range of concentrations from 3.10^{-4} to 3.10^{-3} M/l: the phototactic movements were accelerated as compared to the controls the maximum acceleration corresponding to the concentration of 10^{-3} M/l (Fig. 19*c* and Fig. 20). The maximum acceleration of the reaction to darkness produced by ATP was not equal but closely approached to the acceleration caused by strong red light.

Measurements of the gas exchange (Fig. 20*a*) indicated that respiration was accelerated in concentrations ranging from 3.10^{-4} to 5.10^{-3} M/l while photosynthesis dropped in concentrations from 3.10^{-3} up amounting to 80% of the initial value at 5.10^{-3} M/l. Contrary to the other inhibitors used in this investigation only ATP inhibited phototactic movements while impairing only a little the ability to photosynthesis.

The curves illustrating the relationship between the arrangements of chloroplasts and the intensity of light show that the effect of ATP in concentrations higher than 10^{-3} M/l was opposite to the effect of DNP. With the rise in the ATP concentrations the curves shift in the direction of weaker light intensities indicating that the sensivity to light was increasing so that within the range of lower light intensities

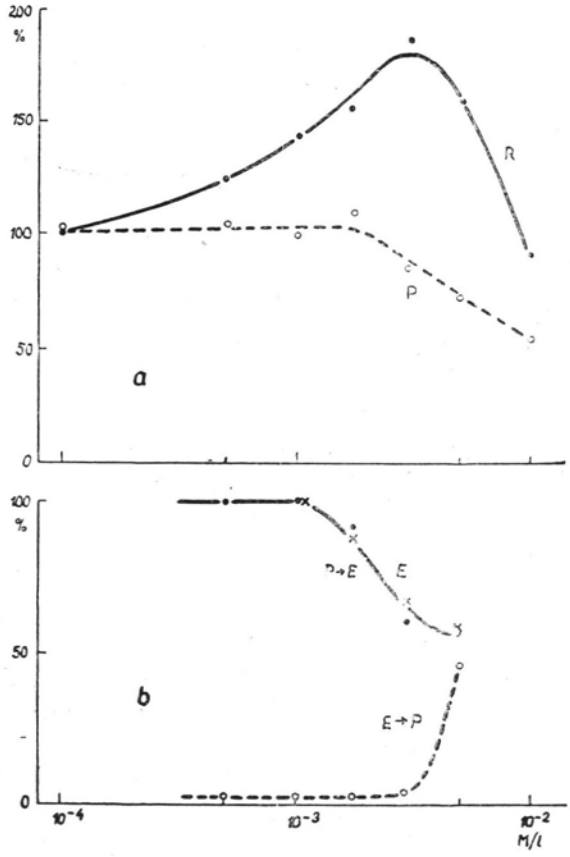

Fig. 20. Influence of ATP concentration on the gas exchange (*a*) and on the chloroplast arrangement (*b*). Details as in fig. 6

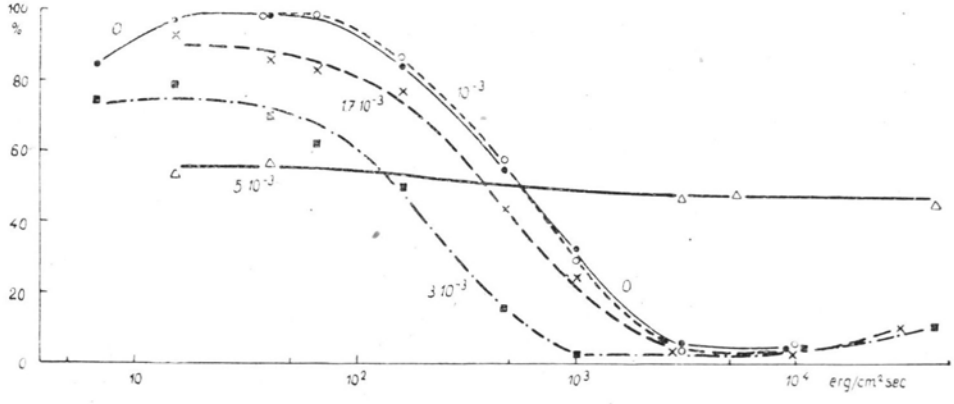

Fig. 21. Curves showing how chloroplast arrangements depend on light intensity in different ATP concentrations. Details as in Fig. 9.

