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The action spectrum for the light depended movements of chloroplasts in *Lemna trisulca* L.

by

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I. INTRODUCTION

The knowledge of the action spectrum is essential for the understanding of the mechanism underlying the chloroplast movements associated with light. However, the available information about the activeness of the particular regions of radiation in the movements of chloroplasts is in some respects contradictory.

Apart from the in a way exceptional phototactic chloroplast movements in some algae — e.g. *Mougeotia* or *Mesotaenium* — which are mainly induced by red light (Senn 1908, Haupt 1959b, Haupt & Thiele 1961), the short-wave part of the spectrum is generally regarded as the most active region of the visual radiation in the chloroplast movements of all plants. There is, however, some contradiction on this point between the results reported in the literature: whereas most authors (Faminzin 1867, Schmidt 1870, Senn 1908, Voerkel 1933, Zurzycka 1951, Babuskin 1955a) agree that only the short-wave radiation is active in the movements from the position of darkness to that of weak light (+ phototaxis), there is much disaccord about the action spectrum in the movements induced by strong light (— phototaxis). Earlier investigations seem to indicate that the action of red light though weaker than of blue light, is nevertheless different from the effects of darkness (Boehm 1859, Frank 1871). Senn (1908) believed that also in the latter case only the short-wave region was active and that the effect of red light was exactly the same as that of darkness. He found that the only exception here were some species with red-brown chromatophores (*Chromulina, Striatella, Neotia, Orobanche*) in which tactic movements were caused by red light.

On the ground of experiments with *Funaria* Voerkel reported that in negative phototaxis the main influence is exerted by long-wave UV radiation the part played by visual radiation being negligible or none. Linsbauer and Abramowicz found that both red and blue light was active on the movements brought out in *Lemna trisulca*

by strong irradiation. The activeness of the red radiation in this species was confirmed by Zurzycka (1951) whose observations indicated that the movements of chloroplasts in red light differed strikingly from those in darkness. A specific influence of red light was also observed by Zurzycki and Zurzycka (1951) in *Selaginella*. On the other hand, Babuskin (1955a) found that pure red radiation obtained by accurate filtration eliminating all infra-red rays was inactive in the chloroplast movements of tobacco leaves; he suggested that the effects observed by Zurzycka had been caused by an admixture of infra-red rays, since in his experiments such a composition of light induced chloroplast movements. Finally, Seybold (1956) demonstrated that the elimination of the short-wave rays (less than 470 mμ) from sun light completely cancelled the activeness of sun light in the movements of chloroplasts in *Begonia*, even though infra-red rays were not eliminated.

The fundamental qualitative differences in the results of the researches just reviewed do not allow us to form a clear picture of the spectral activeness of light. The question thus arises whether it is the spectral sensitivity in various species that differs so drastically or whether the assumption is false that the movements induced by strong light always consist in the same physiological processes (Haupt 1959a). In view of these different possible interpretations it was thought desirable to investigate the action spectrum in the movements of chloroplasts with special stress being laid on the long-wave region.

II. MATERIAL AND METHODS

The experiments were carried out on fronds of *Lemna trisulca* L. The plants were obtained from various natural stands in small, natural water reservoirs in the neighbourhood of Cracow. Each batch obtained from a homogeneous population was placed in an aquarium filled with tap water. Since most stands of *L. trisulca* have water rich in humus substance a piece of peat was placed in every aquarium (about one cu. decimetre to 5 litres of water). The aquaria were protected from direct sunlight. The experiments were made one to four weeks after the plants had been brought to the laboratory. All the plants in each of the experimental series came (unless otherwise stated) from the same population. In this way the obtained results, especially those concerning the absolute sensitivity, are comparable for each series of experiments in the particular parts of the work, but not when various series are considered. All the experiments were made during the summer months (June to September) of the years 1959 to 1961.

The fronds used for the experiments were young, vigorous, 3 to 5 millimetres long. They were placed in the usual way together with their maternal shoot in a drop of tap water on microscope slides. During the longlasting experiments (several hours) the slides were kept in a moist chamber.

Every leaf has a gradient of the different sensitivities of chloroplasts to light. The movements in the border cells and in the cells from the tip of a leaf are slower and less clearly marked, whereas the

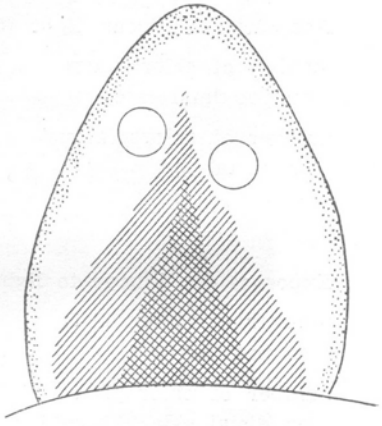

Fig. 1. Schematic diagram of a leaf of *Lemna trisulca* showing the particular zones. Dotted — border zone; lined — double layer mesophyll zone; cross-lined — multi-layer zone. The circles mark the areas where observations were made

mesophyll tissue from the centre and the basal part of a frond being composed of more than one layer of cells is unsuitable for accurate observations. The observations were thus made in the parts of leaves with a single layer of mesophyll cells near the axis of the leaflets and near the part with two or multi-layer mesophyll (Fig. 1). Owing to the presence of raphide cells it was possible to identify again the already examined cells and to repeate the observations in the same parts of a leaf, sometimes even in the same cells.

A new method, different from that in earlier researches, was devised for determining the chloroplast arrangements. It consisted in counting the chloroplasts separately on the side facing towards (*proximal*) and on the one facing away from (*distal*) the source of light. For this purpose the chloroplasts in the flat position were counted twice in every cell, first on the proximal and then, after readjusting the sharpness to a new plane, on the distal side of the cell (Fig. 2). The quantitative arrangement of chloroplasts was determined as the percentage of chloroplasts in the flat position (on the proximal and the distal sides of a cell) with regard to their total number; this was denoted as % *E*. Counts of the total number of chloroplasts were made in the same part of a leaf after its adaptation to weak light. The leaves were adapted to weak light before an experiment when the initial arrang-

ement of chloroplasts was to be the flat one — i .e. epistrophe — or after an experiment when other initial arrangements were needed. For adaptation the leaves were exposed to white light of 60 lx (40 W 220 V lamp at a distance of 120 cm.) during two hours. After this treatment practically all the chloroplasts moved from the side walls to the upper and under sides of a cell where they could be easily counted.

Here is an example of the counts:

Adaptation to weak light (full epistrophe):

number of chloroplasts on proximal side	12,	12,	10,	9,	12,	13,	13,	9,	9,	14—	112
number of chloroplasts on distal side	13,	9,	9,	14,	6,	10,	9,	5,	8,	9—	92
											204=100%

Exposure for 1 hour to light: 429 mμ, 610 erg/cm^2 sec.:

number of chloroplasts on proximal side	2,	1,	2,	2,	1,	3,	2,	4,	4,	3—	24
number of chloroplasts on distal side	6,	9,	8,	9,	6,	6,	5,	11,	6,	7—	73
											97=47.5%

In experiments on the influence of light the slides were irradiated on the microscope table through the illuminating system of the microscope. This procedure made possible the concentration of light giving very high intensities. However, since the beam of light thus obtained was not composed of parallel rays only, the question arose how this optical system affected the arrangement of chloroplasts. To answer this question three series of irradiations with white light (the spectral characteristic is given in Part IV) were carried out using a Lumipan microscope and a lighting system with 1) a 0.16 aperture (practically all the rays were then parallel), 2) a 0.8 aperture (this was used in all the other experiments), and 3) fully dispersed light obtained by introducing an opal plate in the place of the slide. As is to be seen from the curves in Fig. 3 illustrating the results of this experiment the arrangements of chloroplasts were almost identical in the case of the aperture of 0.16 and 0.8, but differed completely in diffuse light. In this last case in medium light intensities the chloroplasts were mainly grouped on the proximal side of the cells, though some of them stayed on the side walls, so that full epistrophe was not reached. In strong

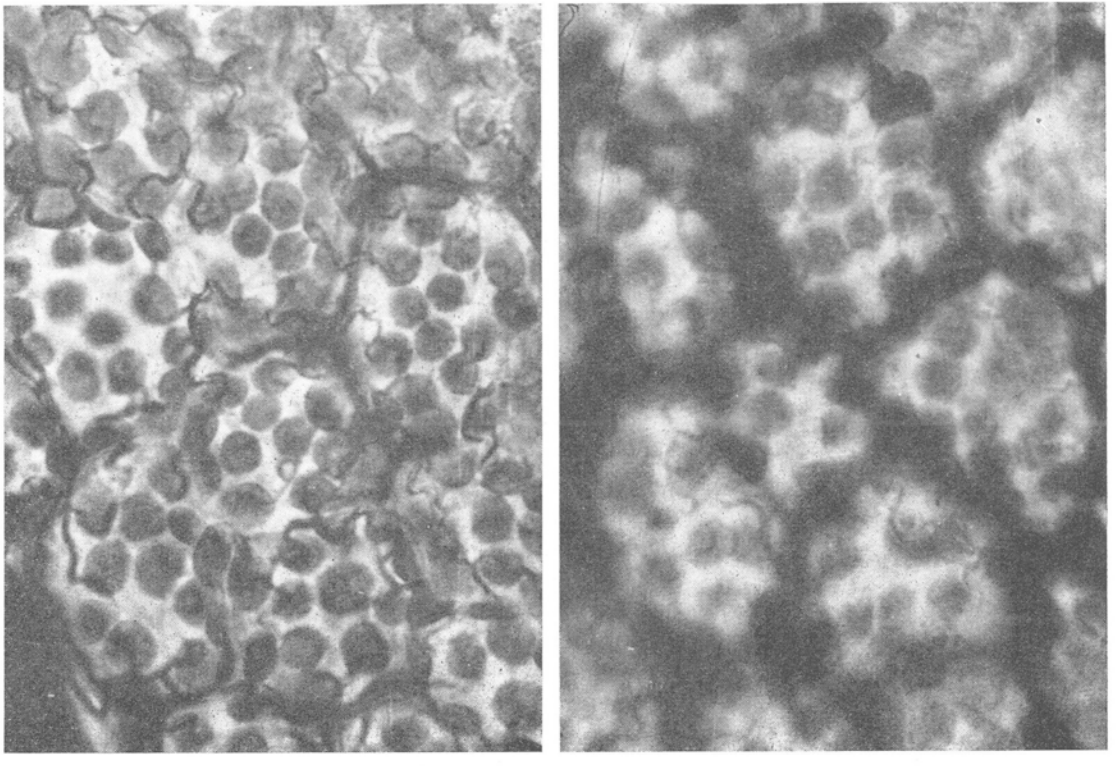

Fig. 2. Leaf cells of *Lemna trisulca* with chloroplasts on the proximal and distal cell walls

light the chloroplasts moved to the side walls, but some of them stayed on the distal side of the cell, in particular along the rim forming a ring of chloroplasts in the flat position. This state of things was caused by the specific distribution of the light intensity gradient within a cell exposed to diffuse light, since then the light on the side walls

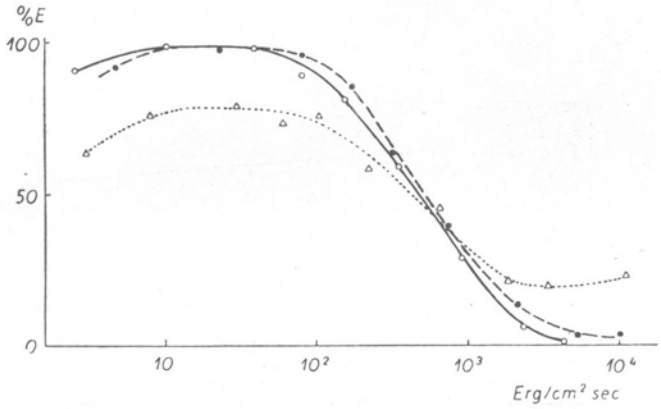

Fig. 3. The dependence of chloroplast arrangements (%E) on light intensities. Starting position: epistrophe. Irradiation time: one hour. Irradiation with white light using apertures 0.16 (—), 0.8 (- - -), and diffuse light (....). Each point represents the average of five measurements

was relatively stronger and only the central part of the distal side of the cell was very strongly lighted. The two curves corresponding to the aperture of 0.16 and 0.8 show that a change of the light aperture from 0 to 0.8 had no essential influence on the distribution of light intensity within a cell (see Zurzycki 1961a): irradiation through the microscope system gives results comparable with the results that would be obtained in a parallel beam of light.

Measurements of the absolute intensity of radiation were made with a micro-thermocouple of sensitivity 9.28 μV/mW and a Zeiss string galvanometer of internal resistance 5.5 Ω. The technical details of the measurements were described in an earlier report (Zurzycki 1961a). The intensity of UV radiation was measured with a thermopile (Kipp &Zonnen) of sensitivity 118 μV/mW equipped with a quartz window. In those few instances where the intensity of light was defined in luxes the photometric values were calculated from absolute measurements after establishing the erg/lx relation by gauging the light source with both a thermopile and a precise luxmeter.

The temperature in all experiments was 20—23°C.

III. THE ARRANGEMENT OF CHLOROPLASTS IN DARKNESS

The researches on chloroplast movements in *Lemna trisulca* continued for many years in this laboratory have shown that the movements taking place in darkness (the weak light → darkness reaction) are highly variable and very distinctly depend on the strain of plants as

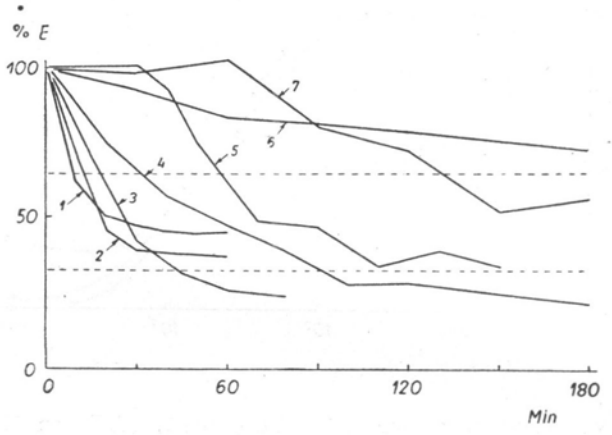

Fig. 4. The course of the chloroplast displacements in darkness. Initial position: epistrophe. x-axis — time in hours, y-axis — %*E*. Each curve illustrates the behaviour of a different strain. Curves *1, 2, 4* & *5* were plotted from counts on one side of the cell, curves *3, 6* & *7* according to the method described in text. The dashed line marks the dispersion of %*E* calculated theoretically for a uniform distribution of chloroplasts in the cell

well as on various difficult to define inner factors associated with the physiological state of the plant. The curves in Fig. 4 illustrate a wide range of examples of chloroplasts movements characterizing the variability of the course of this reaction. In spite of this wide variability the results obtained for one population were highly concordant.

