Arrangement of chloroplasts and light absorption in plant cell.

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Introduction

The biological meaning of the phenomenon of chloroplasts movements within the cell is not well understood though these movements are very common. As the varying intensities of light are the main cause of the movement it is probable that these changes in position are the mechanism by which the plant adjusts itself to the light conditions of the environment (S e n n 1908), and on one hand they enable the possibly maximum absorption of weak light for photosynthesis while on the other they guard the chloroplasts from the damaging effects of too strong light.

In his monograph S e n n (1908) shows on numerous examples that the displacement of chloroplasts occurs as a rule between those parts of the cell which are illuminated more brightly (due either to direct light or to concentration of light rays) and those which are less illuminated (due to local absorption or refraction). This however does not explain all cases (e. g. the *Mesocarpus* type). It also seems that in leaves infiltrated and immersed in water the differences in light intensities in the different parts of cells are to weak to be taken as the only cause of the phototactic phenomenon.

When comparing the arrangement of chloroplasts in cells which are in optimum light conditions (epistrophe) to those in maximum light conditions (parastrophe)* it appears that in parastrophe the directly illuminated surface of chloroplast diminishes while at the

* Terms epistrophe parastrophe are used not in the sense of a specific morphological arrangement (F r a n k 1872, S e n n 1908) but as the arrangement which corresponds to the optimum and maximum intensities of light.

same time the layer of chloroplast becomes thicker and a considerable part of the cell is emptied of them and becomes transparent to light.

Such changes in the arrangement of chloroplasts can influence profoundly the amount of light energy absorbed within the cell.

The purpurose of the present paper is to investigate the changes in the amount of light absorbed in dependence on the arrangement of chloroplasts.

Theory

When looking on a cell in the same direction in which light falls it appears that in the optimum intensity of light all or nearly all the surface of cell is covered by chloroplasts, while when in bright light the part of the surface covered with chloroplasts is much smaller and at the same time their layer becomes thicker (Plate I). This phenomenon is illustrated on Fig. 1.

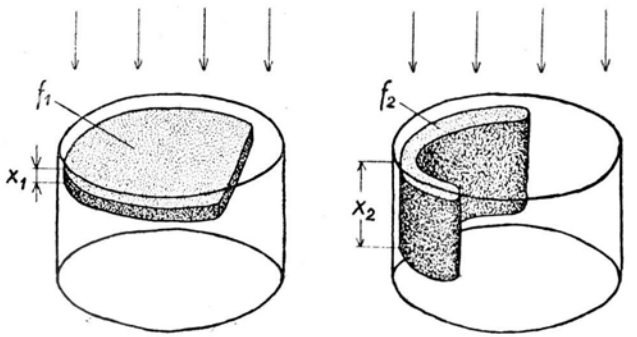

Fig. 1. Diagram illustrating changes in arrangement of chloroplasts under the influence of light. Left — arrangement in optimum light conditions (epistrophe) and right — in strong light (parastrophe).

What are the changes in energy absorbed when the chloroplasts displace themselves from one position to another?

Let i be the intensity of light falling on a unit of the surface area

f — the area of the surface of chloroplasts directly illuminated (or rather the area of the projection of that surface on a plane perpendicular to the rays of light)

x — the average thinckness of the chloroplast layer measured in the direction of light rays.

Assuming that[2]

[2] The equity of the assumptions will be dealt with in the course of the discussion.

(a) light is absorbed only by chloroplasts and passes through the remaining parts of the cell without loss
(b) the chloroplasts is optical homogenus
(c) the absorption of light in chloroplasts follows the Beer-Lambert law

the amount of energy absorbed can be expressed by

$$I_a = I_0 - I_t$$

where I_a is the amount of energy absorbed
„ I_0 „ „ „ „ „ entering the cell
„ I_t „ „ „ „ „ leaving the cell

as

$$I_0 = i \cdot f$$
$$I_t = I_0 e^{-Ecx} = i \cdot f e^{-Ecx}$$

where E is the coefficient of extinction for given wave length and given concentration of the absorbing matter
c is concentration of the absorbing matter

then

$$I_a = i \cdot f \cdot (1 - e^{-Ecx})$$

in this equation f and x are correlated to each other. Assuming that
(d) the volume of the chloroplast does not change when the chloroplast is displaced or changes its shape

we obtain

$$f \cdot x = \text{const} = a$$

$$x = \frac{a}{f}$$

$$I_a = i \cdot f \cdot \left(1 - e^{-Ec\frac{a}{f}}\right).$$

In this expression I_a is only a function of f.

Considering that

$$\frac{de^{-Ec\frac{a}{f}}}{df} = \frac{Eca}{f^2} \cdot e^{-Ecaf}$$

derivative of this equation is

$$\frac{dI_a}{df} = i\left(1 - \frac{Eca}{f} \cdot e^{-\frac{Eca}{f}} - e^{-\frac{Eca}{f}}\right)$$

It can easily be shown that the value of this result is always positive because if

$$i\left(1-\frac{Eca}{f}\cdot e^{-\frac{Eca}{f}}-e^{-\frac{Eca}{f}}\right)>0$$

then as $i>0$

$$1-\frac{Eca}{f}\cdot e^{-\frac{Eca}{f}}-e^{-\frac{Eca}{f}}>0$$

or

$$e^{-\frac{Eca}{f}}>1-\frac{Eca}{f}$$

This in fact is so because

$$e^{-\frac{Eca}{f}}=1-\frac{Eca}{f}+\frac{(Eca)^2}{f^2\cdot 2!}-\frac{(Eca)^3}{f^3\cdot 3!}+\ldots+\frac{(Eca)^n}{f^n\cdot n!}.$$