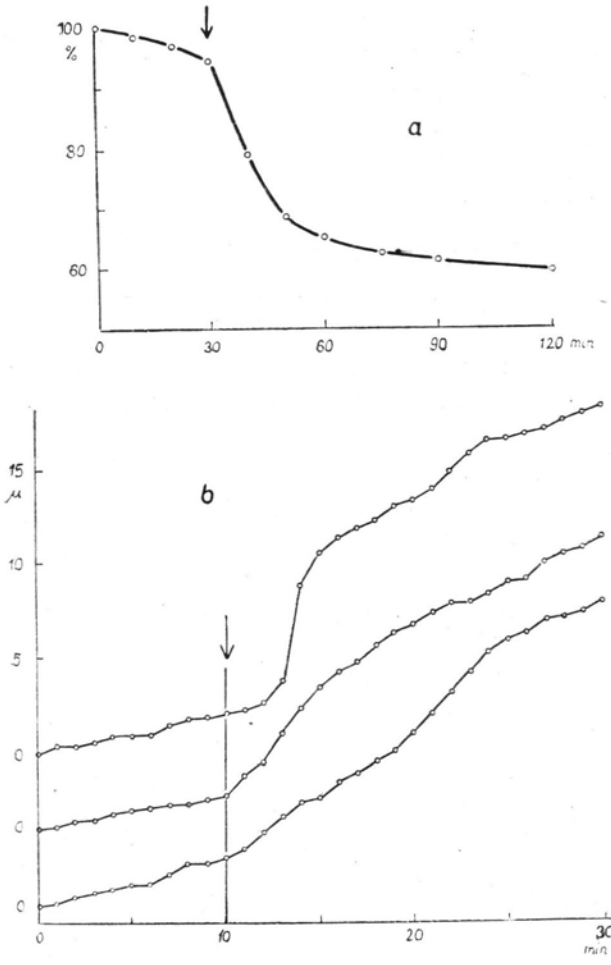

Fig. 22. Movements of chloroplasts in darkness. While the displacements were going on (at the time mark by arrows) 10^{-3} M/l ATP solution was introduced.

a — Course of the displacements. X — time in minutes, Y — %E. *b* — time distance curves for three chloroplasts. Details as in Fig. 4.

the full weak light arrangement could not be maintained. The 5.10^{-3} M/l ATP solution entirely abolished the sensivity to light or prevented the chloroplasts from attaining the phototactic arrangements. The effect of ATP resembled under many respects the effect of additional irradiation with strong red light (Zurzycki 1964). Similarly as in the case of additional irradiation with red light the treatement with ATP accelerated the movement towards the position of darkness and made the chloroplasts more sensitive to blue light. The results of cinematographic analysis (Table 2) show that not only the reaction itself was accelerated but also the movements of the particular chloroplasts on their paths traced in the cell became more rapid.

The last series of experiments consisted in introducing of ATP while the chloroplast displacements were taking place in darkness. When 10^{-3} M/l ATP solution

Table 2
Average speeds of chloroplasts under different conditions (μ/min)

Weak red light (about 300 erg/cm^{2}sec)	0.26
Strong red light (about 120000 erg/cm^{2}sec)	0.59
Weak red light + 10^{-3} M/l ATP solution	0.51

was introduced into the medium of the cells kept in darkness while the very slow movements of chloroplasts were taking place, very rapidly the rate of the displacements to the side walls became accelerated so that already 5 min. after ATP had been introduced the acceleration was clearly noticeable (Fig. 22a); twenty minutes later the more or less stationary darkness arrangement of chloroplasts was attained. The analysis of cinematographic recordings shows that in weak red light (about 300 erg/cm^2 sec) used for making the photographs the average speed of chloroplasts was 0,217 μ/min. When ATP was introduced the movements accelerated sharply; this acceleration lasted from 5 to 10 minutes and then the chloroplasts slowed down again, but their average speed even then was 0,57 μ/min., i.e. much higher than it had been before ATP treatment.

DISCUSSION

Anaerobic conditions making respiration impossible, if maintained too long, are noxious to *Lemna* leaves. A similar noxious effect of anaerobiosis was reported in *Mougeotia* by Fetzer (1963). The analysis of chloroplast displacements in the early phase after anaerobic conditions were applied indicates that in these conditions the typical phototactic arrangements can neither be maintained nor attained. This is consistent with what Senn (1908) observed in *Funaria*; he found that when *Funaria* leaves were placed in nitrogen or hydrogen the chloroplasts assumed the apostrophe arrangement and the ability to react to light disappeared. Prolonged anaerobiosis causes the complete inhibition of phototactic movements and this effect is analogous to the inhibition under anaerobic conditions of protoplasmic movements in some kinds of cells having cell walls (Kelso and Turner 1955, Zurzycki 1951). Although much prudence is needed in drawing any conclusions from the experiments with anaerobiosis in this investigation, it seems that the energy derived from glycolysis is insufficient to satisfy the demand of the mechanism controlling phototactic movements.