In some kinds of shoots there is an interval, called the lag period, during which after the onset of darness the chloroplasts remain for some time in the flat position before their delayed movement begins (Fig. 4 curves 5 and 7). A similar lag period was reported in *Funaria* by Voerkel (1933, Fig. 10). In *Lemna* the movement of chloroplasts almost always begins immediately after the onset of darkness and already 5—10 minutes later the displacements are sufficient for numerical recording. The rate of the drop in the value of % *E* differs greatly in various experiments and does not seem to be connected with the occurrence or not of the lag period (see Fig. 4 curves 3, 5, 6 and 7). Contrary to what was reported by Stahl (1880) and Senn (1908)

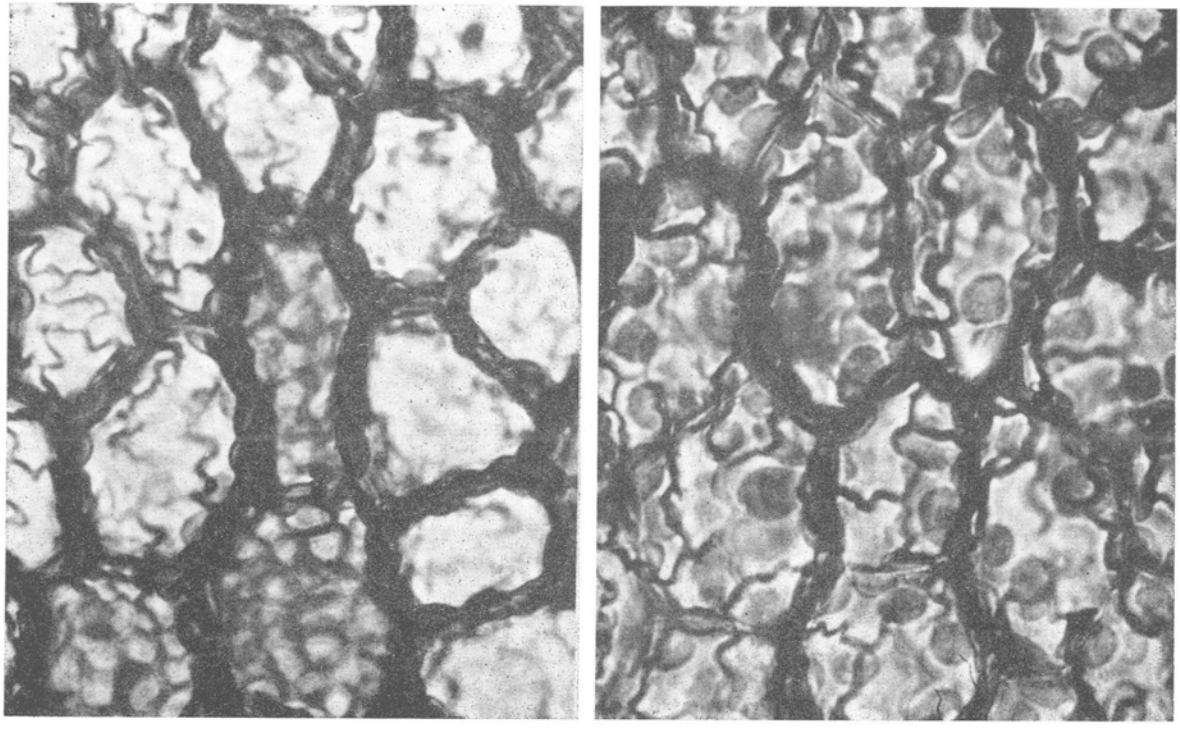

Fig. 5. The arrangement of chloroplasts in darkness. Left: classical apostrophe; all the chloroplasts are on the walls contacting with other cell walls in the mesophyll tissue. Right: the usual darkness arrangement; chloroplasts are distributed on all the cell walls

the movement of the chloroplasts does not cause the disappearance of them all from the outer walls of the mesophyll. The classical apostrophe described by those authors is quite exceptional, whereas the normal arrangement in darkness is the uniform distribution of the chloroplasts on all the cell walls so that the value of $\%\, E$ ranges 25 to 60 (Fig. 5).

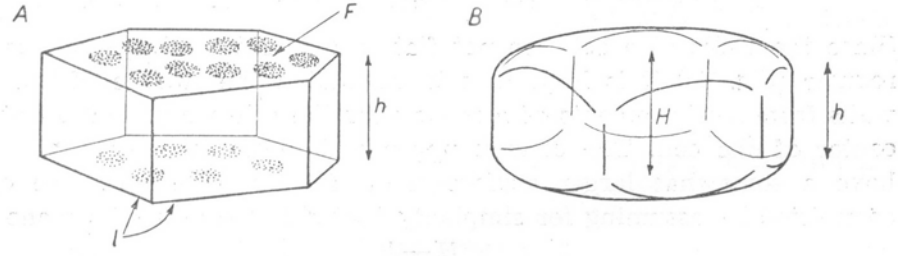

Fig. 6. A) Simplified diagram of a mesophyll cell: F — base of solid, l — circumference of base, h — height. All the chloroplasts in the flat position. B) More accurate diagram of cell: h — height of side walls, H — maximum height of cell

The time necessary to reach this state depends on the rate of the movements and may be only one hour in some cases and five or even ten hours in others. Once this state is reached it is retained for at least 24 hours of darkness (Fig. 4).

The question arising in connection with what has just been said is whether the "incomplete apostrophe" is caused by a tendency of the chloroplasts to avoid the outer mesophyll walls or by their uniform distribution on all the cell walls (what S e n n called peristrophe)? The answer to this question was sought by analysing the spacial relations within the cell. Let us imagine a simplified cell from the single layer mesophyll in the form of a solid with a hexagonal base (Fig. 6a) and let F be the area and l the circumference of the base, and h the height of the solid; let n stand for the total number of chloroplasts. If the distribution of the chloroplasts is uniform on all the walls, then their number on the upper and lower faces must decrease as compared to full epistrophe according to the ratio $\frac{2F}{2F+lh}$ and, therefore, $\%\, E$ for this chloroplast arrangement will be

$$X = \frac{100}{1+\frac{lh}{2F}}.$$

Thus, knowing the dimensions of a cell we can calculate the value of $\%\, E$ corresponding to the uniform distribution of the chloroplasts on all the walls of the cell.

The values of F and l were measured on drawings of cells made with an Abbe apparatus and the value of h was calculated by measuring with the micrometer screw of the microscope the height of the side cell walls at several points of the cell.

Here is an example of the results thus obtained:

$$F = 1490\,\mu^2,\ l = 144\,\mu,\ \text{mean}\ h = 26\,\mu,\ X = 44.5\%.$$

Since the walls of a cell are not flat and its actual shape is thus not regular (Fig. 6b) it is important to calculated the surface of the side walls from measurements of h made near the side walls and not at the center of the cell. The convex upper and lower faces of a cell also have a somewhat larger surface than if they were flat. The error committed by assuming for simplicity that F is the area of a plane and not of a convexity of the height $\frac{H-h}{2}$ is not greater than three to six per cent, which has no practical significance for the final result.

Table 1 lists the values of X (i.e. of $\%\,E$ calculated on the assumption that the distribution of chloroplasts on all the cell walls is uniform) and the actual values of $\%\,E$ counted after one and after three hours of darkness in six different populations of *L. trisulca* from various stands.

As is to be seen in the table the distribution of the chloroplasts on all the cell walls is uniform when the values of $\%\,E$ range from 35 to 55 and when the numbers of chloroplasts on the upper and the lower walls are equal. This arrangement indicates that within a cell there is no taxis or gradient that could cause the groupping of chloroplasts. In the majority of cases the actual arrangement of chloroplasts was within the limits established for a uniform distribution (see Fig. 4). Only in a few cases $\%\,E$ in darkness was less than 30 which meant that the chloroplasts manifested a preference to group by the side cell walls.

Table 1

Theoretically uniform distribution and actual distribution of chloroplasts in *Lemna* cells

Material from	Calculated % E		Actual % E	
	Mean	Extremal values	After 1 h.	After 5 h.
Botanical Garden	42.1	35.6—45.0	69.5	52.5
Mydlniki	38.8	32.8—52.0	55.8	54.5
Koło Tynieckie	53.4	39.0—64.0	56.5	48.2
Szarow	41.1	38.5—44.5	72.2	66.3
Płaszow	46.1	42.2—50.2	41.0	22.3

It is to be expected theoretically that the value of $\%\,E$ would be higher in the flat cell near the leaf margins and lower in the thicker ones near the center of a leaf. Moreover, it is necessary to stress that

the calculations made above are correct only when in full epistrophe all the chloroplasts are in the flat position and none of them stay on the side walls. If the chloroplasts in a cell are so numerous that in favourable light conditions there is not enough room for them on the upper and lower walls, as often is the case in autumn leaves, then the value of $\%_0 E$ for a uniform distribution is much higher than it would result from the size of the cell. This may be one of the reasons why in autumn leaves the movements taking place in darkness are not very well marked.

IV. THE INFLUENCE OF WIDE SPECTRAL REGIONS ON THE CHLOROPLAST ARRANGEMENTS

Methods. The irradiation was with rays from five spectral bands in the visual and the neighbouring regions. The characteristics of the spectral regions and the details of the filter systems used to isolate the necessary radiation are assembled in Table 2. Fig. 7 illustrates the relative transmittance of the filters. The source of radiation in regions I and II was a mercury Hanau Q 700 lamp

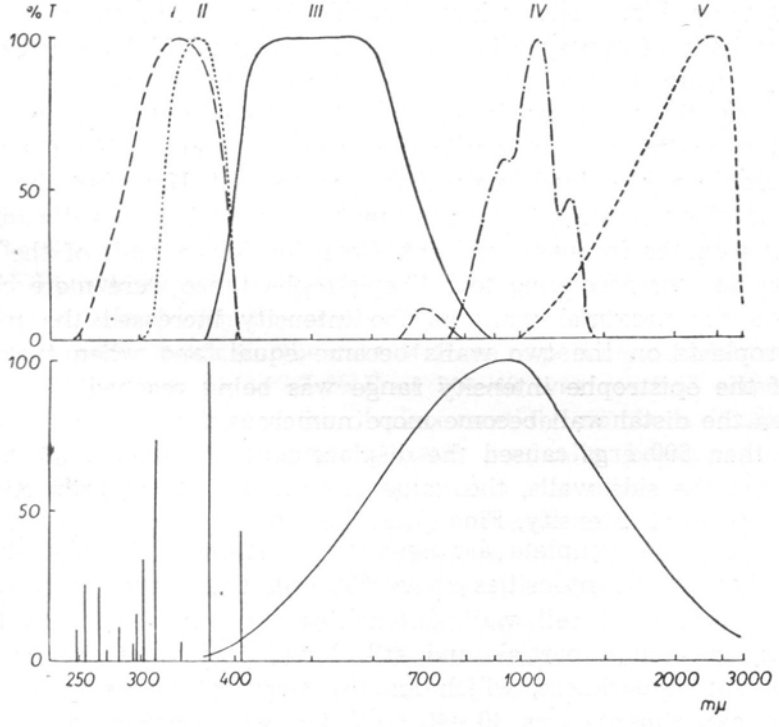

Fig. 7. The relative transmittance of the system of filters listed in Table 2 (top) and the relative spectral emission of the sources of radiation: the Q 700 mercury lamp (according to the maker's specification) and the low-voltage lamp for colour temperature 3000°K (bottom)

Table 2

Optical characteristic of a applied wide spectral regions

No.	Definition	Range in mμ	Filtres
I	Wide UV	250—400	UG 11—2 mm.
II	Near UV	300—400	UG 11—2 mm. + glass 3 mm. + $CuSO_4$ 100 g/l 1 cm.
III	Visible	370—850	GG 18—4 mm. + KG 1—5mm.
IV	Near IR	750—1400	UG 7—2 mm. + H_2O 1 cm.
V	Far IR	1200—3000	UG 7—2 mm. + BG 23—2 mm.

and in regions III—V a 6V15W low-voltage lamp. The spectral analysis of the radiation emitted by these lamps is shown in Fig. 7 (bottom curve). For UV irradiation the slides were covered with a quartz plate and placed in a chamber with the needed filters fitted in its window; no convergent optical system was applied in this case. In the other spectral regions a Lumipan microscope (aperture 0.8) and the necessary combination of filters introduced into the optical system were used. Differences in the intensity of irradiation were obtained by introducing Schott's neutral filters (series NG) in the case of the spectral region III and blackened copper wire grids in the other case.

Results. Fig. 8 shows how the chloroplast arrangement obtained after one hour of exposure to visual light (region III) depended on the intensity of irradiation. The initial arrangement of chloroplasts was obtained by the preliminary exposure to white light of 60 lx. When exposed to white light of low intensity (50—600 erg = 10—150 lx) all the chloroplasts remained in the flat arrangement. However, the distribution of chloroplasts on the proximal and distal cell walls differed depending on the intensity of light. Near the lower limit of the range of intensities corresponding to full epistrophe there were more chloroplasts on the proximal wall; as the intensity increased the number of chloroplasts on the two walls became equal, and when the upper limit of the epistrophe intensity range was being reached the chloroplasts on the distal wall become more numerous. Irradiation intensities greater than 600 ergs caused the displacement of some of the chloroplasts onto the side walls, the value of $\%E$ decreasing proportionally to the increase of intensity. Finally, at the intensity of about 50 000 ergs (= 10 000 lx) the complete arrangement of strong light (parastrophe) was reached. In all intensities above 600 ergs there were more chloroplasts on the distal cell wall. Intensities ranging 50—8 ergs (about 10—2 lx) induced a partial, and still lower intensities the complete arrangement of darkness, which for the strain of plants used in this series of experiments was 30—40 $\%E$ and was reached in one hour. A characteristic trait of the darkness arrangement was the equal number of chloroplasts on the proximal and the distal walls of the mesophyll cells.

The results obtained with the darkness and the strong light arrangements as the starting positions were in both cases the same. These experiments were carried out in only a few light intensities and as the results were highly conformable the complete curves were not

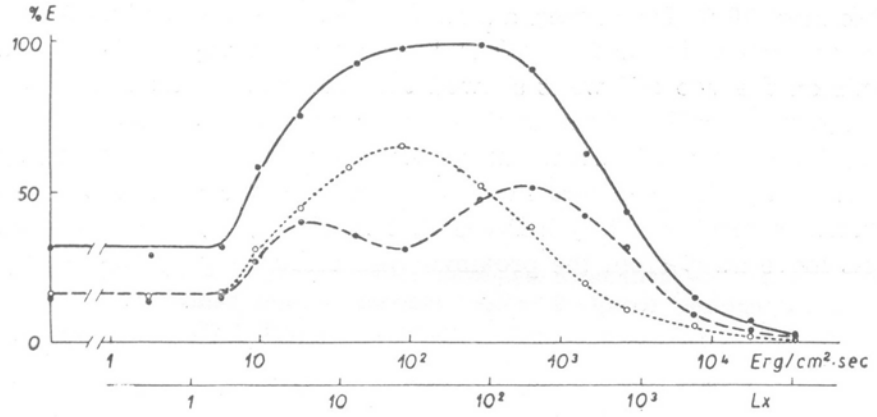

Fig. 8. The dependence of the chloroplast arrangements (y-axis — %E) on light intensity (x-axis). Initial position: epistrophe. Time of irradiation: one hour. Every point represents the average of five recordings. Solid line — total per cent of chloroplasts in flat position: dashed line — per cent of chloroplasts on distal side; dotted line — per cent of chloroplasts on proximal side

plotted. This confirms the earlier observations (Zurzycka & Zurzycki 1954) that the preliminary irradiation has no influence on the final effect of exposure to white light.