The limiting value of the function I_a when $f=0$ is $I_a=0$
when $f=\infty$ is $I_a=iEca$

The diminution of the area f with the simultaneous increase of the thinckness of the absorbing layer x, is always followed by the diminution of energy absorbed. If an arrangement of a constant concentration of the absorbing matter (chlorophyll) is considered then the dependance of the changes in energy absorption on changes in area f is dependent on the extinction coefficient. Fig. 2 demonstrates an example of relative changes in light energy absorbed in a plate measuring initially 100 $\mu^2 \times 1\,\mu$ and diminishing gradually its directly illuminated surface (initially 100 μ^2) while simultaneous no decrease in volume and a consequent growth in its thickness takes place.

Moreover the following assumptions were made:

(e) that chlorophyll concentration was 6%
(f) the extinction coefficient was such that its logarithms were 3,5, 4,0, 4,5, 5,0.

It was also assumed that the plate of the initial shape absorbs 100% of energy. It appears from the graph that when the extinctions are small even a considerable diminution of the surface area and of thickening of the absorbing layer has only slight influence on the change in light absorption. It is not till the area becomes very small that the amount of light absorbed diminishes and then it does so abruptly. The greater the extinction the greater is the influence of

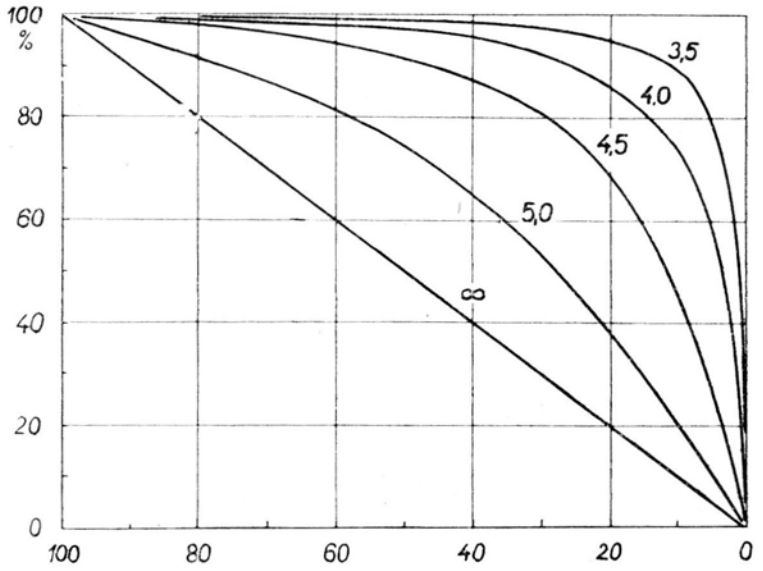

Fig. 2. Relative changes in absorption by a plate the surface area of which was originally f = 100μ² and the thickness x = 1μ while its volume remains constant. Numbers indicate the logarithm of extinction

the decrease in area. In the case of E = ∞, i. e. in the case of the substance which is wholly not transparent, the amount of energy absorbed would be directly proportional to the decrease in area.

So far it was assumed that the intensity of light i falling on the cell is constant. However when the chloroplasts are in the epistrophe arrangement the intensity of light is weaker then it is when they are in parastrophe.

Let

I_e, i_1, f_1, x_1 be the values of I_a, i, f, x in epistrophe
I_p, i_2, f_2, x_2 „ „ „ „ I_a, i, f, x in parastrophe

Then the amount of light absorbed in epistrophe is

$$I_e = i_1 \cdot f_1 \cdot (1 - e^{-Ecx_1})$$

and in parastrophe

$$I_p = i_2 \cdot f_2 \cdot (e^{-Ecx_2})$$

The amounts I_e and I_p are dependent not only on the extinction coefficient but also on the values of i_1, i_2, f_1, f_2, x_1 und x_2

If the energy absorbed both in epistrophe and parastrophe was the same, i. e. if the ratio

$$\frac{I_p}{I_e} = 1$$

or nearly equal one then it would be permissible to say that the system regulating the absorption of energy is perfect. On the other hand the more is the ratio $\frac{I_p}{I_e}$ greater than one and the more it approaches the value of the ratio $\frac{i_2}{i_1}$ the less efficient is the mechanism regulating the absorption of energy. When the chloroplasts do not change their position within the cell then

$$\frac{I_p}{I_e} = \frac{i_2}{i_1}$$

Experiments

To check what is the degree of efficiency of mechanism regulating by the movement of chloroplasts the absorption of light energy

1. the changes of f and x in 10 typical cells in epistrophe and parastrophe were investigated.

2. the intensities of light in which epistrophe and parastrophe occurs were estabilished

3. this efficiency i. e. the ratio $\frac{I_p}{I_e}$ for several values of extinction was calculated.

Investigations were done on 4 different species i. e. *Mougeotia sp.*, *Selaginella Martensii* Spring, *Funaria hygrometrica* Sibth, and *Lemna trisulca* L.