This supposition is confirmed by the results obtained in experiments with sodium fluoride. By stopping the dehydrogenation of 2-phosphoglyceric acid sodium fluoride acts as a typical inhibitor of glycolysis in most experimental objects when it is applied in concentrations ranging from 10^{-5} to 10^{-3} M/l. But in *Lemna* the

chloroplast movements are not stopped by this inhibitor till its concentration is increased to a non-physiological level. A similar effect was reported by Fetzer (1963) for *Mougeotia*. The circumstance that the inhibition of glycolysis has no influence on the course of phototactic displacements points to the minor significance of the energy derived from glycolysis in the mechanism of chloroplast movements. Contrary to what has been observed in slime molds where glycolysis is an important source of energy for protoplasmic streaming (Kamiya 1959), in *Lemna* as well as in *Mougeotia* phototactic movements are independent of this metabolic process. The acceleration of some types of phototactic displacements in the more concentrated NaF solutions seems to be due to the liquifying effect which the Na and F ions have on the protoplasm (Zurzycka and Zurzycki 1951). Gimesi and Pozsar (1955) reported an analogous influence of sodium fluoride on protoplasmic movements in *Elodea*.

According to Gaffron (1944, 1945) ortho-phenantroline applied in solutions of even very low concentration inhibits photosynthesis. This effect probably consists in the blocking of the intermediate products between the primary photosynthetic reaction on the one hand and oxygen and hydrogen on the other, thus stopping the primary reaction (Simonis 1960). Fetzer found that already at the concentration of 10^{-5} M/l o-phenantroline had an inhibiting effect on the phototactic response to strong light in *Mougeotia*. *Lemna* is less sensitive than *Mougeotia*, but also in *Lemna* concentrations of o-phenantroline higher than 10^{-4} M/l have a distinct effect.

The specific action of hydroxylamine constists in that it inhibits the photosynthetic liberation of oxygen (Gaffron 1942, 1944). In the case of *Lemna* no influence of hydroxylamine on the rate of respiration was observed (Zurzycki and Zurzycka 1955) and owing to this property of specifically blocking the photosynthetic process hydroxylamine is a very useful research tool. In *Mougeotia*, however, hydroxylamine has proved to be an inhibitor also of respiration (Fetzer 1963).

2,4 dinitrophenol is an inhibitor well known for its ability to uncouple phosphorylation from oxidation. It is not certain how this inhibitor acts on photophosphorylation. Urbach and Siminis (1962) believe that photophosphorylation is independent of DNP and a similar independence is reported by Avron (1964) for a related compound, dichlorophenol. It has been demonstrated that in muscles (Perry 1960) and in myxomycete plasmodia (Nakajima see Kamiya 1959) DNP activates ATP-ase and lowers the level of ATP in cells (Takeuchi and Hatano see Kamiya 1959). This phenomenon may have its explanation in the opposite effects of ATP and DNP on the arrangement of chloroplasts (light curves, agglomeration of chloroplasts) observed in this investigation. The strong influence that dinitrophenol has on protoplasmic movements has been reported by several workers (see Kamiya 1959). Fetzer (1963) has demonstrated the inhibiting effect that this compound has on chloroplast movements in *Mougeotia*. The influence of DNP on chloroplast movements observed in *Lemna* is associated with its influence on respiration in a similar way as in *Mougeotia*, but *Lemna* cells appear to be more

sensitive to this influence. The significance of pH for the effectiveness of the biological influences on DNP was observed by Marcus and Mayer (1963).

Many workers have reported the acceleration of protoplasmic movements caused by ATP in cells having cell walls (Takata 1958, in *Acetabularia*; Sandan 1959, in *Nitella*; Kulesza 1961, in *Elodea*; Tageeva and Dubrov 1961, in *Allium*). The action of ATP usually consists in the periodical acceleration of the movements, the acceleration disappearing after 15 to 30 minutes. In the present experiments the acceleration of some types of phototactic movements lasted much longer and could be observed several hours after ATP was first supplied. The higher ATP concentrations inhibit the displacements of chloroplasts similarly as they inhibit protoplasmic movements (Sandan 1959) and the movements of flagella (Marcus and Mayer 1963).