Fig. 9 illustrates the typical time displacements of chloroplasts induced by white light of different intensities and by darkness. The placing of a leaf with the chloroplasts in the flat position in darkness caused a rather quick (in the case of these experimental plants) displacement so that the value of %E of 30—40 was reached already after one hour. In the course of the displacement the numbers of chloroplasts on the proximal and the distal wall gradually became equal (Fig. 9 — 1). The exposure to strong light of a leaf adapted to weak light (Fig. 9—2) stimulated very rapid displacements ending by the complete strong light arrangement. The movement of chloroplasts from both the proximal and the distal wall to the side walls began immediately, but was more rapid from the proximal wall. When a leaf, in which the arrangement of strong light had been induced, was exposed to weak light the chloroplasts quickly returned to the flat arrangement and the displacement was completed within 30—40 minutes. In the first stage of this displacement the chloroplasts began to move to the wall, which was distal in strong light, regardless of the direction of the actual source of light (Fig. 9—3 & 4); in the later stage they were regroupped

to assume the arrangement characteristic for the given intensity and direction of weak light. The response was essentially the same when a leaf first exposed to strong light was placed in darkness (Fig. 9—*5* & *6*). At first there was a rather rapid return to the flat arrangement attaining at the most 70 % *E* and then a slower drop of the value of % *E* to the level characteristic for the darkness arrangement. Separate chloroplast counts on the two cell walls showed that the movements were quicker and the well marked transient maximum of % *E* was reached sooner on that wall of a cell which during exposure to strong light had been the distal one. Two factors may be responsible for such a behaviour. S e n n's researches (1908) indicate that in strong light all the chloroplasts move away from the proximal wall assembling by the boundary

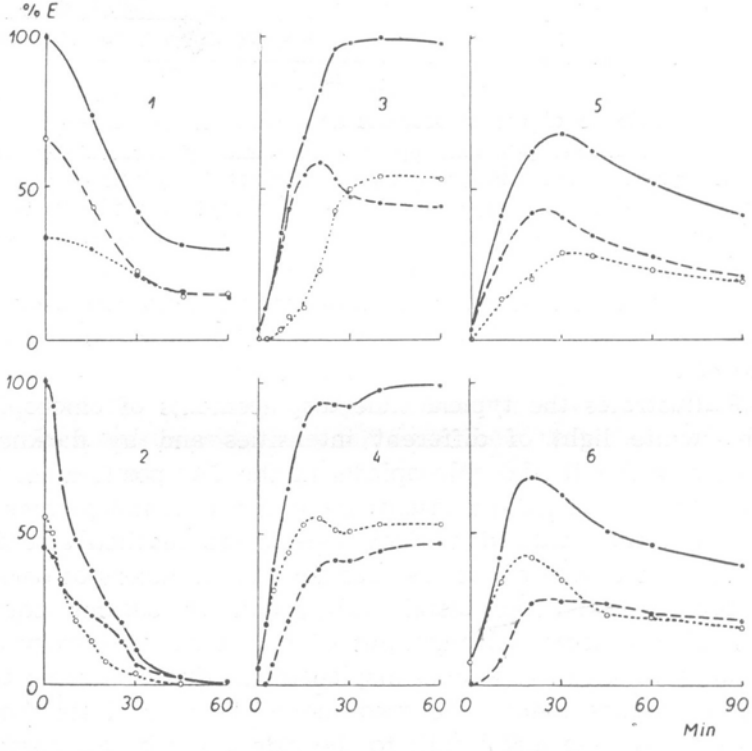

Fig. 9. The time course of the chloroplast displacements. The denotations are the same as in Fig. 8. The initial position in graphs *1* and *2* was epistrophe: graph *1* — displacements in darkness; graph *2* — displacements induced by strong light (10 000 lx). The initial position in graphs *3* and *4* was parastrophe, intensity of illumination 60 lx: former exposure to strong light was either from the opposite direction than to weak light (graph *3*) or from the same as to weak light (graph *4*). Graphs *5* and *6* show displacements in darkness the initial position being parastrophe; the denotations are the same as for the graphs *3* and *4*

of the side walls with the distal wall and partly trespassing onto the distal wall; owing to this arrangement when they return to the flat position their path to the distal wall is shorter and easier. Another

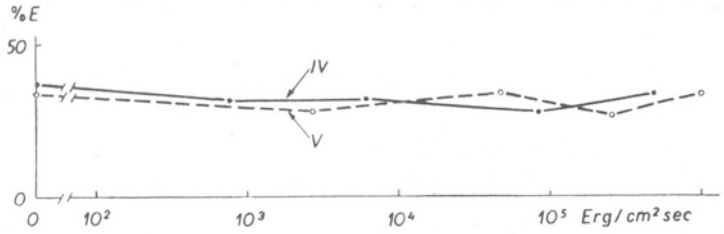

Fig. 10. Chloroplast arrangements after infra-red irradiation: solid line — spectral region IV; dashed line — spectral region V. Initial position: epistrophe. Time of irradiation: one hour

possibility (though both factors may act simultaneously) is that strong light has a specific effect on the proximal cell wall making difficult the return of the chloroplasts to this most strongly and directly irradiated wall of the cell. In view of M o u r a v i e f f's recent experiments

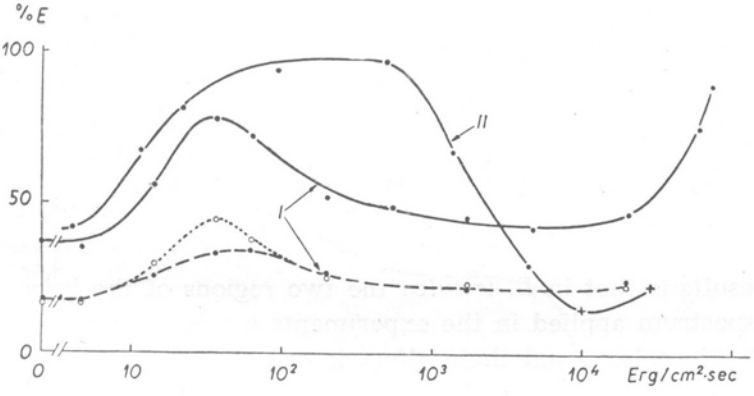

Fig. 11. Chloroplast arrangements obtained after UV irradiation. Solid line I — spectral range I; solid line II — spectral range II. Other denotations as in Fig. 8. Initial position: epistrophe. Time of irradiation: one hour

(1960), in which he demonstrated that the endoplasm moved away from the cell walls exposed to the strongest light, the latter explanation seems highly probable.

The irradiation of a leaf previously adapted to weak light with infra-red radiation (spectral regions IV and V) ranging 10^3 to 10^6 ergs gave the same effect as darkness (Fig. 10). Similarly, there were no

differences between the time course of displacements in cells kept in darkness and in cells exposed to infra-red radiation (see Part VI of this report). In leaves previously adapted to darkness, i.e. when apostrophe was the starting position, infra-red radiation induced no significant rearrangement of the chloroplasts. The conclusion to be drawn from

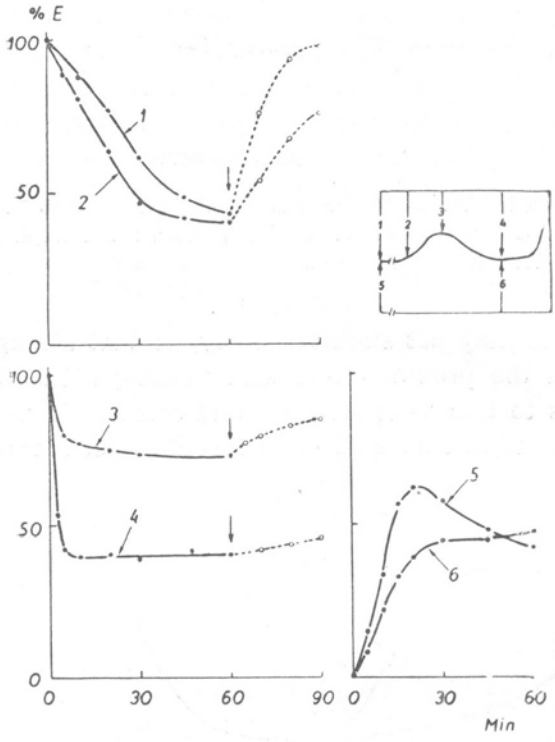

Fig. 12. The course of chloroplast displacements induced by UV irradiation of spectral region I. Initial position for curves *1*, *2*, *3*, and *4* was epistrophe, and for curves *5* and *6* parastrophe. The top right-hand corner graph illustrates the applied intensities (cf. Fig. 11). The dotted lines show the behaviour of chloroplasts in white light of 60 lx

these results is that in *L. trisulca* the two regions of the infra-red part of the spectrum applied in the experiments are inactive in the movements of chloroplasts and their effect is exactly the same as the effect of darkness.

Radiation of the near UV region, composed of hardly any other than the 366 mμ band, had the analogical effect as white or blue light (Fig. 11); up to a certain intensity of the radiation the chloroplast arrangement was epistrophe and higher intensities induced the movement of chloroplasts to the side cell-walls. Full parastrophe was never attained because the high intensities caused the aggregation of chloroplasts; aggregation was also induced by white light but not till very high intensities were applied (Zurzycki 1957).

Radiation from the region I comprised besides the near UV also

the biologically very active rays of wave lengths 250—300 mμ. The exposure of leaves to radiation of this composition led to very characteristic effects (Fig. 11 & 12): when the intensities were very weak the chloroplasts assumed the typical arrangement of darkness, but even in the intensity as low as 4,6 ergs the time necessary for the chloroplasts to assume the darkness arrangement was shorter than in the controls and after one hour of this irradiation the return to the flat position in white light was much slower. In higher intensities there was a tendency to maintain epistrophe (the value of % E increased, there were more chloroplasts on the proximal wall). The value of % E reached the maximum (80%) when the radiation intensity was about 30 ergs and dropped again in higher intensities. In the intensity range of 100 to 20 000 ergs the value of % E remained about 45 the chloroplasts being evenly distributed on the proximal and the distal wall. This state was reached after 5—10 minutes of irradiation, a very short time indeed, and after one hour the chloroplasts were no longer capable of returning in the usual way to the position of weak light. Finally, in intensities above 30 000 ergs the chloroplasts were stopped on their starting positions, whence the increase of % E. Analogical results were obtained when the starting position was that of strong light (Fig. 13), the only difference being that the inhibition of all displacements already

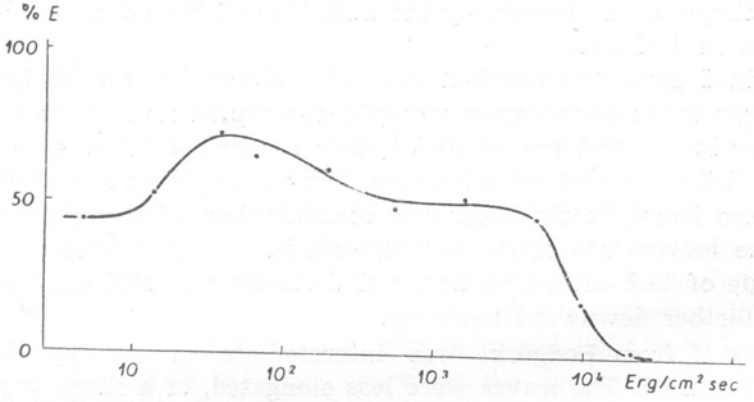

Fig. 13. Chloroplast arrangements after UV irradiation of spectral region I. Initial position: parastrophe. Irradiation time: one hour

began at the intensity of about 10 000 ergs. These observations are very similar to what was reported by Voerkel (1933), but a detailed comparison is impossible since that worker measured irradiation intensities in relative values only.

V. THE INFLUENCE OF RED LIGHT

Methods. The techniques of irradiation were the same as the experimental procedure described in Part VI. The red light was obtained from a 672 mμ interference filter and the control observations in blue light were carried out using a 485 mμ filter.

Results. In the preliminary experiments it was found that, whereas the chloroplast arrangements obtained in blue light were analogical to those in white light (see Part IV), irradiation with red light never led to full epistrophe, regardless of the applied intensity of irradiation. Moreover, in the applied intensities (up to 50 000 ergs) of the red light the arrangement of strong light was never fully reached. Nevertheless, the arrangements obtained in response to red light were in most cases dependent on the intensity the value of $\%_0 E$ being higher in the range of weak intensities. However, this effect was not always reproducible and in some experimental series no significant differences in the value of $\%_0 E$ after one hour of irradiation were apparent over the whole range of the applied intensities of red light.

In view of these discrepancies so strongly marked in the preliminary experiments as well as of the analogies between the effect of red light and darkness mentioned in the literature and the considerable variability of the displacements taking place in darkness (see Part III) it was decided to examine the influence of red light on the behaviour of three different strains of *L. trisulca*. The strains differed from each other by the shape of the fronds, by the structure of the cells, and by their reactions in darkness.

Strain I from Szarów had distinctly elongated, thin pale leaves. The single layer assimilating mesophyll occupied nearly the whole of the older leaves and the whole of the younger leaves. The cells were large and the chloroplasts numerous, but owing to the size of the cells they were loosely distributed; the concentration of the pigments was low. The movements of the chloroplasts in darkness were very slow: the value of $\%_0 E$ after five hours of darkness was still only about 75 with a further downward tendency.

Strain II from Podgórki was intermediate in its type between strains I and III. The leaves were less elongated, of a shape typical for the majority of *L. trisulca* populations. The part of the leaves with the single layer mesophyll was smaller. The cells were of medium size and the concentration of pigments in the chloroplasts was higher than in strain I. The movements of chloroplasts in darkness were relatively slow: the value of $\%_0 E$ after five hours was about 50 with a further, very slow downward tendency (ater 12 hours $\%_0 E$ was 40—45).

Strain III from Podolany was characterized by very short leaves with a very narrow marginal zone of single layer mesophyll. The cells

were relatively small with numerous, dark green chloroplasts. The displacements in darkness were rather rapid: after one hour $\%E$ was about 50 dropping to about 35 during the next few hours.