1. Measurement of the surface area f were made by drawing with an Abbe's apparatus under a microscope (objective 45 x and eyepiece 15 x) outlines of the cell and chloroplasts was estimated and calculated in μ^2. The thickness of the chloroplast layer was either obtained from direct measurement in every cell *(Mougeotia)* or the average thickness calculated from more then twenty chloroplasts on a cross-section of a leaf *(Selaginella)* was accepted or finally

by measuring the average thickness of chloroplasts and allowing for necessary corrections *(Funaria, Lemna).*

Mougeotia. In every cell of this alga there is one plate shaped chloroplast, which both in weak light and in optimum light conditions is placed perpendicularly to the light rays. In bright light the plate shaped chloroplast makes a turn of 90° and places itself paralelly to the light rays (S t a h l 1880, S e n n 1908). The breadth of the chloroplast in one position is its thickness in the other and vice versa, consequently an accurate estimation of x_1 and x_2 is very simple. The data on the area f and the thickness x for cells of *Mougeotia* in epistrophe and parastrophe and also the percentage the surface area changes in parastrophe in relation to the surface area in epistrophe are given in Table I. As it can be seen from this table when the chloroplast turns by 90° the surface area f diminishes to approximatly 1/4 of what it was before, at the same time the thickness of chloroplast increases approximatly four times. In comparison to the other plants investigated the changes of f and x resulting from the change in the arangement of chloroplasts is the greatest in *Mougeotia.*

TABLE I

Mougeotia sp.

i_1=25000 $\quad \frac{i_2}{i_1} = 3{,}2$

i_2=80000

No of cell	Epistrophe		Parastrophe		f_2 %	I_p/I_e			
	f_1 in μ^2	x_1 in μ	f_2 in μ^2	x_2 in μ		log E=4.5	log E=4.0	log E=4.5	log E=5.0
1	2106	5.0	479	22.0	22.8	2.72	1.97	1.11	0.76
2	2180	4.4	480	20.0	22.0	2.77	2.02	0.99	0.74
3	2410	4.6	505	22.0	21.0	2.29	1.96	1.09	0.70
4	2308	5.2	492	24.4	21.4	2.54	1.87	0.90	0.70
5	4100	4.8	957	20.5	23.9	2.74	2.01	1.16	0.78
6	2275	4.6	518	20.0	22.8	2.73	2.02	1.16	0.76
7	2035	5.0	518	19.6	25.5	2.74	2.08	1.22	0.84
8	2470	4 9	545	22.2	22.1	2,55	1.95	1.09	0.74
9	3410	5.3	850	22.0	24.4	2.14	2.09	1.23	0.82
10	3252	4.6	745	20.5	22.8	1.83	2.06	1.16	0.77

Selaginella Martensi. In *Selaginella* the movements of chloroplasts in the epidermis were investigated (P r i l l i e u x 1874, S e n n 1908, Z u r z y c k i and Z u r z y c k a 1951). In each cell of this plant there is one chloroplast which in weak light lines its cup shaped bottom. In bright light the chloroplast move to one of the side walls of the cell. The surface area of the chloroplast was obtained from diagrams made with Abbe apparatus while its thinckness in epistrophe was taken to be the average thickness of chloroplasts from 20 cells in a cross section of a leaf. The thinckness of the chloroplasts in parastrophe was calculated from the obtained values of f_1, f_2 and x_1 by assuming its constant volume. E. g. $f_1 = 594\ \mu^2$, $f_2 = 310\ \mu^2$, the average thickness of the chloroplasts in epistrophe is 7,3 μ then

$$x_2 = \frac{f_1 \cdot x_1}{f_2} = \frac{594 \cdot 7.3}{310} = 14.0\,\mu.$$

In Table II are given the values in epi- and parastrophe of f and x calculated for 10 cells of *Selaginella.* From this table it appears that the diminution of surface area in parastrophe is far smaller in this plant than in *Mougeotia* and on the average it is one half of the surface area in epistrophe. Correspondlingly the thickness of the chloroplasts in parastrophe is twice as big.

Funaria hygrometrica. In leaf cells of this moss there are numerous disc like chloroplasts the shape and the size of which is characteristic also for higher plants. In epistrophe they place themselves on the top and the bottom walls of the leaf cells, while in strong light they move to the side walls and arrange themselves parallely to the light rays (F a m i n t z i n 1867, S e n n 1908, V o e r k e l 1934). The difficulty in estimating f and x is that in epistrophy the chloroplast are arrranged in two layers one over the other of which one partly covers the other. When drawing in the arrangement of chloroplasts in the outline of the cell first the upper layer was drawn in and than after lowering the tube of the microscope the arrangement of the bottom chloroplast layer was sketched on the same drawing. Planimetry of the surface of the chloroplasts illuminated directly and the surface of the bottom chloroplasts shaded by the upper plasids was made separately. The area of the surface illuminated directly was taken to be f, while the area of the shaded surface served to calculate the corrections of x. The thickness of one layer was calculated from the average thick-

TABLE II

Selaginella Martensi

$i_1 = 500$ $\quad \frac{i_2}{i_1} = 30$

$i_2 = 15000$

No of cell	Epistrophe		Parastrophe		f_2 %	I_p/I_e			
	f_1 in μ^2	x_1 in μ	f_2 in μ^2	x_2 in μ		log E=3,5	log E=4,0	log E=4,5	log E=5,0
1	594	7.3	310	1.40	52.3	28.40	24.55	18.89	15.78
2	580	7.3	317	1.34	54.7	28.64	25.10	20.07	16.52
3	634	7.3	243	1.91	41.8	27.13	21.45	24.37	11.58
4	513	7,3	203	1.85	39.5	27,26	21.75	14.79	11.96
5	486	7.3	202	1.76	41.6	26.71	22.30	15.38	12.56
6	513	7.3	229	1.64	44.7	27.89	23.05	16.50	13.50
7	445	7.3	283	1.15	63.6	29.06	26.48	23.00	19.24
8	405	7.3	216	1.37	53.3	28.75	24.86	19.27	16.12
9	418	7.3	217	1.41	51.7	28.30	24.54	18.78	15.68
10	358	7.3	203	1.29	56.6	28.65	25.44	20.22	17.17