The energy necessary for the chloroplast movements in darkness may be derived solely from the metabolic processes (respiration, glycolysis), since all possibilities of light being a source of energy are then excluded, or the movements may be associated with a tendency to equalize a state of disequilibrium, or tension, caused and maintained by the previously existing light conditions. The movements in darkness, however, continuing uninterruptedly also when the chloroplasts are uniformly distributed (about 50% E) and the nature of these movements in the later phase being similar as in the early phase of the displacements (Zurzycki 1962b, see also Fig. 4*a*), it seems natural to assume that the main part in the supply of energy for these movements is obtained from the dissimilation processes, primarily by oxygen respiration. This interpretation is supported by the experiments with hydroxylamine, which indicate that, when oxygen respiration is undamaged, the chloroplast movements in darkness reamin unchanged, though the other types of displacements may be seriously disturbed. On the other hand any damage to the respiration processes has a strong influence on the movements in darkness. The degree of damage to the apparatus of respiration necessary to stop chloroplast movements in darkness depends on the manner in which the inhibitor acts, but the slowing down of the rate of the gas exchange (of the uptake of oxygen) does not indicate to what extent the respiration apparatus is damaged. For instance the movements stop when the respiration is 50% of the normal rate in sodium fluoride, 30% in o-phenantroline, and 100% in DNP; in this last case the movements become weaker much earlier, in much lower DNP concentrations, while at the same time the greater intake of oxygen points to the uncoupling of oxidative phosphorylation.

In strong red light the movements of chloroplasts are essentially the same as in darkness, but they are more rapid (the photokinetic effect). In an earlier paper (Zurzycki 1962b) the suggestion was advanced that the response to red light may have been associated with the photosynthetic production of ATP. The present investigation brings some details supporting this hypothesis: (a) solutions of inhibitors strongly inhibiting photosynthesis and with no (NH_2OH) or little (o-phenantroline) damaging influence on respiration abolish the specific effect of red light and make the rate of chloroplast displacements to be the same as in darkness in spite of irradiation with red light; (b) DNP, which inhibits oxidative phosphory-

ıation more strongly than photophosphorylation, blocks almost all chloroplast movements in darkness in concentrations as low as 6.10^{-5} M/l, though in red light chloroplast movements are still distinguishable when the concentration of DPN is twice as high; (c) when ATP is applied from outside to cells kept in darkness the speed of chloroplast displacements becomes similar to the speed of chloroplasts in strong red light.

In the case of the typical phototactic reactions (the reactions to strong light and to weak light) the equalization, if any, of the state of disequilibrium can exert any significant influence only in the early stage of displacements. In the later stages, when a new state of disequilibrium is building up, energy must be supplied from the respiration process and/or from the process of photosynthesis. The reaction to weak light is more sensitive to the action of the inhibitors used in this investigation than the reaction to strong light. A similar difference in sensivity was observed by Fetzer (1963) in *Mougeotia*. There may be various reasons of this difference.

(a) During the one hour period of adaptation an inhibitor may prove to be more harmful in strong light than in weak light. The evidence against this view is provided by that in the case of all the inhibitors in this investigation the reaction to weak light is always more sensitive and also by that after an adaptation period shortened to 30 or even 20 minutes the course of the displacements is unchanged as compared to one hour adaptation.

(b) In the reaction to strong light a certain part in the supply of energy may be played by ATP formed in the process of photophosphorylation, but any major significance of this source of energy is possible only when the rate of photosynthesis is high. The intensity of blue light used to obtain the weak light arrangement was very low, only 20 to 40 erg/cm^{2}sec, i.e. a few hundred times lower than the intensity corresponding to the compensation point. In this weak light intensity the rate of photosynthesis is negligeably low. In the present experiments the reaction to strong light was brought out by blue light of intensities approaching or surpassing the compensation point and under these conditions the additional influence of photosynthesis seems probable. On the other hand, the influence of a strong inhibition of photosynthesis on the movements in light of high intensity is not as strong as is the case with the reaction in red light (see Fig. 10). Moreover, irradation with additional red light during the reaction induced by blue light of high or medium intensity affects only the final stage of the reaction and not the rate of chloroplast displacements (Zurzycki 1964). All this evidence, though it does not exclude the possibility of photophosphorylation being an additional factor in the energy supply for phototactic displacements, seems to indicate that the difference between the reaction to weak and to strong light cannot be fully explained by the process of photosynthetic phosphorylation.

(c) Finally, there is the possibility that light acts directly on the respiration apparatus and that this action consists in the stimulation of the intensity of respiration (and of the output of energy) by strong light. The rate of gas exchange in hydroxylamine seems to indicate that in *Lemna* a substantial intensification of the respiration process in strong light actually takes place.