The fronds of these three strains are illustrated in Fig. 14 and the chloroplast arrangements after one and after five hours of irradiation with red light of different intensities are shown in Fig. 15. The diffe-

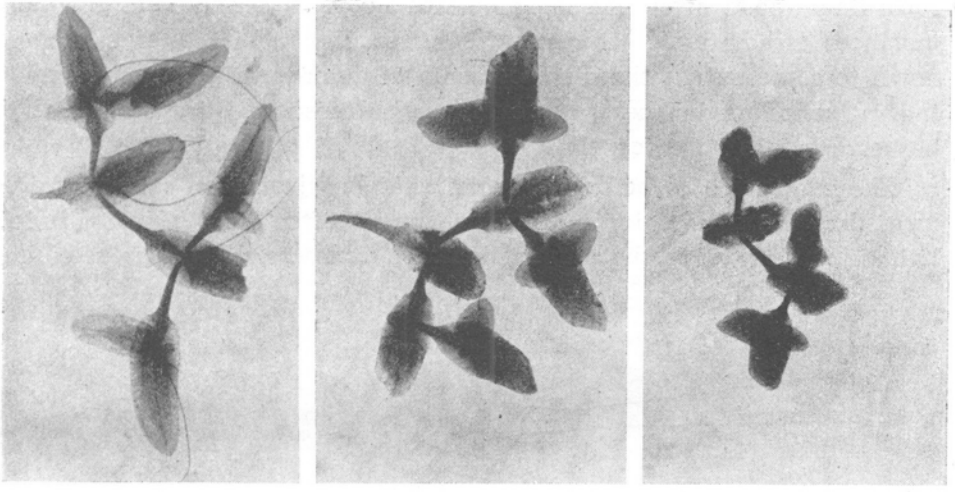

Fig. 14. Fronds of the three strains used for experiments with red light. Left — type I from Szarów; centre — type II from Podgórki; right — type III from Podolany

rences in the shape of these curves may serve as an example of the discrepancies of the results obtained in the preliminary experiments. The relation between the intensity of light and the arrangements of chloroplasts is manifested by both curves plotted for strain I. A similar relation is shown in the case of strain II after one hour of irradiation but not after five hours. Finally, no distinct relation between the chloroplast arrangements and the light intensity is shown by either of the two curves for strain III. The explanation of this apparent difference in the behaviour of the particular strains in red light is to be looked for in the analysis of the time course of the displacements (Fig. 16). In strain I, where the displacements towards the darkness arrangement were very slow, red light of high intensities distinctly accelerates this process, which is shown by the downward trend of the $\%E$ curves plotted after one hour and after five hours of irradiation (Fig. 15). In the leaves of strain II red light of high intensities also accelerates the movements of chloroplasts, but their final arrangement is the same as in darkness; this arrangement not being reached till after five hours of darkness, the curve showing how the chloroplast arrangement

depends on the intensity of red light displays this relation only in experiments lasting one hour. In strain III the darkness arrangement is reached already after one hour, but since also in this strain red light accelerates the movements of the chloroplasts towards the arrangement of darkness, reducing the time in which it is reached to about half an hour, the acceleration is not reflected by the curves plotted from recordings made after one hour or five hours of irradiation. In many instances irradiation with very weak red light (5—100 ergs) slowed down to some extent the displacements of chloroplasts, as compared to the influence of darkness, however, the differences were too small to be regarded as significant.

The conclusion to be drawn from what has just been said is, therefore, that the final chloroplast arrangement after irradiation with red light is analogical to the arrangement in darkness. However, red light

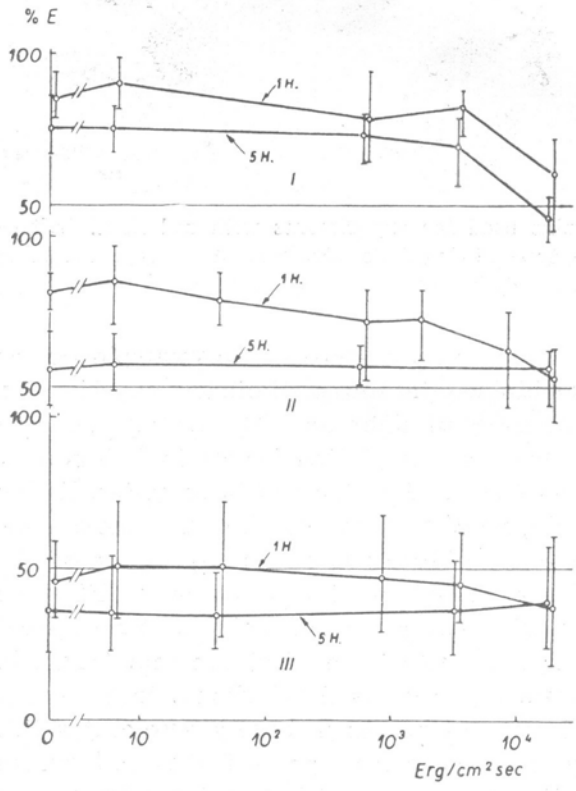

Fig. 15. The dependence of chloroplast arrangements on the intensity of red light ($\lambda = 672$ mμ) after one and five hours of irradiation. Every point represents the average from 10—12 measurements. The vertical lines mark the extreme dispersions

is not inactive: having no significant influence on the phototactic arrangement it clearly affects the kinetics of the movements: it strongly accelerates them in the high intensity range and perhaps decelarates them in the low intensity range.

The proportion of chloroplasts on the distal and proximal cell-walls after irradiation with red light manifested much greater discrepancies than was the case in darkness. Usually, the number of chloroplasts on these two walls was more or less the same, but often, specially in

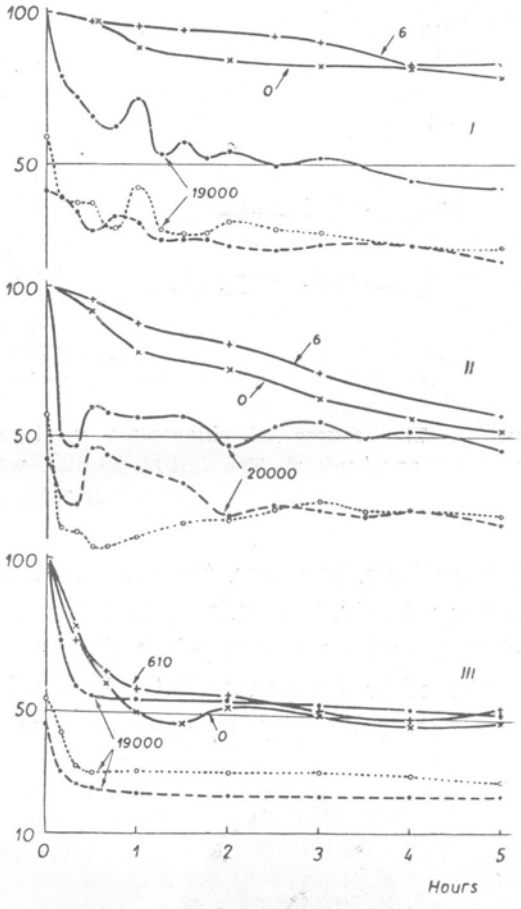

Fig. 16. The course of chloroplast displacements in darkness and in red light ($\lambda = 672$ mμ) in the three experimental strains. The figures mark the light intensities in ergs/cm²sec. The differences between the proximal and the distal sides of the cells are shown in the case of the highest irradiation intensity

the high intensity range, the chloroplasts were very distinctly grouped on one of the walls, either the distal or the proximal according to the intensity of light; these concentrations of chloroplasts could either gradually disappear (Fig. 16 — II) or persisted for a long time (Fig. 16 — III). The selectivity of the applied filters being very high this effect cannot be attributed to an addition of short-wave radiation. An explanation of this effect, which was not necessarily caused by the tactic influence of light, will be suggested in the light of the results reported in Part VII.

Irradiation with red light, when the starting position had been that of darkness or of strong light, gave results confirming the non-tactic

influence of long-wave radiation. When chloroplasts in apostrophe were irradiated with red light their displacements were insignificant, which was reflected by only slight oscillations of $\%E$ and sometimes by a greater number of chloroplasts on the distal or the proximal walls;

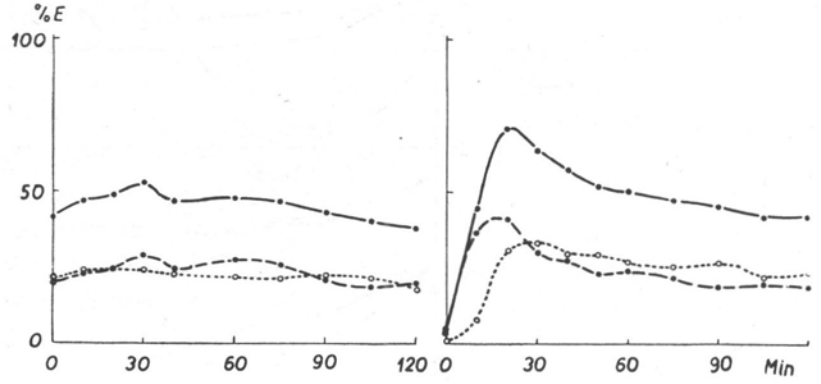

Fig. 17. The course of chloroplast displacements in red light ($\lambda = 672$ mμ, $I = 18\,000$ ergs/cm²sec). The initial position were darkness (left) and parastrophe (right)

on the whole, however, the arrangements of the chloroplasts remained unchanged (Fig. 17). Chloroplasts in the position of strong light irradiated with red light behaved in the same way as in darkness (Fig. 17).

The other differences between the influence of red and blue light were manifested by the spacial distribution of this influence. To

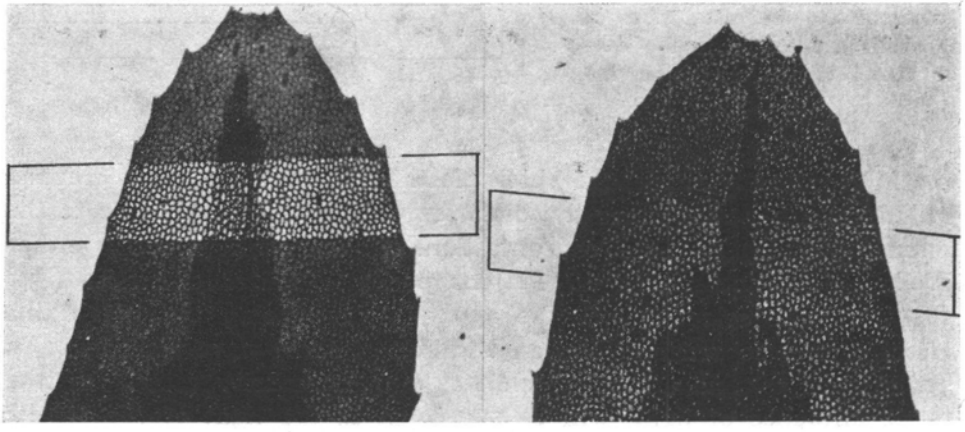

Fig. 18. The effects of local irradiation with light from a narrow slit. Left: $\lambda = 485$ mμ, $I = 1350$ ergs/cm²sec; right: $\lambda = 672$ mμ, $I = 21\,000$ ergs/cm²sec. Initial position: epistrophe. Time of irradiation: one hour

demonstrate this difference a very narrow beam of light was used for irradiation. For this purpose a leaf was placed directly over the slit of a diaphragm attached to the upper face of the slide. Blue light applied in this way caused chloroplast displacements only in the irradiated part of the leaf (Fig. 18 — *1*). The limitation of the displacements was so strict that in the strongly lit part of a cell some chloroplasts could be in the profile position while others in the shaded part of the same cell were in the flat position. A similar difference in the displacement of chloroplasts within one cell was observed by Borodin (1869) and according to Virgin (1951) the influence of light on the response of chloroplasts to centrifugation was analogical. On the other hand the irradiation through a slit with red light sufficiently strong to accelerate chloroplast displacements was much less restricted to the irradiated part of the leaf. The zone of action had no strict boundary and the accelerating influence seemed to spread to the neighbouring unlit parts of the tissue, in particular towards the leaf base (Fig. 18 — *2*).

VI. THE ACTION SPECTRUM

Methods. To examine the effects of irradiation and the time course of the displacements the monochromator with interference filters described in an earlier report (Zurzycki 1961a) was used. With this equipment it was possible to irradiate simultaneously ten preparations using different wave lengths. Single linear interference filters with the following maxima were used in the experiments: 362, 382, 400, 429, 442, 452, 470, 485, 504, 523, 541, 558, 622, 682, 715, and 787. The transmission curves of these filters accounting for the residual light are shown in Fig. 19. The curves were plotted from measurements made with a Uvispek spectro-photometer (Hilger). As the source of light 100 W or 750 W, 110 V projection lamps were used.

In view of the observed qualitative differences in the response to short-wave and long-wave radiation different methods were applied for defining the action spectrum in phototaxis and in photokinesis.

Within the short-wave region the measure of the activeness of light was assumed to be that intensity of light which caused the displacement of half of the chloroplasts. In the reaction darkness arrengement → weak light arrangement leaves kept in darkness for 12 hours were exposed to irradiation for one hour and then the chloroplast arrangement was recorded. The average values of % *E*, calculated from five or six repetitions for each intensity, were plotted on graphs (Fig. 21). In the experimental material the position of darkness was about 50% *E* whereas the position of weak light was 100% *E*: the light intensity, determined by interpolation on the graphs, in which the value of % *E* would be 75 was assumed to be measure of the activeness of light for the apostrophe → epistrophe reaction. Similarly, in the epistrophe → parastrophe reaction, in which the starting position was 100% *E* and the final one 0% *E*, the measure of the activeness of light was the intensity giving 50% *E*. In both instances the mean errors and the extreme dispersions of the values of the thus conceived measure of activeness were calculated graphically from the points of intersection of the

75% line or the 50% line with the lines joinig the limits of the mean errors or of the extreme dispersion for the particular measurements (Fig. 21).

The nature of the effect that the long-wave part of the spectrum has on chloroplast movements made the speed with which the displacements took place, and not the final arrangement, the significant factor in determining the activeness of this spectral region. From the recordings of the time course of the reactions in different intensities of various wave lengths determinations were made of the reaction time i.e. the time necessary to achieve half of the

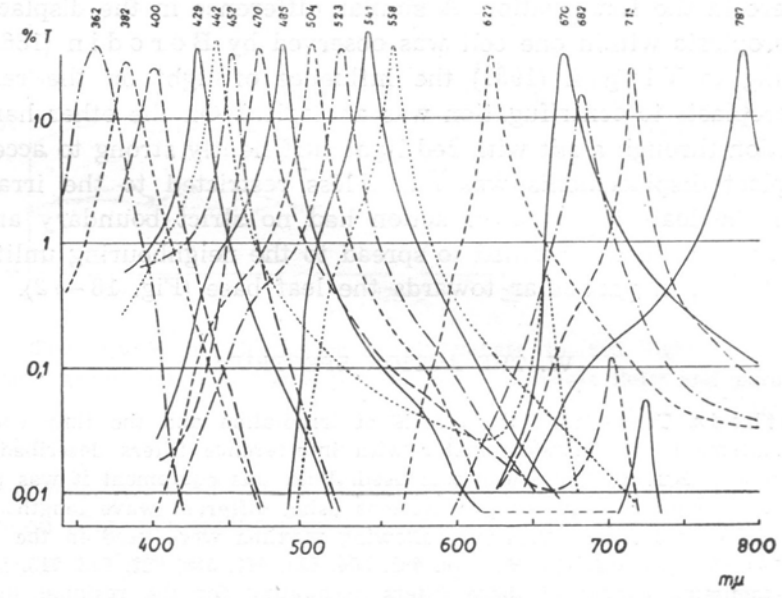

Fig. 19. Transmittance curves for the interference filters used for the experiments. Figures at the top mark λ max.

displacement, which in this case was 75 %E. The reaction time was established for every intensity as the average of five or six repetitions. The mean reaction times were plotted against the intensity; the curves thus obtained are shown in Fig. 24. As is to be seen from the curves the reaction time in weak intensities of light was about 60 minutes and in high light intensities it dropped to the minimum of about 10 minutes. The measure of the kinetic activeness of light has been assumed to be the light intensity in which the acceleration of the reaction equaled half of the maximum acceleration, i.e. the intensity in which 75 %E was obtained during 35 minutes $\left(\frac{60+10}{2}\right)$. This intensity was read off the curves by interpolation.