ness of 20 chloroplasts. In order to simplify the calculations the bottom shaded area was treated as if it spread evenly with uniform thickness under the whole of the surface f and increasing its thickness. E. g. the area of directly illuminated chloroplasts is $783\,\mu^2$. the area of the shaded surface is 103 μ^2 the average thickness of chloroplasts was calculated to be 1,8 μ The volume of the shaded layer of chloroplasts will therefore be $103 \cdot 1{,}8 = 185\,\mu^3$. If this layer wase spread evenly under the directly illuminated chloroplasts layer the thickness of such a layer would be $\frac{185}{783} = 0{,}24\,\mu$. In this example results are f = 783 μ^2 and x = 1,8 + 0,24 = 2,04 μ. Such an approximation is not free from error, but as some of the other assumption on which the calculation is based are also not very exact, this error can be neclected. In Table III are given the quantitative values obtained for 10 cells in the manner described above. The value of f_2 was obtained by direct measurement and of x_2 from the equation $x_2 = \frac{f_1 x_1}{x_2}$. As it appears from data obtained the diminution of the surface f in *Funaria* is smaller then in *Selaginella* and is as a rule 1/2.

TABLE III

Funaria hygrometrica

$i_1 = 5000$ $\quad \frac{i_2}{i_1} = 13$

$i_2 = 65000$

No of cell	Epistrophe			Parastrophe		f_2 %	I_p/I_e			
	f_1 in μ^2	f II layer	x_1 in μ	f_2 in μ^2	x_2 in μ		log E=3,5	log E=4,0	log E=5,0	log E=5,0
1	783	103	2.04	392	4.08	50.0	12.60	12.21	10.97	8.86
2	720	61	1.95	425	3.30	58.8	12.76	12.45	11.62	10.53
3	898	109	2.02	459	3.95	51.1	12 80	12.24	10.85	8.33
4	817	159	2.15	452	3.88	55.3	12.78	12.26	11.04	8.76
5	1000	162	2.09	587	3.56	58.8	12,48	12.15	11.28	8.94
6	695	101	2.06	331	4.33	47.6	12.90	12.13	10.50	7.84
7	851	170	2.16	500	3.68	58.7	12.63	12.32	11.38	9.33
8	412	54	2.04	263	3.19	63.9	12.95	12.62	11.64	9.85
9	439	97	2.20	317	3.05	72.3	12.60	12.51	11.95	10.60
10	574	85	2.07	344	3.46	60.0	12.91	12.30	11.23	9.37

TABLE IV

Lemna trisulca

$i_1 = 800$ $\quad \frac{i_2}{i_1} = 15$

$i_2 = 12000$

No of cell	Epistrophe			Parastrophe		f %	I_p/I_e			
	f_1 in μ^2	f II layer	x_1 in μ	f_2 in μ^2	x_2 in μ		log E=3,5	log E=4,0	log E=4,5	log E=5,0
1	473	117	2.37	338	3.32	71.5	149.9	145.7	135.8	120.5
2	676	128	2.26	392	3.90	58.0	147.4	141.7	129.0	103.4
3	446	173	2,63	216	5.43	48.5	145.7	127.9	116.2	85.4
4	729	190	2.39	351	4.97	48.2	145.8	138.8	118.2	87.3
5	783	212	2.42	351	5.40	44.9	148.1	136.0	114.7	81.7
6	784	204	2.39	446	4.20	56.9	146.1	142.5	125.2	100.5
7	594	181	2.48	364	4.05	52.3	148.3	140.7	128.5	105.2
8	540	72	2.15	311	3.73	57.5	146.5	142.6	128.9	104.1
9	553	138	2.47	364	3.77	65,8	148.2	144.6	133.7	112.6
10	551	108	2.29	297	4.25	53.7	147.2	141.7	124.5	97.4

Lemna trisulca. The shape, the arrangement and the displacement of chloroplasts are in this plant the same as in *Funaria* (S t a h l 1880, S e n n 1908, Z u r z y c k a 1950), the same were also the methods used for measurement and calculation of f and x. The data are given in Table IV. The diminution of the surface area f relatively to the surface area in epistrophy is here as in *Funaria* 1/2.

2. To investigate the dependence of the arrangement of chloroplasts on the intensity of light the apparatus described in a previous paper (Z u r z y c k i and Z u r z y c k a 1951) was used. The plant material were placed in a drop of water in a humidity chamber according to G a u t h e r e t ' s method. As a saurce of light either a low voltage 35W 12V electric lamp or a projection 300W 110V lamp were used. The intensity of light was measured with a photometer. The arrangement of chloroplasts was drawn in the same cells before and after the cell was subjected to each of the investigated intensities of light. The area of the surface of chloroplasts in epistrophy was taken to be 100% and to this value was related the area after illumination. The results are illustrated by fig. 3. The sensitivity of chloroplasts to light varies greatly in the