In the case of almost all the inhibitors applied in this investigation the complete inhibition of photosynthesis is associated with the abolition of the ability of the chloroplasts to assume phototactic arrangements. If the apparatus of the movements is not substantially injured by the inhibitor, then the chloroplasts tend to assume a uniform distribution (about 50% E), and if the injury to the apparatus of the movements is serious, no displacements of chloroplasts take place. An exception to this rule is provided by ATP, which blocks phototactic displacements while photosynthesis is still taking place. This may point to the very close relation of ATP with the mechanism of the movements. In the case of all the other inhibitors photosynthesis is essential for the phototactic arrangements to be attained, though only traces of this process — e.g. in very weak light or under very strong inhibitory action — are sufficient, even if the rate of photosynthesis is so low that it probably has no influence on the production of energy in any significant amounts. The supposition thus arises that photosynthesis is necessary primarily to give the chloroplasts a "sense" of the light conditions, i.e. it is an indispensable condition of the tonal stimulus (Haupt 1959). According to this approach the complete abolition of the reaction to weak light by hydroxylamine is the result of the inhibition of photosynthesis being so strong (weak light + inhibitor) that the chloroplasts are not capable to react to the light conditions, whereas in strong light (strong blue light or additional red light) the residual photosynthesis still going in spite of the action of the inhibitor allows the chloroplasts to "sense" the light gradients.

SUMMARY

The influence of various inhibitors of particular metabolic processes on the principal types of chloroplast displacements in *Lemna trisulca* was investigated. The inhibitors were sodium fluoride, o-phenantroline, hydroxylamine and 2,4 dinitrophenol. Moreover the investigation covered the displacements of chloroplasts in anaerobic conditions and under the influence of adenosine triphosphate.

It was found that energy derived from glycolysis had no influence on the chloroplast movements. The typical phototactic arrangements could neither be attained nor maintained after oxygen respiration had been cut off. The displacements in darkness were found to be connected with the energy derived from respiration. The photokinetic action of red light consisted in the photosynthetic formation of ATP. The action of red light could be suppressed by strong inhibition of photosynthesis and conversely, similar effects as those caused by strong red light could be induced in darkness by supplying ATP from outside.

So far as phototactic reactions were concerned the reaction to weak light was found to be more sensitive, in any given concentration of an inhibitor, than the reaction to strong light. This phenomonon may be explained by the additional action of ATP derived from photophosphorylation on the apparatus of the movements and/or by the stimulating effect strong light has on the respiratory system.

Phototactic movements stopped at the point at which photosynthesis was completely blocked, but even the smallest traces of photosynthesis were sufficient for the movements to take place.

The suggestion is advanced that the least traces of photosynthesis are the indispensable condition for the existence of the tonal stimuli, which allows the chloroplasts to respond to light intensities in the phototactic reactions.

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Energia ruchów chloroplastów u Lemna trisulca L.

STRESZCZENIE

Zbadano wpływ kilku inhibitorów, blokujących określone reakcje metaboliczne, na zasadnicze typy przemieszczeń chloroplastów u *Lemna trisulca*. Zastosowano następujące inhibitory: fluorek sodu, o-fenantrolinę, hydroksylaminę i 2,4 dwunitrofenol. Zbadano również przemieszczenia chloroplastów w warunkach beztlenowych i pod wpływem ATP.

Stwierdzono, że energia czerpana z glikolizy jest bez znaczenia dla ruchów chloroplastów. Typowe układy fototyktyczne nie mogą zostać osiągnięte ani utrzymane po wyłączeniu oddychania tlenowego. Przemieszczenia w ciemności związane są z energią czerpaną z oddychania tlenowego. Działanie fotokinetyczne światła czerwonego polega na fotosyntetycznej produkcji ATP. Efekt czerwieni można znieść przez silne zahamowanie fotosyntezy i na odwrót w ciemności można uzyskać podobne efekty jakie wywołuje silne światło czerwone przez doprowadzenie ATP z zewnątrz.

W obrębie reakcji fototaktycznych stwierdzono większą wrażliwość reakcji światła słabego w stosunku do reakcji światła silnego dla określonego stężenia inhibitora. Zjawisko to można tłumaczyć dodatkowym działaniem ATP pochodzącym z fotofosforylacji na aparat ruchowy lub (i) pobudzającym działaniem silnego światła na aparat oddechowy.

Przemieszczenia fototaktyczne ustają z chwilą całkowitego zablokowania fotosyntezy, ale do ich przebiegu wystarcza obecność bardzo małego natężenia tego procesu. Wysunięto przypuszczenie, że obecność choćby znikomej fotosyntezy jest warunkiem wystąpienia bodźców tonalnych umożliwiających chloroplastom reagowanie na natężenie światła w reakcjach fototaktycznych.