Results. The curves in Fig. 20 supply examples of the chloroplast displacements from the initial arrangements of darkness and of weak light for various intensities of light of wave-lengths 452 and 523 mμ. As is to be seen the stable arrangement of chloroplasts, which depended on the light intensity and the wave-length, was in all cases reached

during less than one hour and subsequently did not change significantly in spite of further irradiation. Because of this in later experiments on the action spectrum of phototaxis the time of irradiation was always one hour. Only in very high intensities of short-wave radiation the regroupment of chloroplasts sometimes lasted more than one hour (Fig. 20). In these very high intensities, however, the arrangement assumed by the chloroplasts was not typical (aggregation) and was not studied in detail in this work. Fig. 21 shows how in one of the wave-lengths the arrangement of chloroplasts depended on the intensity and also records the extreme dispersions and the mean errors of the results; all the curves obtained in this way, though without the particular measurements and their dispersions omitted to make the graph more readable, are assembled in Fig. 22. As is to be seen the most

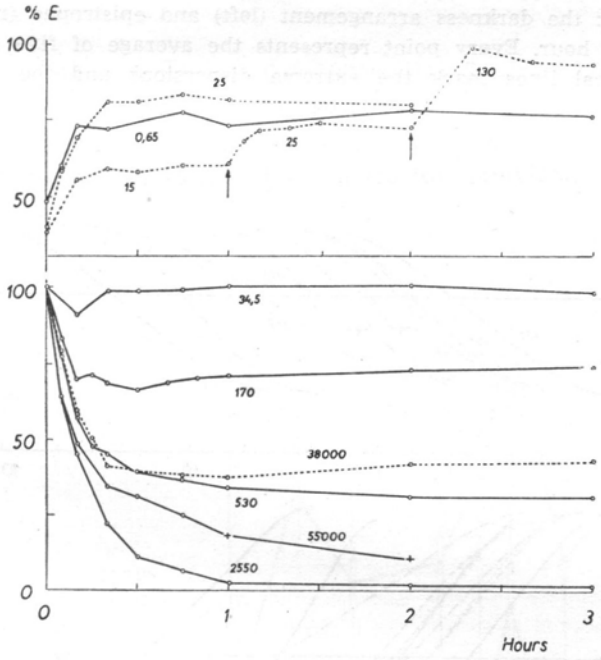

Fig. 20. The displacements of chloroplasts from the arrangement of darkness (above) and from epistrophe (below) as the starting positions. Solid lines show the course of displacements for $\lambda = 452$ and dashed lines for $\lambda = 523$ mμ. The figures by the curves indicate the light intensities in ergs/cm²/sec, + — beginning of aggregation

active in phototaxis were the rays of wave length 452 mμ. In the apostrophe — epistrophe reaction the influence of this wave length was noticeable already when the intensity was 0.1 erg. This means that the absolute sensitivity in *Lemna* was much greater than the sensitivity reported by V o e r k e l for *Funaria* where a response was first

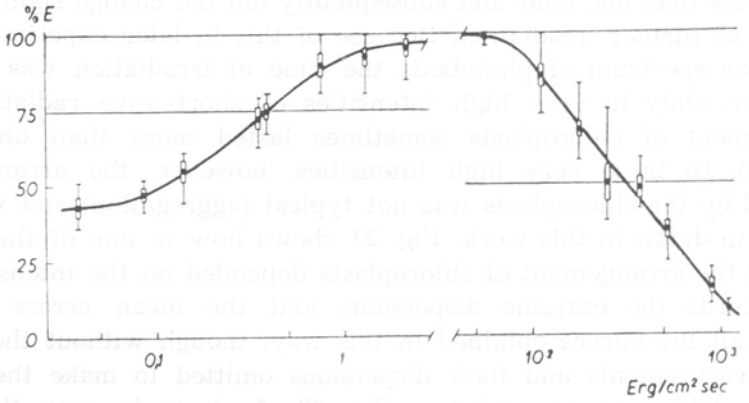

Fig. 21. Dependence of chloroplast arrangements on light intensity for $\lambda = 452$ mμ. Initial positions: the darkness arrangement (left) and epistrophe (right). Time of irradiation: one hour. Every point represents the average of five or six recordings; the vertical lines mark the extreme dispersions and the rectangles the mean errors

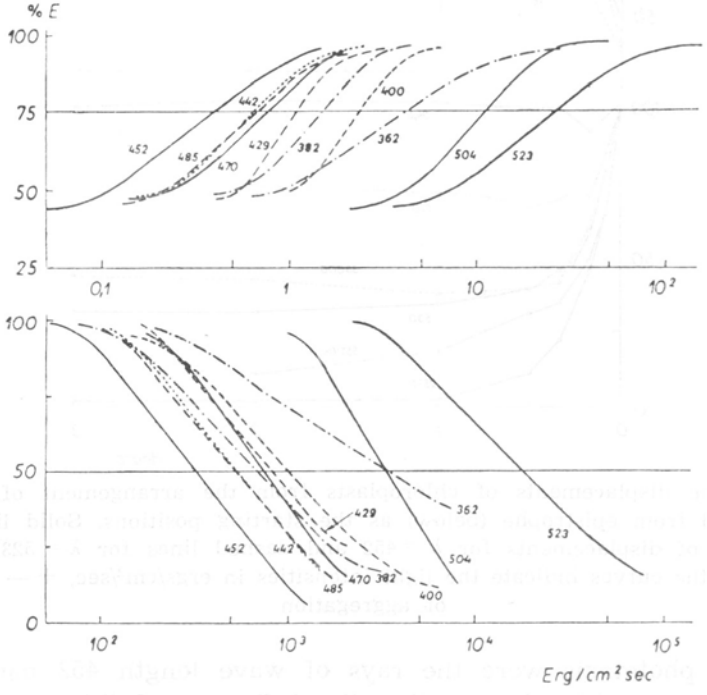

Fig. 22. Dependence of chloroplast arrangements on light intensity for various spectral bands. The initial positions were the darkness arrangement (abowe) and epistrophe (below). The figures by the curves indicate the maximum transmittance of the filters

Table 3

Radiation intensities inducing the half displacements in phototaxis

λmμ	Reaction: darkness-weak light		Reaction: weak-strong light	
	ergs/cm²sec	Quanta/cm²sec10^9	ergs/cm²sec	Quanta/cm²sec10^{12}
362	4.1	750	3030	604
382	1.45	280	630	122
400	2.3	404	960	194
429	1.05	228	790	171
442	0.62	138	520	116
452	0.39	89	330	75
470	0.72	171	715	170
485	0.66	162	535	131
504	11.0	2800	3350	852
523	25.0	6600	18000	4750
541	530	14450	—	—

brought out by the intensity of 0.000004 cal/cm²hour (≡2.9 ergs/cm² sec.). The curves in Fig. 22 illustrating the dependence of the chloroplast arrangements on the light intensity are parallel in almost all wave lengths. Only the inclination of the curves for band 362 mμ is distinctly

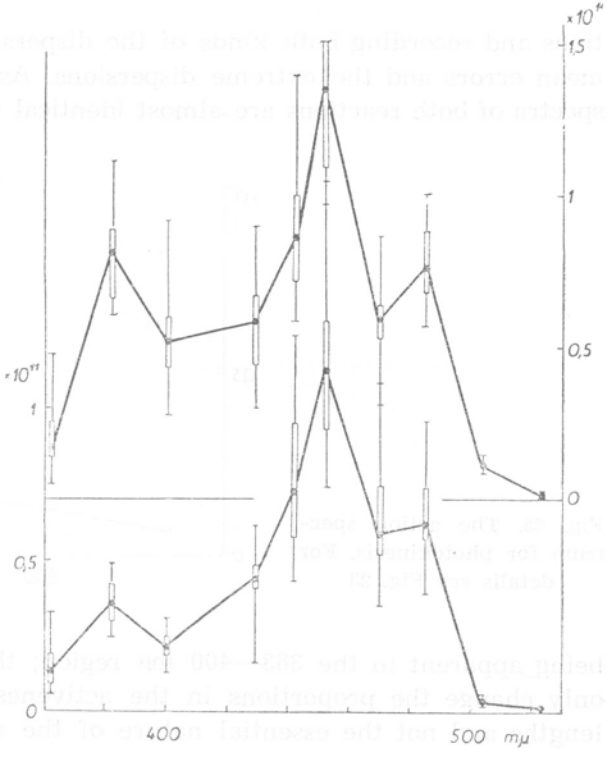

Fig. 23. The action spectrum for the phototaxis of chloroplasts. x-axis — the wave length, y-axis — the reciprocal of the light intensities in quanta/cm² sec, producing one half of a reaction. The left scale refers to the apostrophe → epistrophe reaction and the right one to the epistrophe → parastrophe reaction. The mean errors and the extreme dispersions are marked as in Fig. 21

different, which seems to indicate that the action of this wave length was somewhat different than of the other spectral regions.

Table 3 lists the light intensities accepted in this work as the measure of the activeness of light and Fig. 23 shows the action spectrum plotted from the data in this Table accounting for the quantifica-

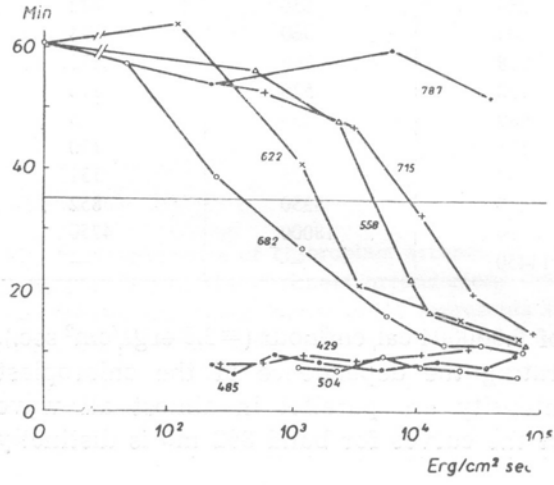

Fig. 24. The dependence of the reaction time on the light intensity. x-axis — light intensity, y-axis — reaction time in minutes. The figures by the curves indicate the wave lengths. Every point represents the average of five or six recordings

tions and recording both kinds of the dispersion in the results, i.e. the mean errors and the extreme dispersions. As is to be seen the action spectra of both reactions are almost identical the only slight differences

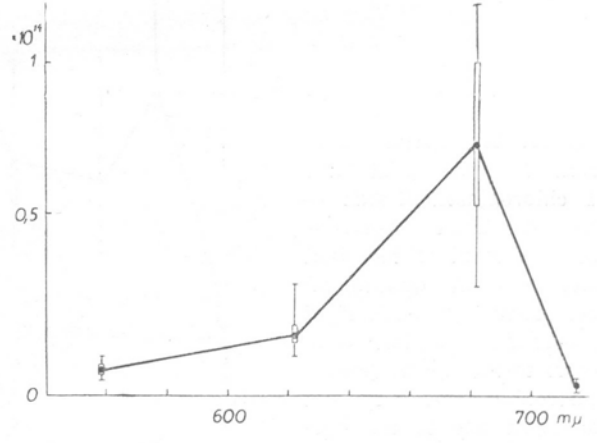

Fig. 25. The action spectrum for photokinesis. For detalis see Fig. 23

being apparent in the 383—400 mμ region; these differences, however, only change the proportions in the activeness of the particular wave lengths and not the essential nature of the action spectra.

The action spectrum of phototaxis has three peaks at 382, 452, and 485 mμ. The present results show a marked discrepancy with those obtained by B a b u s k i n who published so far the only action spectrum for the phototaxis of chloroplasts. Although the positions of some of the peaks of activeness are in both cases similar (e. g. the peaks at 480 and 450 mμ), nevertheless according to B a b u s k i n the highest relative activeness occurs at 420 and 430 mμ, whereas in *Lemna* it occurs at 450 mμ. On the other hand, the present spectrum is very similar to the action spectrum obtained by V i r g i n (1952, 1954) for the "viscosity" of the protoplasm as measured by the response of chloroplasts to centrifugation.

As has been mentioned above, the measurements in the long-wave range were based on the time of the reaction. Fig. 24 shows how the reaction time depend on the light intensity for five wave lengths in the 557—780 mμ region. As shown by the graph the most active in the acceleration of the displacements is the red radiation in the band 682 mμ, whereas the band 780 mμ has no accelerating effect. Using the measure of the activeness of light defined earlier in this work it has been possible to plot the action spectrum for the kinetic activeness (Table 4, Fig. 25). This spectrum has a well marked peak at about 680 mμ and showed a gradual drop in the activeness of light with the shortening of the wave length. When the infra-red range was reached the activeness of light very rapidly dropped to zero.

The reaction times for the long- and the short-wave regions are very difficult to compare, because the latter induced tactic arrangements in which the final arrangement depended on the intensity of irradiation. However, if the reaction time is defined as the time necessary

Table 4

Radiation intensities causing the half acceleration of displacement (i.e. reducing the reaction time to 35 minutes)

λmμ	ergs/cm²sec	Quanta/cm²sec 10^{12}
558	4400	1235
622	1600	500
682	290	137
715	8900	3200

to attain half of the final arrangement, then the reaction time in the 400—520 mμ region does not significantly depend on the intensity of light; in the epistrophe → parastrophe reaction it was about eight

minutes ranging five to ten minutes (see Fig. 20 and Fig. 24). Only in blue light of the highest intensities causing the chloroplasts to aggregate already in the course of their displacement the average reaction time increased to about ten minutes ranging eight to fifteen minutes.

VII. THE CINEMATOGRAPHIC ANALYSIS OF THE CHLOROPLAST MOVEMENTS

M e t h o d s. The cinematographic technique was used to examine the kinetics of the movements of the particular chloroplasts. The displacements of chloroplasts were photographed on a 16 mm. film using the frame by frame equipment Usually the acceleration was 1/100 (1 frame every 6.25 sec.) and accelerations of 1/500 (1 frame every 37.5 sec.) were exceptional and were only applied when the movements were very slow. The monochromatic light was obtained from an interference continuous running filter (Schott — band filter) the transmittance of which for the region of wave lengths used in the experiments is shown in Fig. 26.