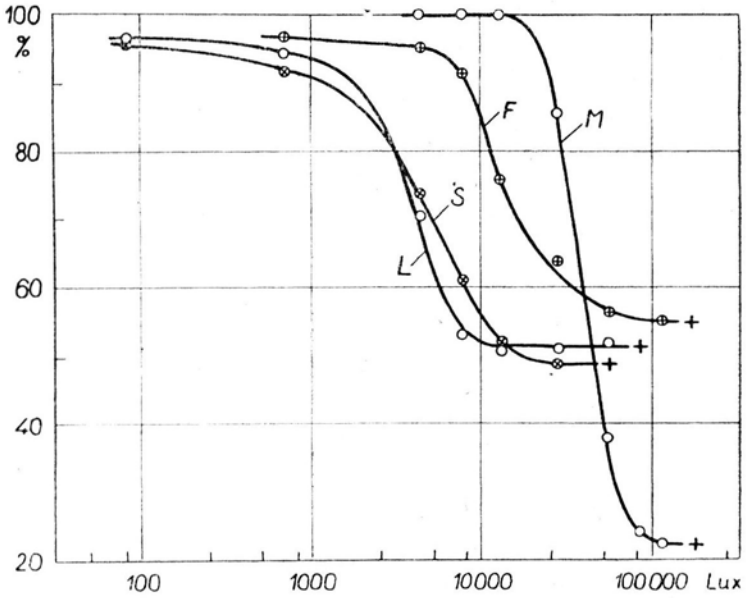

Fig. 3. The dependence of surface area f expressed in % (ordinates) on the intensity of light in lux (abscissae). The cross indicates the harmful action of light on chloroplasts.

different plants. The most sensitive are *Lemna* and *Selaginella*, considerably less sensitive is *Funaria* and the least sensitive is *Mougeotia*. In *Lemna* light intensity of 12 000 lux is sufficient to cause complete parastrophe while in *Mougeotia* this same intensity induces no movement of chloroplasts. In Table V are given the highest intensities of light in which the epistrophe still exists in the cell (i_1) and the lowest intensities which can induce parastrophe (i_2).

TABLE V

	i_1	i_2
Mougeotia	25 000	80 000
Selaginella	500	15 000
Funaria	5 000	65 000
Lemna	800	12 000

This method of estimating the sensitivity of chloroplasts to light can probably be used in ecological researches as one of the objective methods of photophilous and photophobic requirements of plants.

3. From (1) the data obtained about f and x in epistrophe and parastrophe and from (2) data denoting intensities of light proper to epistrophe and parastrophe the efficiency of the mechanism of light energy absorption was calculated i. e. the relation between the ratio of the energy absorbed in parastrophe to the energy absorbed in epistrophe for various extinctions. The results are tabulated in separate columns of Tables I—IV. In each table the ratio of the intensities of light causing parastrophe and epistrophe is also given. This ratio denotes how many times the absorption would increase if the arrangement of chloroplasts did not change.

As it should have been forseen from the introductory considerations a notable change in the absorption of energy when the chloroplasts change their position occurs only in those waves which are much absorbed (marked extinction), while the absorption of waves which are little absorbed remains almost the same regadless of the arrangement of chloroplasts. In green cells therefore the phototactic movements can cause a greater or a lesser absorption mainly of blue and red light while the rays of green light regardless of the arrangement of chloroplasts are always absorbed almost proportionally to the intensity of light.

It is only in *Mougeotia* that the mechanism regulating the amount of energy absorbed is really efficient. Those rays of which the absorption is greatest in parastrophe absorbed less than in epistrophe even when the intensity of light is three times stronger. The absorption in parastrophe of the waves somewhat less absorbed does not increase or increases only slightly. The efficiency of this mechanism is conditioned both by the small increase in the intensity of light which induces parastrophe as compared to the intensity of light proper to epistrophe and by the considerable changes in f and x when the arrangement of chloroplasts changes. The values of $\frac{I_p}{I_e}$ for all cells are nearly the same which is due to the considerable uniformity of the investigated material.

In three other plants the amount of light absorbed increases inspite of the movement of chloroplasts caused by the increase of light intensity. This increase in absorption is for waves little absorbed nearly as great as the increase in light intensity and for waves much absorbed far smaller then the increase of intensity. E. g. in *Selaginella* the intensity of light to cause the change from epistrophe to parastrophe must increase thirty times, while the absorption (for extinction log E = 5,0) increases only 12—15 times, in *Funaria* when the intensity of light increases 13 times the absorption increases 8—10 times; in *Lemna* those values are: the increase in light intensity 15 times, increase in absorption 8—12 times. The results obtained for different cells often vary considerably. The greater is the increase whith — in the cell of the surface area f and the thickness x — the more marked is the regulation of the energy absorbed.

Fig. 4. illustrates schematically the changes in the amount of light energy absorbed (waves of which log. E = 4,5) in relation to the changes in the arrangement of chloroplasts in average *Mougeotia* and *Funaria* cell. At first and till the chloroplasts begin to move the absorption increases almost proportionally to the increase of intensity of light. Within the limits of intensities of light in which the transposition of chloroplasts is not complete the increase in absorption is lesser or nearly ceases in dependence on the efficience of the mechanism regulating the absorption. When complete parastrophe is reached the absorption again becomes proportional to the increase in the intensity of light. However as according to

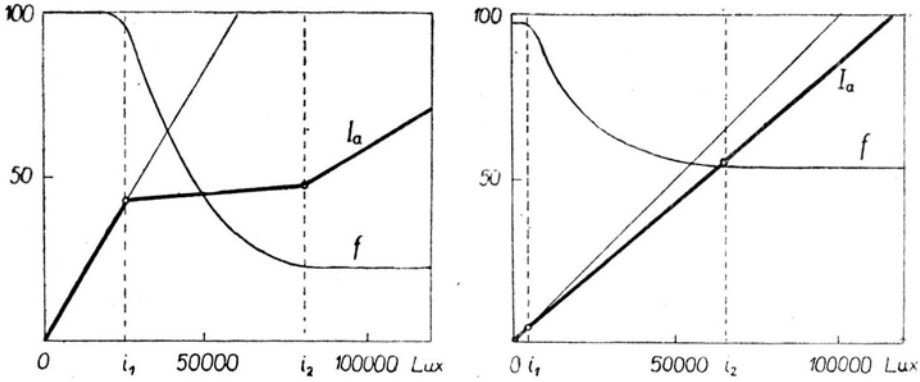

Fig. 4. Changes in absorption of light in relation to intensity of light, left — *Mougeotia*, and right — *Funaria*. Abscissae — intensity of light in lux, ordinates — changes in absorption and in surface area f.

the introductory reasoning

$$f_1 \cdot (1 - e^{-Ecx_1}) > f_2 \cdot (1 - e^{-Ecx_2})$$

the coefficient of proportionality is smaller in parastrophe than it is in epistrophe. If it is considered that maximum extinction values for live chloroplasts are probably approximately such that $\log E_{max} = 4{,}5$ and that average extinctions are far smaller then it follows that mechanism regulating the amount of evergy absorbed by the transposition of chloroplasts is little effective. *Mougeotia* would be an exception as its mechanism is quite efficient.

Discussion

The first experimental data on the influence of the arrangement of chloroplasts on the absorption of light in a plant cell are given by S t a h l (1909) who copied leaves on light sensitive paper and found that those leaves in which the chloroplasts are arranged vertically let through far more photochemically active light then leaves the chloroplasts of which are in the flat position. An attempt to deal with this problem quantitatively by thermoelectric measurements was made by S c h a n d e r l and K a e m p f e r t (1933) in their investigation on the transparency of leaves to white and coloured light. Unfortunatly they worked only leaves with numerous cell layers *(Tradescantia, Pelargonium, Adiantum* and *Coleus)* in which the changes in the arrangement of chloroplasts are less marked and probably have less influence on the absorption of

light than in leaves with only one or few cell layers. Also the wide range of transparency of filtres used make the interpretation of their results difficult. However the results obtained by Schander l and Kaempfert show that, when chloroplasts move from epistrophe to parastrophe, the absorption of the leaf diminishes (in the leaves experimented with by 2—15%, and on the average by approxim. 8%) and this diminution in absorption is most marked in blue light and far less in yellow-green and red light. This in priciple is in accordance with the present considerations.

It was possible to calculate the relation between energy absorbed in epistrophe and energy absorbed in parastrophe only when several assumptions were made. The correctness of these assumptions will now be discussed:

(a) The assumption that light passes through the cell without any loss of energy is not strictly true. If however we consider only the visible range of the spectrum and disregard its infrared and ultraviolet wave lengths then the absorption of this radiation by the parts of the cell with no pigment is extremely small in comparison to the light absorbed in chloroplasts. Seybold (1932) estimates, on the basis of measurements made with the epidermis of *Allium*, that one layer of cells absobes approximately 3% while one chloroplast of *Sagittaria* absorbs approximately 30% of the light falling on it. The absorption of light by the protoplasm and vacuole throughout the cell is therefore only 1/10 of what is absorbed in one chloroplast only. As in a cell there usually are either one or more layers of chloroplasts, or the chloroplasts is very thick *(Mougeotia, Selaginella)*, this ratio is probably less than 1/10. It follows that the absorption of light whithin the cell can be overlooked in the reasoning and the resulting error will be negligible.

(b) The assumption that chloroplasts are optically homogenous — necessary for enabling the application Beer-Lambert's law — is the most optional of all the assumptions made. The data obtained so far on the structure of chloroplasts show that 1° the chloroplast is composed of albuminous stroma and of grana in which probably are located all the chloroplast pigments. (Doutreligne 1934, Heitz 1934, Granich and Porter 1947). To determine the concentration of chlorophyll in chloroplasts it is assumed that the pigment is distributed evenly throughout the plastid, though actually the concentration in the grana is greater; the pigments are more concentrated, but the thickness of the layer they form is correspondingly thiner. This concentration of pigments has

no fundamental influence on the amount of light absorbed because as c increases x diminishes proportionally and so the expression e^{-Ecx} remains constant. 2⁰ The lamellar arrangement of albumin and lipoid strata and also the directive arrangement of lipoid particles (Frey-Wyssling and Steimann 1948) cause the chloroplasts to birefract light rays (for literature see Frey-Wyssling 1948). The birefrigeance however has no influence on the absorption of coloured and not polarized light and so the capability of chloroplasts to birefract light is no source of error in the present considerations. 3⁰. According to Hubert's (1935) and Frey-Wyssling's (1937) schema the mutual arrangement of chlorophyll and carotene particles is strictly defined. The porphyrin part of the chlorophyll is stuck onto the albumin layer while the phytyl chain reaches perpendiculary to the porphyrin ring into the hydrophobic layer. Such a mutual relation in the arrangement of pigments would have an important influence on the absorption of light. In the flat position (epistrophe) the absorption would mainly be caused by chlorophyll particles and less by carotenes while in the profile position (parastrophe) the opposite would be the case, and absorption would be due mainly to carotenes and only partly to chlorophyll. This schematic structure of chloroplasts is only hypothetic, though investigations on the influence of coloured light on phototactic chloroplast movements seem to corroborate it (Zurzycka 1950). The lack of any quantitative evidence on the possible difference in absorption of light by the inner structure of chloroplasts made it impossible to take this structure into account when the absorption of light was calculated. However it should be stressed that if such differences were existant they could cause fundamental changes both in the quantitative and qualitative relation of light absorbed in the flat and profile arrangements.