The already mentioned independence of the rate of the displacements on the irradiation with infra-red rays made possible the filming of the reaction taking place in "physiological darkness", which was presumably identical with the reactions in darkness. The system of filters used for this purpose was UG 7—4 mm. + H_2O — 5 cm. the lamp being supplied well below the nominal

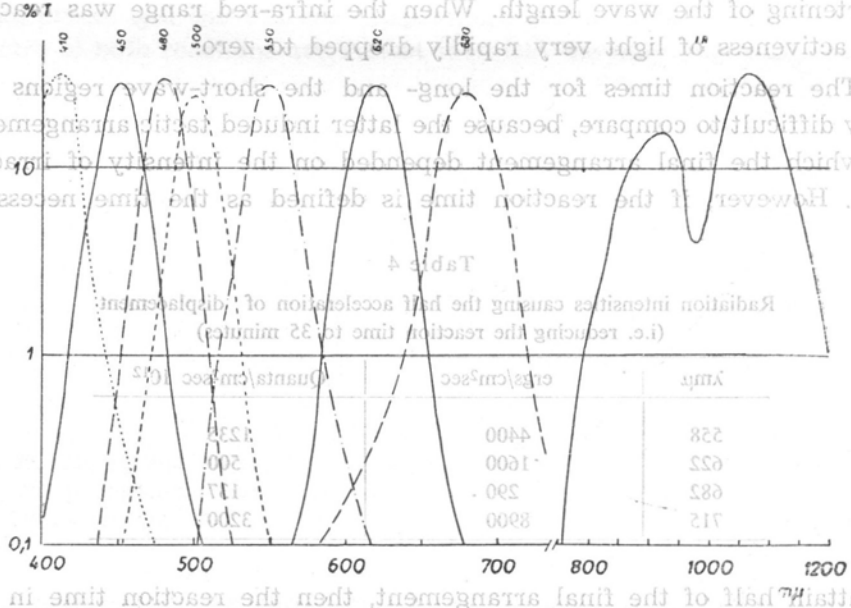

Fig. 26. Transmittance curves of the interference filters used for making motion films. On the extreme right — the transmittance curve of the filters used for mation films in infra-red radiation

voltage. The intensity of the irradiation in the place of a slide was about 8500 ergs. The negative film was High Speed Infra Red (Kodak).

The films were analysed by projecting them frame by frame onto a screen and marking on the screen the positions of the particular chloroplasts. In this way both the paths and the speeds of the particular chloroplasts during their displacements could be determined. To make the drawings readable only the paths of the chloroplasts centres were traced (Zurzycki & Zurzycka 1953).

Results. Let us first consider the kinetics of the displacements taking place in the bands: 480 and 670 mμ and in the infra-red region

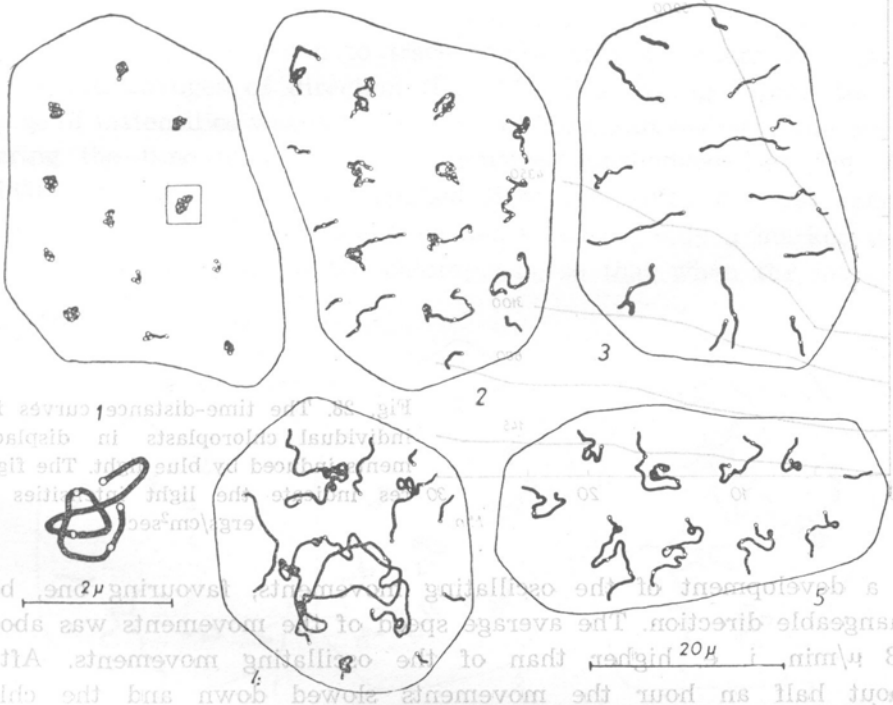

Fig. 27. The paths of chloroplasts traced in blue light (λ = 480 mμ) of different intensities. *1*) 145 ergs (displacements during 60—90 minutes of exposure), *2*) 680 ergs, *3*) 3550 ergs, *4*) 5900 ergs, *5*) 10 800 ergs/cm²sec. The points mark the positions of chloroplasts every 10 minutes

(Fig. 26). In blue light of low intensity (145 ergs) the epistrophe arrangement was maintained, though the chloroplasts were not motionless: they moved with small irregular jerks in various directions out of their main position the amplitude of these oscillations being 1—2.5 μ. The average speed of these movements was 0.1 μ/min. (Fig. 28), but there were no significant changes in the arrangement of chloroplasts, which remained typical for epistrophe (Fig. 27). A higher light intensity resulted in a slight drop of % *E* and caused better marked displace-

ments (Fig. 27—2): the chloroplasts lying near the border of a cell were displaced with a flowing motion along undulated or almost straight paths onto the side walls, while the chloroplasts lying nearer the centre began moving in a rather complicated way, which was as

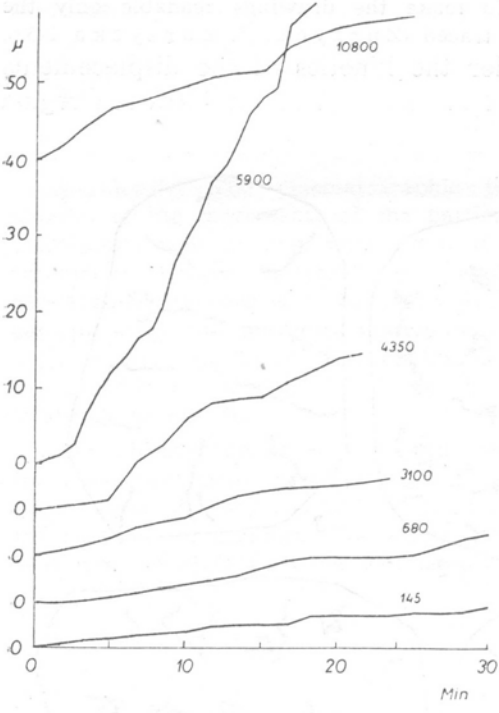

Fig. 28. The time-distance curves for individual chloroplasts in displacements induced by blue light. The figures indicate the light intensities in ergs/cm²sec

if a development of the oscillating movements, favouring one, but changeable direction. The average speed of the movements was about 0.3 μ/min. i. e. higher than of the oscillating movements. After about half an hour the movements slowed down and the chloroplasts, now less numerous on the observed cell-wall, established their positions; they continued to oscillate (the speed now dropped to 0.17 μ/min.) similarly as in epistrophe, but the amplitude of the oscillations seemed slightly larger and the movements in one fixed direction were more frequent. In still higher intensities inducing an almost complete parastrophe all the chloroplasts moved along almost straight or slightly undulated paths towards one of the side walls usually the nearest one. Loops and changes of direction were sometimes seen but only near the side walls and, so far as can be judged from the films, they occurred only when there was no more room on the side walls (Fig. 28—3). The average speed of the movements was about 0.5 μ/min.; the chloroplasts moved at a more or less uniform rate till they got near

a side wall when their speed dropped. On the screen the chloroplasts seem to be gently flowing off towards the side walls. The intensity of light giving this pattern of displacements was the weakest in the range producing full parastrophe. In higher intensities the pattern of the movements changed entirely. In the range above 4500 ergs only the chloroplasts lying nearest the border moved along more or less straight paths to the side walls at an average speed of 6.0 μ/min.; the others moved with the same speed during the first 2—5 minutes of irradiation and then their speed drastically increased and became very changeable (Fig. 28).

The chloroplasts began to trace complicated meanders with many loops and changes of direction (Fig. 27). The average speed in this range of instensities was very high, 3—4 μ/min., but owing to the meandering the time of the reaction remained unchanged (see Fig. 24). Finally, the highest of the applied light intensities did not change essentially the pattern of the movements causing only a marked drop of the average speeds of the chloroplasts, so that when the intensity

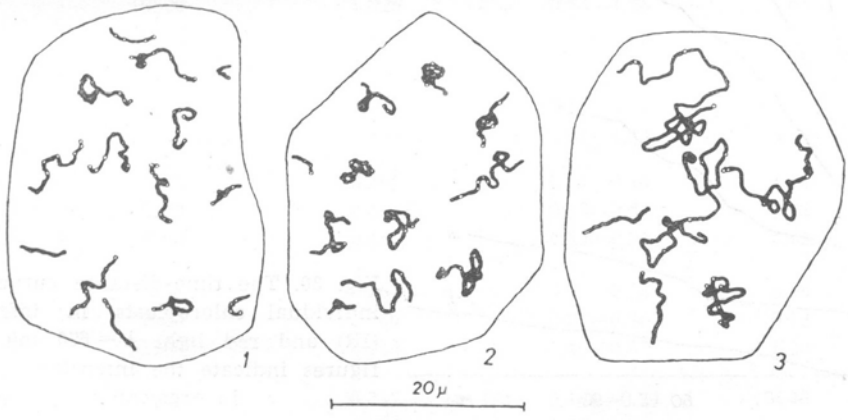

Fig. 29. The paths traced by chloroplasts in infra-red and red light. *1*) infra-red radiation, 8500 ergs/cm²sec; *2*) λ = 670 mμ, I = 570 ergs/cm²sec; *3*) λ = 670 mμ, I = 53 800 ergs/cm²sec. The points mark the positions of chloroplasts every 10 minutes

was 10 800 ergs the speed dropped to one μ/min. In this intensity the aggregation of the chloroplasts began already after 20—30 minutes of irradiation.

The exposure of leaves with chloroplasts in the epistrophe position to infra-red radiation induced, in the material used in these experiments, very slow chloroplast movements. (Note: the plants were of the

same strain as in Part VI, see Fig. 24). The paths of the chloroplasts (with the exception of the chloroplasts lying close to the side walls) were looped and meandering, but the pattern of the movements was not an oscillation round some fixed position and for all the changes of direction the chloroplasts had a progressive motion. The average speed was about 0.1 μ/min. varying between transient accelerations (to about 0.26 μ/min.) and moments of immobility (Fig. 29 — *1* and Fig. 30).

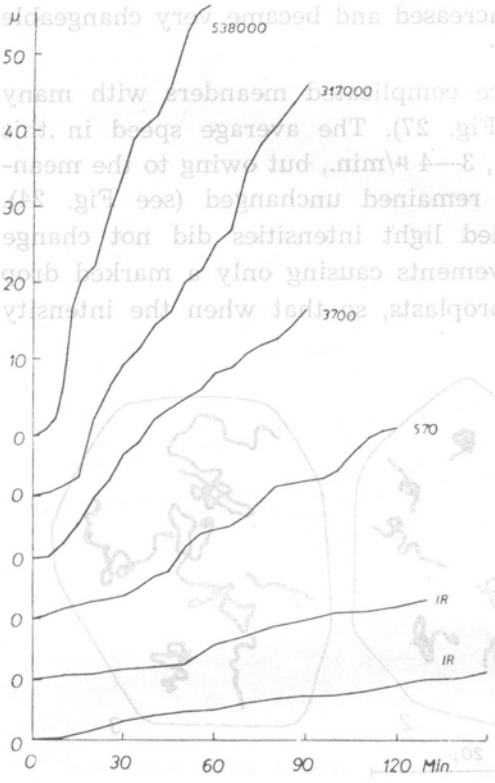

Fig. 30. The time-distance curves for individual chloroplasts in infra-red (IR) and red light $\lambda = 670$ mμ. The figures indicate the intensity of light in ergs/cm²sec

The displacements in red light were very similar to those caused by infra-red radiation, but the movements had much more vigour: the loops traced in the temporary changes of direction, not clearly apparent in infra-red radiation owing to the slowness of the movements, now became very distinct. The higher the intensity of the red light the greater became the average speed of the chloroplasts (Fig. 30), rising to 0.64 μ/min. with transient maxima of up to 2.5 μ/min., and the more complicated were the paths (Fig. 29). The characteristic traits in the behaviour of the chloroplasts in red light were their frequent returns from the side walls onto the upper wall and their wanderings there

Table 5

The speed of chloroplasts movements in μ/min. in different light intensities and for different wave-lenghts

λ mμ	Intensity ergs/cm²sec	Average speed of 8—10 chloroplasts	Dispersion of the average speeds of individual chloroplasts	Maximum transient speed
410	400	0.25	0.21—0.32	0.40
	1420	0.45	0.38—0.56	1.52
450	148	0.263	0.18—0.48	0.76
	1100	0.454	0.36—0.58	1.1
	6500	2.23	1.52—3.16	5.4
480	42	0.083	0.065—0.11	0.21
	680	0.322	0.26—0.35	0.76
	1100	0.45	0.31—0.51	1.20
	3100	0.515	0.48—0.56	2.4
	3550	0.684	0.52—1.10	2.3
	4350	3.12	2.20—4.16	5.4
	5900	4.64	3.72—5.44	6.44
	10800	1.026	0.84—1.26	5.04
500	970	0.305	0.28—0.40	0.90
	3500	0.593	0.42—0.86	1.38
	7500	1.03	0.58—1.36	3.72
	14300	1.40	1.02—1.84	4.56
555	950	0.265	0.21—0.34	1.14
	3000	0.46	0.38—0.57	1.08
	20500	0.64	0.54—0.76	1.62
620	537	0.262	0.15—0.34	0.58
	1000	0.280	0.21—0.36	0.72
	31500	0.540	0.46—0.67	1.20
670	570	0.237	0.164—0.29	0.40
	3700	0.387	0.27—0.51	1.04
	31800	0.532	0.28—0.62	1.86
	53800	0.640	0.39—0.91	2.52
IR	8500	0.0916	0.052—0.168	0.26

during a fairly long time. This behaviour was never observed in the high intensities of the short-wave region.

The analysis of the displacements at other wave lengths lead to the following conclusions. The patterns of movements described for 480 mμ were characteristic for the whole 400—500 mμ region, though at every wave length different intensities brought out the particular kinds of

movements: the oscillations, the flowing, and the disorderly meanders at high speeds. The flowing type was the least distinct at 500 mμ. On the other hand, in the region 550—700 mμ the representative pat-

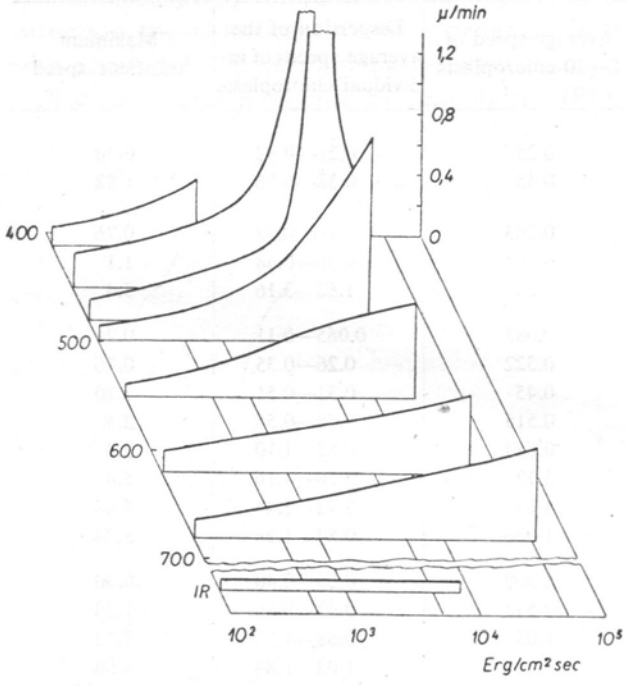

Fig. 31. The dependence of the average speed of chloroplasts (y-axis) on light intensity (x-axis), and wave length (z-axis)

tern was the one described for 680 mμ, but again for every wave length the speed of chloroplasts depended differently on the intensity of light.

The relation between the average speed of the chloroplast movements and the intensity of irradiation for different wave lengths is shown in Fig. 31 and in Table 5.

VIII. THE INFLUENCE OF LIGHT ON CHLOROPLASTS MOVEMENTS AND ON OTHER PHYSIOLOGICAL PROCESSES

A. The centrifugation of chloroplasts

Methods. The close resemblance between the action spectra in phototaxis and in the light induced changes of the viscosity of the protoplasm (Virgin 1952) as well as the observed dependence of the response of chloroplasts to centrifugation on their arrangement (Zurzycki 1960) seemed to indicate that both these effects could have much in common. The aim of this investigation was not to establish the action spectrum for the changes in the behaviour of chloroplasts during centrifugation but merely to compare the light intensities causing these changes with the light intensities inducing the changes of the

chloroplast arrangements. The plant material in the experiments was the same as in Part VI of this work. The experimental procedures were based on an earlier investigation (Zurzycki 1960). The slides with the fronds were kept for 12 to 18 hours in darkness to induce apostrophe and then having been irradiated during one hour according to the procedure described in Part VI they were centrifuged for 45 minutes at 2500 r.p.m. (= 838 g). Immediately after centrifugation counts were made of the undisplaced chloroplasts on the upper, lower, and side walls. The leaves were then adapted to weak light of 60 lx and after three hours the full epistrophe arrangement was defined in the usual way. The leaves in which the return to epistrophe was not complete were eliminated. With this procedure it was possible to determine the proportion of chloroplast undisplaced by centrifugation, i. e. the proportion of chloroplasts with a C_0 greater than the applied centrifugal force.

Here is an example:

Centrifugation after irradiation with light of 500 mμ and intensity 570 ergs/cm²/sec.

Number of chloroplasts undisplaced by centrifugation

on the proximal wall	50
on the distal wall	49
on the side walls	22
	121

Number of chloroplasts after adaptation to weak light

on the proximal wall	127
on the distal wall	96
	223

Thus the per cent of chloroplasts undisplaced by centrifugation was 54.2.

Results. The experiments were carried out for four wave lengths: 400, 452, 504, and 672 mμ. The results are illustrated by the curves in Fig. 32. After centrifugation of leaves previously kept in darkness the per cent of undislocated chloroplasts was on the average 35.30 (the

Table 6
Light intensities increasing the percentage of undisplaced chloroplasts to 60

λmμ	Light intensity	
	ergs/cm² sec	quanta/cm² sec 10^9
400	4	808
452	0.5	112
504	30	7560

extreme values were 25.0 and 47.6 per cent). The proportions of chloroplasts undisplaced by centrifugation after exposure to very weak light of all wave lengths were within the same limits. Rising light intensities in the short-wave range increased the proportion of undis-

placed chloroplasts, whereas irradiation with red light had no significant influence on this process over the whole investigated range of intensities, i.e. up to 21 500 ergs/cm²/sec. When taking into account that

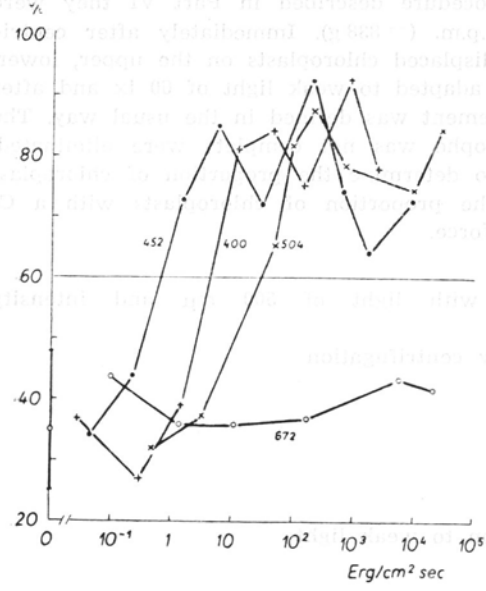

Fig. 32. The dependence of the per cent of chloroplasts undisplaced by centrifugation (x-axis) on light intensity (y-axis) and the wave length of light (figures by the curves) used for the initial irradiation

after darkness and after exposure to very weak light the average proportion of undislocated chloroplasts was 35 per cent, whereas after exposure to short-wave radiation it increased on the average to 85 per cent, and that we assumed as the measure of the activeness of light the intensity causing the proportion of undisplaced chloroplasts to remain unchanged at the level of 60 per cent $\left(\frac{35+85}{2}\right)$, then interpolating the curves in Fig. 32 we can define the activeness of the particular wave lengths in this process (Table 6).

B. Photosynthesis

Methods. The measurements of the rate of photosynthesis were carried out at 23°C on isolated leaves using a capillary respirometer (Zurzycki 1955, Starzecki 1961) and the light filters described in Part VI of this report. Light intensities were measured with a thermopile (Kipp & Zonnen).

The recorded (see below) absence of any strict relationship between photosynthesis and the arrangement of chloroplasts in monochromatic light in contrast to the high degree of this relationship observed in earlier experiments with white light (Zurzycki 1955, Babuskin 1955b) persuaded the author of the need to repeat the experiments in white light of different spectral composition:

1) in light of colour temperature about 2000 K° obtained from a 250 W 220 V projection lamp working on an 82 V current,

2) in light of colour temperature about 3000 K° obtained from the lamp working on the nominal current, and

3) in light of colour temperature about 6000 K° obtained in the same way as in point 2 using a BG 26—6 mm. filter and a special pale violet filter 2 mm. thick.

The relative spectral distributions in these three sources of light were defined from measurements with a Zeiss mirror monochromator as compared to a standard lamp with the colour temperature 2850 K°. As is to be seen in Fig. 33 the three sources of light considerably differed by the proportion of energy in the short-wave regions: the proportion was highest in source 3 and lowest in source 1. The total energy of light was measured for the 400—700 mμ

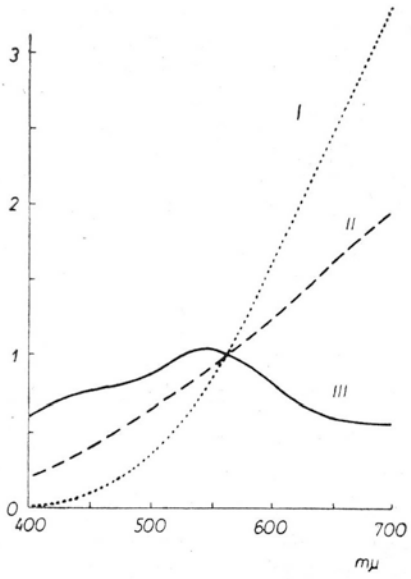

Fig. 33. The relative spectral distribution of energy in the spectra of the three kinds of white light used for the experiments. The curves are equalized for $\lambda = 560$ mμ

part of the spectrum using a thermopile and filters GG 18—2 mm. and RG 8—2 mm. The grading of energy was obtained by interposing grid filters. The values of the erg/lx ratio for light from sources 1—3 were respectively 10.02, 4.14, and 2.72.

Results. The light curves of photosynthesis in monochromatic light are shown in Fig. 34. The optical system of the respirometer did not allow the use of high light intensities and because of this the saturation point of photosynthesis could be established only for the band 682 mμ. In the short-wave region only the beginning of the curves could be established. The radiation intensities inducing the photosynthesis rate equal to 1/4 of the maximum (which approximately corresponded to the compensation point) were established for each wave length from the graph and so were the intensities corresponding to the point of intersection of the extended first section of the light curve of

photosynthesis with the maximum value of photosynthesis. The latter of these intensities constituted an arbitrarily accepted measure of the saturation of photosynthesis; in most instances the actual saturation points corresponded to somewhat higher light intensities and could not be established. The values of the compensation point and of the arbitrary saturation point are plotted in Fig. 35.

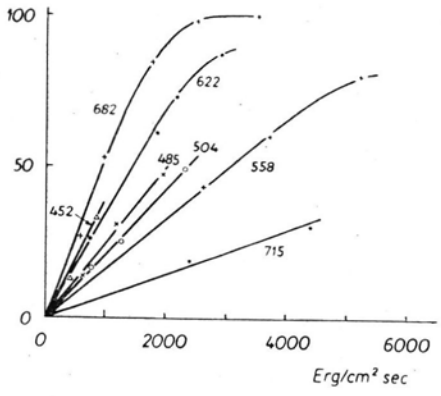

Fig. 34. The dependence of the intensity of actual photosynthesis (y-axis) on light intensity (x-axis) for various wave lengths (figures by the curves)

When the curves illustrating the relation between different physiological processes in the leaves of *L. trisulca* are compared with the wave length and the intensity of radiation (Fig. 35) it is seen that there is a close parallelism between the apostrophe-epistrophe reaction and the ability of chloroplasts to be displaced by centrifugation. Both the action spectra and the ranges of the active intensities are very similar, which seems to indicate that the movement of chloroplasts is related to their ability to be displaced by centrifugation and that the two processes have a common controlling mechanism. On the other hand the curve illustrating the epistrophe-parastrophe reaction shows no relation with photosynthesis. In the case of the particularly active wave lengths (e.g. 452 mμ) full parastrophe is attained in light intensities well below the compensation point of photosynthesis, whereas in the band 523 mμ light intensities sufficiently high to cause the saturation of photosynthesis are still insufficient to induce the complete strong light arrangement of chloroplasts. Finally, the curve illustrating the kinetic activeness of the long-wave part of the spectrum bears some relation to the curve of photosynthesis. Throughout the long-wave part of the spectrum the light intensities defined in Part VI as the measure of the kinetic activeness of light lie inbetween the compensation point and the arbitrary saturation point of photosynthesis.

The experiments with white light show, as was to be foreseen, the

great significance of the short-wave part of the spectrum in influencing the arrangement of chloroplasts (Fig. 36). The higher the proportion in the spectrum of the tactically active rays the lower is the total energy needed (in the region 400—700 mμ) to rearrange the chloroplasts. Owing to the high proportion of short-wave radiation in the spectrum light with the colour temperature 6000°K induces parastrophe at inten-

Fig. 35. The dependence of various photobiological proceses taking place in *Lemna trisulca* cells on light intensity (x-axis) and the wave lengths (y-axis): *1*) the action spectrum of phototaxis in the apo. → epi reaction, *2*) the action spectrum of phototaxis in the epi. → para. reaction, *3*) the action spectrum in photokineses, *4*) irradiation intenstity reducing by half the ability of chloroplasts to be dislocated by centrifugation, *5*) the curve of the compensation point, *6*) the curve of the arbitrary saturation point

sities six times lower than light with the colour temperature 2000°K. This means that the arrangements of chloroplasts in white light depend not on the total energy of light but on the share in it of the short-wave radiation. On the other hand, the dependence of photosynthesis upon the various kinds of white light is diametrically opposite (Fig. 37). The highest rate of photosynthesis was induced by light from source 1 with a high share of red rays and the lowest by light from source 3. The intensities of the three investigated kinds of white light causing the characteristic changes of chloroplast arrangements and in the rate of photosynthesis are listed in Table 7. Although, a change in the colour

temperature of white light has a totally different effect on the chloroplast arrangement than on photosynthesis, nevertheless, in the case of the colour temperature of light from source 3 there seems to be a certain correlation between the two processes. This correlation consists

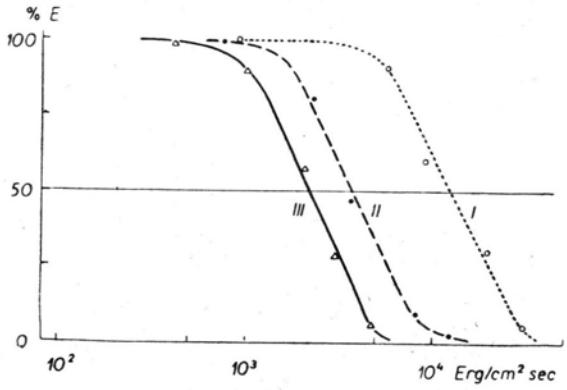

Fig. 36. The dependence of the chloroplast arrangements (y-axis) on light intensity (x-axis) in the three kinds of white light

in that the epistrophe arrangement was retained up to the intensity approximately corresponding to the compensation point or even beyond it and full parastrophe was reached in intensities causing the saturation of photosynthesis. The fact that this correlation occurs in light having the colour temperature about 6000°K does not seem accidental, since natural white light has a similar composition (the colour temperature

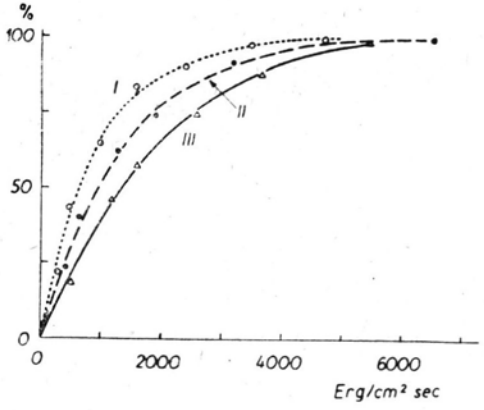

Fig. 37. The dependence of actual photosynthesis (y-axis) on light intensity (x-axis) in the three kinds of white light

of direct sun rays is 5600°K). We may thus conclude that, though the changes in the tactic arrangement of chloroplasts are controlled by the short-wave part of the spectrum, the sensitivity of chloroplasts to light is of the kind making them reach the parastrophe arrangement

Table 7

Intensities of white light giving a pre-defined intensity of photosynthesis and characteristic phototactic arrangements. Light intensities in ergs/cm²sec. (400—700mμ)

	Types of white light		
	I	II	III
Photosynthesis:			
25% of real photosynthesis (comp. point)	250	400	650
Arbitrary saturation	1150	2000	2200
Actual saturation	4000	5000	6000
Phototaxis:			
100% *E* (beginning of strong light displacements)	4500	1200	600
50% *E*	12500	3700	2100
0% *E*	35000	10000	5500

more or less simultaneously with the saturation point in the process of photosynthesis, but only if the colour temperature of light is close to 6000°K.