(c) To calculate the energy absorbed by chloroplasts the Beer-Lambert's law was applied to the light absorption of chloroplasts. The application of this law to chlorophyll solutions in different disolvents of various concentrations and also to chloroplast suspensions was often investigated (for literature see Hubert 1935, Sapożnikow 1951) and on the whole it has been found to be applicable to weak concentrations of chlorophyll. Wakkie 1934 (cited after Frey-Wyssling 1937) found divergences from this law for strong chlorophyll concentrations. which were caused by the maximum of absorption exceeding

6800 Å. However in chloroplasts in which the chlorophyll concentration remains constant and only the absorbing layer thickens this cannot happen. Seybold (1932) investigating the absorption of light in a different numbers of leaf layers of *Sagittaria montevidensis* found that the absorption of light of different colours was in agreement with Beer-Lambert's law. These results make admissible the assumption that as in several layers of laeves light is absorbed according to Beer-Lambert's law it will also be so absorbed by one layer of chloroplasts and the possible error will be very slight.

(d) Calculations of the thinckness of the absorbing layer in parastrophe *(Selaginella, Funaria* and *Lemna)* were based on the assumption that the volume of chloropasts does not change when they change their position. The few observations of the influence of light on the shape of chloroplasts (Senn 1908, Gicklhorn 1932) indicate that strong light causes sametimes what is called a contraction i. e. a change in shape of the chloroplast. As these observations are delt with only descriptively it is impossible to ascertain whether this change of shape is followed by change in volume. Even if such were the case a change in volume would introdue no error in the calculations, because intensities which could cause contraction were estimated as harmful and measurement were done only with weaker intensities of light.

(e) Euler, Bergmann and Hellstrom (1934) calculated the amount of chlorophyll contained in leaves of *Elodea densa* of which the surface area was know and after calculating from this result the amount of chlorophyll in one medium size chloroplast came to the conclusion that the concentration of chlorophyll varies from 5—6,5% — on average 6% — of the fresh chloroplast mass. Godniew and Kaliszewski (1940) obtained very similar results and estimated the average concentration of chlorophyll in chloroplasts of the leaves of *Mnium medium* moss to be 5,85%. The results obtained from chemical analysis of isolated chloroplasts (Menke 1939, 1940, Comar 1942) are difficult to interpret as they are obtained from dry not fresh material.

From the above results the concentration of chlorophyll in chloroplasts was taken to be 6%.

(f) It is hard to estimate the degree of extinction in chlorophyll of living chloroplasts though some informative data can be obtained from investigations on the absorption of light in chloro-

phyll solutions. Data concerning a chlorophyll solution in ether obtained from two sources are given in Table VI. The logarithm of the coefficient of extinction in a 1 cm deep molar solution (molecular weight 900) was taken as unit of extinction.

TABLE VI

	Log of extinction	Wave lenght λ	Source
E maks.	5,0 (chl. *a*)	~ 430	Heierle 1935
	5,1 (chl. *b*)	~ 450	
	5,4 (chl. *a*)	428	Comar i Zscheile 1942
	5,5 (chl. *b*)	455	
E min.	3,5 (chl. *a*)	~ 480	Heierle 1935
	3,5 (chl. *b*)	~ 500	
	3,4 (chl. *a*)	470	Comar i Zscheile 1942
	3,4 (chl. *b*)	500	

Any conclusion on the absorption in chloroplasts can be drawn only from the work of R a b i d e a u, F r e n c h and H o l t (1946) who investigated among others the absoption spectrum of a suspension of chloroplasts calculating at the same time the amount of chlorophyll in the suspension. Data based on the work of the above writers and calculated in the same manner as for Table VI are given in Table VII. Four coefficients of extinction the logarithms of which were 3,5 4,0 4,5 5,0 were taken into account when the absorption by chloroplasts was calculated. On the basis of the results obtained by R a b i d e a u, F r e n c h and H o l t it can be accepted that the minimum extinction in chloroplasts is such that its logarithm is approximately 3,5 and the logarithm of the maximum extinction is between 4,0 and 4,5.

When comparing in the four plants the efficiency of the mechanism regulating — by means of chloroplasts movements — the absorption of energy it appears that this mechanism — with one exception i. e. *Mougeotia* — is not very effective. This is probably due to the fact that in *Selaginella, Funaria* and *Lemna,* chloroplasts move to the side walls of the cell they find themselves in the parts of the cell which are lighted less brightly (S e n n 1908). It follows that the amount of light absorbed is influenced not only by

TABLE VII

	Log of extinction	Wave lenght λ	Material
E Maks.	4,23	436	Spinacia
	4,25	691	
	4,33	~ 420	Spinacia
	4,22	~ 676	
	4,38	~ 420	Dolichos
	4,38	~ 680	
E min.	3,66	578	Spinacia
	3,48	~ 540	Spinacia
	2,73	~ 750	
	3,75	~ 540	Dolichos
	2,81	~ 750	

the change of the shape of the absorbing layer but also by the lesser intensity of light which reaches the chloroplasts when they are on the side walls of the cell. In *Mougeotia* the chloroplast moves about the center of the cell and the intensity of light which reaches it both in epistrophe and parastrophe is the same as the intensity which reaches the cell. It is therofere only the change in shape of the absorbing layer that can regulate the energy absorbed, and as it has ben calculated such a system in the case of *Mougeotia* is quite sufficient.

If after complete parastrophe is obtained the intensity of light further increases then the light absorption will increase proportionally to the increase o its intensity. Inspite of this the plant can usually stand even a considerable increase in the intensity of illumination. If the mutual arrangement of chlorophyll and carotene particles is in accordance with Hubert's (1935) and Frey-Wyssling (1937) hypothesis, then light absorbed in parastrophe is of a different nature than light absorbed in epistrophe and so it is possible that inspite of its intensity it is less harmfull to the plant.

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SUMMARY

1. The absorption of light by chloroplasts in different positions in the cell was discussed from the physical point of vue.

2. Quantitative data characteristic for the arrangements of chloroplasts in four plant i. e. *Mougeotia, Selaginella, Funaria* and *Lemna,* were given, and it was found that the surface of chloroplasts illuminated directly diminishes by as much as approximately 75% of the area in *Mougeotia* and by approximately 50% in the case of the remaining plants. In the four plants the changes in the arrangement of chloroplasts dependent on light intensity were examined and it was found that chloroplasts of *Lemna* and *Selaginella* are the most and of *Mougeotia* the least sensitive to light.

3. From the above data the relative changes in absorption of light energy for different coefficients of extinction were calculated.

4. The influence of phototactic movements of chloroplasts on the mechanism regulating the absorption of light energy in plant cells was discussed.

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EXPLANATION OF THE TABLE I:

Table. I. Arrangement of chloroplasts in epistrophe and parastrophe. Objective 40 x, eyepiece 7 x, blue filter.

Top left — *Mougeotia* × 360.
Bottom left — *Selaginella* × 510
Top right — *Funaria* × 430.
Bottom right — *Lemna* × 510.

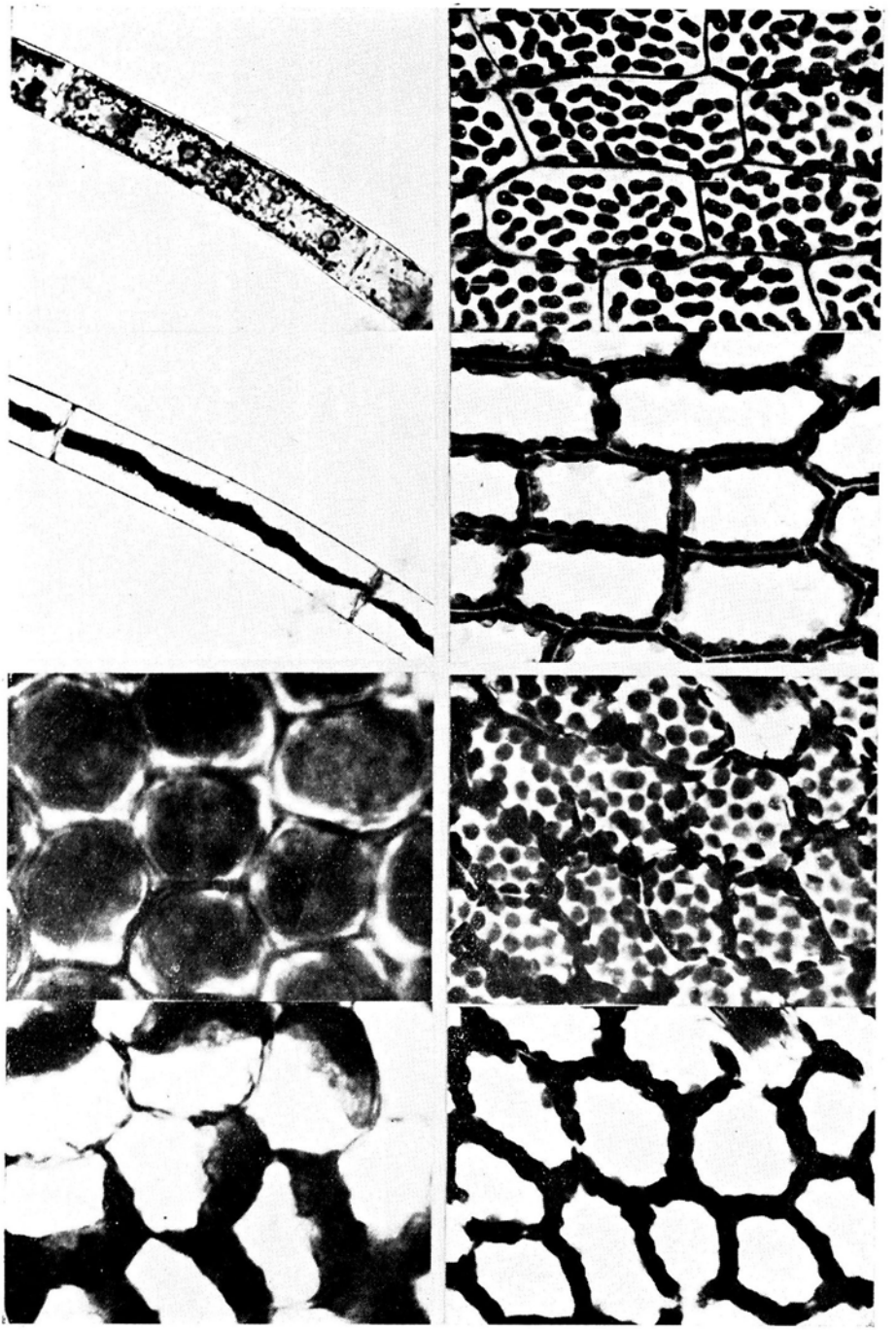

Table I.

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