IX. DISCUSSION

When investigating the influence of the critical spectral regions on the movements of chloroplasts the precise determination of the displacements taking place in darkness is of key importance, since all other chloroplast arrangements are compared to the darkness arrangement. In the case of *Lemna* the arrangement reached in darkness and henceforth persisting during several hours consists in most instances in the uniform distribution of chloroplasts on all the cell walls (S e n n's peristrophe arrangement). Then the cell is probably in a state of relaxation marked by a total absence of taxes. There is evidence to suppose that this effect is manifested also in other plant species. According to S e n n (1908) apostrophe in the cells of spongy and palisade tissue is not reached till after at least three days and is preceded by the much earlier achieved peristrophe arrangement. The uniform distribution of chloroplasts is associated with only a partial decrease in the number of chloroplasts on the walls on which they were all grouped in epistrophe and, thus, it sometimes is difficult to detect, specially if other criteria than the number of chloroplasts in the flat position are accepted to determine the arrangement. E.g. in experiments on the transmittance of light the strong light arrangement of chloroplasts was reflected by a well marked increase of transmittance (B i e b l 1954), but then the distinction between full and partial epistrophe was very difficult (Z u-

rzycki 1961b). When it is remembered that transmittance in the leaves of land plants is influenced by various factors besides the arrangement of chloroplasts (Biebl 1955) then the use of the transmittance criterion in investigation on the action spectrum of light (Babuskin 1955a) must appear highly unreliable.

If the term *phototaxis*, which strictly speaking applies to organisms moving slowly in a medium, can be applied to chloroplasts changing their arrangement within a cell, then photostactic activities can be ascribed to the visual and the near UV part of the spectrum. However, in *Lemna trisulca* no differences were observed between the movements of chloroplasts in the far or near infra-red part of the spectrum and their movements in darkness. This contradicts the results reported by Voerkel (1933) and Babuskin (1955a) who observed the activeness of infra-red radiation. It seems that in their experiments the apparent activeness of infra-red radiation was due to the unspecific influence of heat. Admittedly, Voerkel found that the heating of preparations to 34°C did not induce the same effects as those caused by infra-red radiation, but both he and Babuskin experimented with land plants in the air medium, in conditions which could give rise to local differences of temperature within a cell and could thus produce the grouping of chloroplasts. The water medium of the present experiments reduced the possibility of such local differences of temperature.

A specific influence on the chloroplast arrangements is exerted by a wide range of UV radiation. From the available information it is difficult to decide whether the 240—350 mμ part of the spectrum has any influence on phototaxis and, if so, what this influence may be, but quite certainly an addition of this radiation to the phototactically active near-UV (360 mμ) radiation completely prevents the chloroplasts from assuming the typical phototactic arrangements. The marked acceleration of the chloroplast movements (Part IV) as well as the often demonstrated, sudden, greatly enhanced ability of chloroplasts to be displaced by centrifugation after UV irradiation (Voerkel 1933, Stålfelt 1949) indicate the weak attachment of chloroplasts and the decreased viscosity of the protoplasm. Higher intensities of this radiation completely destroy all chloroplast attachments, which are an essential factor, as will be later demonstrated, in the formation of phototactic arrangements: the distribution of the chloroplasts then becomes uniform on all the cell walls in spite of the simultaneous action of phototactically active rays. The highest of the applied radiation intensities paralyse all movements so rapidly that no changes can take place in the initial arrangement. Voerkel (1933) observed a similar behaviour of chloroplasts in *Funaria* and interpreted it as „Lichtstarre", that is the stopping of all chloroplast movements by the

solidification of the protoplasm, an effect which had been observed by other workers studying the influence of very high light intensities on protoplasmic movements (Pringsheim 1881, Linsbauer 1929).

The particular parts of the visual spectrum have a different influence on the movements of chloroplasts. The typical phototactic arrangements (epistrophe, parastrophe, aggregation) are brought out by the short-wave part of the spectrum, whereas in the long-wave part of the spectrum the final chloroplast arrangement is analogical to the arrangement in darkness. However, the long-wave part of the spectrum is not inactive and accelerates the onset of the final arrangement. By again applying a term borowed from the physiology of algae we may say that red light has a *photokinetic* influence on the speed of chloroplast movements but not on the direction of these movements. A first attempt at explaining along these lines the action of red light was made as early as 1909 by Linsbauer and Abramowicz, but unfortunately their interpretation has not been taken up by other workers and has till now been forgotten. The fact that the action of red light is photokinetic but not phototactic in its character explains why even in one species it may seem to be either active or inactive. Red light seems to be inactive when the final effect of the chloroplast movements is considered (Senn 1908), whereas its activeness is only revealed by considering either the time course of the movements or their effect after a relatively short time as compared to the time of displacements in darkness (Linsbauer and Abramowicz 1909, Zurzycka 1951). The grouping of the chloroplasts on the proximal or the distal (with regard to the light source) cell wall sometimes occurring in red light can probably be explained by the influence of the different intensities of light incident on the two walls on the speed of chloroplast movements. A change of speed caused by local differences in the intensity of light may give rise to the effect of pseudophototaxis (Clayton 1957). The much lower value of % *E* after irradiation with red light as compared to the darkness arrangement reported by Zurzycka (1951) might have been caused by pseudophototaxis, since in her experiments she analysed the chloroplast arrangement on only one cell wall (the distal one).

If the influence of light on the movements of chloroplasts in other plants is similar to what has been observed in *Lemna trisulca* in the course of this work, then presumably the period of time during which the specific action of red light is discernable will be strictly limited; moreover, the most precise techniques for the control of chloroplast movements will be necessary. The land plants do not seem to be very suitable for investigations of this kind, since in darkness the chloroplasts of these plants move very slowly (Senn 1908).

A detailed analysis of the action spectrum in phototaxis shows that the active part ranges 350 to 530 mμ. The action spectrum of this reaction closely resembles the action spectra of the response of chloroplasts to centrifugation (Virgin 1952, 1953), of protoplasmic movements (Bottelier 1934), and of the phototropism in coleoptiles (Schropshire and Withrow 1958, Curry and Thimann 1961). The mechanism underlying all these photobiological processes is presumably the same or at least similar, this supposition being confirmed by the demonstrated strong influence of heteroauxin on the centrifugation of chloroplasts (Stålfelt 1949).

In the long-wave region the action spectrum of kinesis has a peak at 680 mμ. The mutual proportions of the kinetic activeness manifested by various wave lengths of light as well as the range of the kinetically active light intensities seem to indicate that there is a relation with the chlorophyll and photosynthesis. Researches on the action spectrum of photokinesis in algae have shown that also in this case the maximum of activeness is in the red part of the spectrum (Wolken and Shin 1958). If it is true that in algae chlorophyll is the absorbent of light, then probably the kinetic activity of light extends also to the short-wave part of the spectrum and in these wave lengths two processes, taxis and kinesis, are cumulated: the great changes in the speed of chloroplast movements and also in the character of these displacements in blue light of high intensity (Part VII) can be presumably explained by this cumulative effect.

On the ground of the information assembled so far in experiments with *Lemma trisulca* an attempt will now be made at explaining the mechanism underlying the chloroplast movements, in which the reasoning will be based on the following circumstances:

1. In a plant cell there are always more or less active protoplasmic currents of the gliding (turbulent) type with characteristic changes of direction and speed. The existence of these currents was often demonstrated by observations of the movements of microsomes (for list of references see Kamiya, 1959).

2. A chloroplast has the ability to anchor itself on the immobile ectoplasmic gel layer the bond thus formed being rather weak in darkness, very strong in weak light, and the the weakest after irradiation in very high intensities. These changes in the strength of the anchoring bonds are indicated by the results of the experiments with the centrifugation of chloroplasts (Virgin 1951, Zurzycki 1960). They consist in physical changes on the chloroplast ectoplasm contact plane and are only caused by the action of the short-wave part of the spectrum (Virgin 1952, 1954).

3. At the points where the chloroplasts are grouped more endoplasm

assembles, which leads to an unbalanced state in the cell. When the factors causing the grouping of chloroplasts are removed, the state of tension tends to disappear and the endoplasm is again uniformly redistributed.

4. Photosynthesis causes the formation of ATP by way of photophosphorylation; this process is associated with the absorption of light in the system of photosynthetic pigments (Jagendorf, Hendricks, Avron and Evans 1958). During intense photosynthesis some ATP may diffuse to the surrounding protoplasm where it acts as a stimulant on the movements of protoplasm (Takata 1958).

In accordance with point 3 the epistrophe arrangement gives rise to a state of tension within a cell this state being associated with the uneven distribution of the endoplasmic matter. When the factor maintaining epistrophe is removed, e.g. by the onset of darkness, the strength of the bonds holding the chloroplasts in position is reduced (the rate of this process may differ as shown by the variability of the epi.→ apo. reaction) and as soon as this happens the first to move are the chloroplasts lying nearest to the border of the cell; they flow down to the side-walls carrying with them some endoplasm. The other chloroplasts are constantly displaced under the influence of the turbulent plasmatic movements, which are very slow in darkness (see the motion film analysis in Part VII), and depend to a great extent on the physiological state of the cell (see the variability of the speed of displacements in Part III). In the course of displacements the chloroplasts may become more strongly anchored and then their advance is stopped or their speed is reduced. The displacements result in the uniform distribution of the chloroplasts in the cell (if in the mean time no tactic influence is exerted by other stimuli e.g. by chemical agents) and continue without interruption in darkness.

In the long-wave range the displacements follow a similar course, with the only difference that ATP produced by photosynthesis activates the gliding movements of protoplasm and may also accelerate the dissolution of the bonds anchoring the chloroplasts (hence the shortening of the lag period (Zurzycka 1951).

The short-wave and the near-UV part of the spectrum can, according to the intensity of the irradiation, either preserve or rapidly dissolve the bonds anchoring the chloroplasts. At low light intensities the anchoring bonds are preserved and the protoplasmic currents at the most only slightly push out the chloroplasts from their essential positions, to which the chloroplasts elastically return every time (see the oscillations of the chloroplasts, Part VII). Higher light intensities cause the dissolution of the attachments in the parts of the cell where the light is strongest, mainly on the proximal wall (see Part IV): the

chloroplasts move with the protoplasmic currents till they are carried to some less illuminated part of the cell where they again become anchored. Still higher light intensities causing the full parstrophe may have a twofold effect. In the lower range of these intensities the disattached chloroplasts are carried with the surrounding endoplasm to the cell walls where the light is weaker (the flowing of chloroplasts, Part VII), which greatly reduces the thickness of the endoplasm layer in the parts of the cell where the light is strongest. Mouravieff's (1960) investigations point to the possibility of the polar differentiation in the distribution of endoplasm associated with irradiation in the short-wave part of the spectrum. On the other hand, in the higher range of the parastrophe intensities no such protoplasmic movements are induced, but the shock action of light or the cumulation of tactic and kinetic influences causes a sudden acceleration of the gliding movements of the protoplasm. The dissolution of the anchoring bonds is rapid and the changes in the physical properties of the chloroplast/endoplasm contacting layers are of the kind making possible the advance of a chloroplast without any greater resistance on the ectoplasm layer (see Zurzycki 1960); the decrease of the resistances opposing the chloroplast movements is to be inferred from the fact that the average speed of the chloroplasts is not much lower than its temporary maxima (Table 5). The chloroplasts are carried by the gliding movement of the plasma till they reach the places where the ectoplasm layer is less strongly lighted (the side-walls) and where they can get anchored again. This explanation of the movements of the protoplasm seems to be supported by that in the case of the "flowing down" of the chloroplasts their advance on the more strongly lighted wall slows down towards the end of the displacement, whereas, in the case of the "disorderly movements" the chloroplasts are accelerated. In the first case the chloroplasts may be decelerated by the decreasing thickness of the endoplasm layer on the more strongly lighted wall and the resulting higher resistances, and in the latter case the acceleration may be associated with the greater freedom of movements on a wall with fewer chloroplasts but a sufficient amonut of the carrying medium (see Fig. 28). In the highest light intensities the speed of the movements is sharply slowed down, which may be a symptom of the approaching "Lichtstarre". In these very high light intensities the chloroplasts are weakly anchored also on the side-walls (Zurzycki 1960), because even there the ectoplasm layer is strongly lighted, and the tendency of the chloroplasts to get anchored is now reflected by their mutual adhesion one to another with their broad faces, i.e. by their aggregation.

In the strong light arrangement (parastrophe) tensions are developed

within the cell by the local accumulation of protoplasmic matter. When the factor maintaining the parastrophe arrangement is removed the state of tension disappears and this leads to displacements which in their first stage follow exactly the same pattern both in darkness and in red light (see Part IV) and are characterized by straight chloroplast paths of the "flow down" type (Zurzycka and Zurzycki 1957). The carrying of chloroplasts with the gliding movement of the plasma does not begin till the later stage and then it finally ends either by the uniform distribution of the chloroplasts along the cell walls (darkness) or their anchoring in places with the best light conditions (weak light).

The short-wave part of the UV radiation has a destructive influence on the mechanism anchoring the chloroplasts and thus abolishes the ability of chloroplasts to assume phototactic arrangements (see Part IV).

In this approach the often observed in *Lemna trisulca* double nature of the various types of the chloroplast displacements under the influence of such factors as temperature, metal ions, and enzymatic inhibitors would simply consist in that these factors have a different effect on the movements of chloroplasts carried along with the endoplasmic matter than on the movements of individual chloroplasts carried by the gliding protoplasmic currents.

SUMMARY

1. The problem studied in this investigation was the influence of irradiation in various spectral regions and of different intensities on chloroplast movements in cells of *Lemna trisulca* leaves. The distribution of chloroplasts in a cell was established from counts of the number of chloroplasts on the proximal and distal (with regard to the source of light) cell walls.

2. It was found that the course of the displacements from the position of weak light to the position of darkness varied greatly and depended on the material used in the experiments, nevertheless the final result was almost always the uniform distribution of the chloroplasts on all the cell walls.

3. Experiments with five widely spaced wave regions showed that the phototactic activity was exerted by the visual and the near-UV parts of the spectrum. The near- as well as the far infra-red radiation was inactive the displacements being the same as those taking place in darkness. The short UV radiation (240—350mμ) destroyed the ability to assume phototactic arrangements.

4. Radiation in the spectral region 360—530 mμ induced typical

phototactic arrangements. The action spectrum in the reaction from the darkness to the weak light arrangements was exactly the same as in the reaction from the weak light arrangement to that of strong light with one peak at 450 mμ and two lower ones at 485 and 380 mμ. The region 550—715 mμ did not induce the formation of phototactic arrangements but had a regulating influence on the speed of the displacements. The action spectrum of photokinetic activity had a peak at 680 mμ.

5. Motion film analysis showed that in the short-wave region the pattern of the displacements was differentiated into various types of movements according to the intensity of incident light. In the long--wave region the pattern of the movements was essentially the same as in darkness and only the speed of the movements was higher.

6. Experiments with the centrifugation of chloroplasts showed that the weak light arrangement was accompanied by a drop in the ability of chloroplasts to be displaced by centrifugation. The parallelism of the two processes was reflected by the action spectrum as well as by the range of light intensities.

7. The phototactic arrangements were only indirectly related to photosynthesis. The arrangement of chloroplasts was controlled by the short-wave part of the spectrum, but its sensitivity in white light of colour temperature about 6000°K was such that the full strong light arrangement was reached near the saturation point of photosynthesis.

8. On the ground of the assembled results an attempt has been made to develop a working hypothesis explaining the mechanism underlying the movements of chloroplasts.

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