ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXIV — Nr 1
1955

Chloroplasts arrangement as a Factor in Photosynthesis

J. ZURZYCKI

1. INTRODUCTION

The amount of light energy absorbed by a plant cell is of paramount importance for its photosynthetic activity. The factors affecting the amount of absorbed energy can be divided into two groups:

a) the first includes the environmental conditions such as the light intensity and its spectral composition,

b) the second is formed by the ability of the living plant to modify these conditions or to adapt itself to them. This group includes the anatomical structure of the leaf, the concentration of chlorophyll and the arrangement of chloroplasts.

Little attention was so far given to the influence exercised on photosynthesis by the chloroplast arrangements. To our knowledge no quantitative investigations have been yet made on the relation between the assimilation rate and the arrangement of chloroplasts. However, it has been established both experminetally (S t a h l, 1880, S c h a n d e r l and K a e m p f e r t, 1933) and in the course of theoretical consideration (Z u r z y c k i, 1953), that the different positions occupied by the chloroplasts in the cell are not without effect on the amount of absorbed light energy and accordingly changes in the chloroplast arrangement are expected to modify the rate of photosynthesis. The problem is all the more interesting as the chlorophyll concentration and the anatomical structure are almost immutable; while the chloroplast arrangement changes every time the light intensity is modified (S e n n, 1908, Z u r z y c k a and Z u r z y c k i, 1953) and the plant seeks presumably to adapt itself to the new light conditions.

The aim of the present work is to investigate what effect phototactic chloroplast movements have on photosynthesis and in particular:

a) which are the time changes of photosynthetic rate when chloroplasts move under the influence of new light conditions;

b) what relation there may be between the light curve of photosynthesis and the curve illustrating the dependance of chloroplast arrangements on light;

c) to find the quantitative relation between the values of chloroplast arrangements and assimilation if a relation between these two processes exists.

2. MATERIAL

Three species with the ability to displace chloroplasts *(Mougeotia, Lemna* and *Funaria)* and — for control purposes — one deprived of it *(Spirogyra)* were used in the course of our investigations. There were several reasons for making this choice: 1) in these species phototactic movements of chloroplasts were already examined quantitatively (V o e r k e l, 1934, Z u r z y c k a, 1951, Z u r z y c k i, 1952, 1953), and since the displacements were considerable it could be expected that the concomittant changes in the assimilation rate would be pronounced; 2) the species are very convenient for microscopical observations, for they either form filamentous colonies or consist of leaves one *(Funaria)* or few *(Lemna)* layers thick; 3) the leaf area being small the microscopic observation of the whole leaf or at least of the greater part of it is made possible in the course of the experiment.

Funaria hygrometrica S i b t h. The plants were grown in the laboratory from spores. The leaves were cut off from the stalks with a sharp knife and the best developed and least damaged were selected after microscopic inspection. The leaf area ranged from 0.6 to 1.1 mm^2. Usually for measurements two leaves were simultaneously used.

Lemna trisulca L. The plants were obtained from a pond in the Botanical Garden of the University. Young leaves (or more exactly green shoots) consisting almost exclusively of a single mesophyll layer, were isolated from the pocket in the maternal leaf by pressing it lightly with a needle at the place where the leaf is attached. The leaf area ranged from 2 to 7 mm^2.

Mougeotia sp. The plants were collected from ponds in the Fishing Station in Mydlniki near Cracow; the cell dimensions were 26—28/110—180 μ, and in their chloroplasts 7 — 12 pyrenoids were discernible. From the algal wad a small bunch of a short filaments was drawn out with a needle and after microscopical inspection was used for the experiments. The total length of the filaments amounted to 50 — 180 mm.

Spirogyra nitida L i n k was collected from a natural habitata in the vicinity of Cracow. The dimensions of the cells (each with three chromatophores) were 75—96/111—215 μ. From a bunch of long filaments pulled out with a pincette segments 3—5 mm long were cut off with a sharp knife; the number of filaments in the bunch varied between 5—30 and their total length was 25—100 mm.

At the end of the experiments the leaves or the bunches of algal filaments were placed on the glass plate of a magnifying apparatus, and their images, magnified approximately ten times projected on photographic paper. On the photographs the leaf areas or the total lengths of the filaments were measured and formed the basis for the estimation of the amount of material used in the experiment. Several examples illustrating the dimensions and shape of the plant material are given in fig. 1.

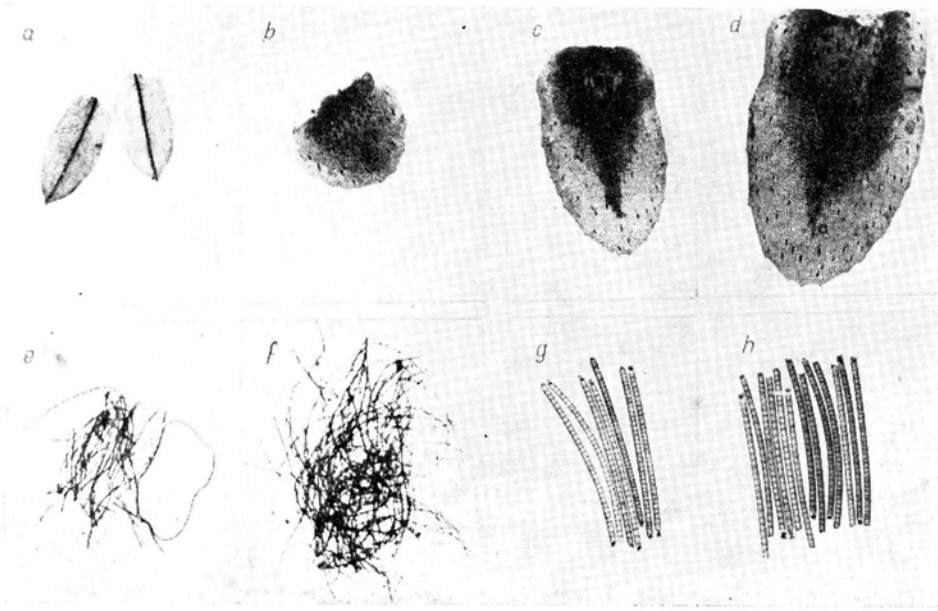

Fig. 1. Plant material used for experiments: a) *Funaria* — total area of two leaves 1,89 mm², b) *Lemna*-leaf area 1.85 mm², c) *Lemna*-leaf area 4.03 mm², d) *Lemna*-leaf area 7.57 mm², e) *Mougeotia*-total filaments length approx. 31 mm, f) *Mougeotia*-total filaments length approx. 110 mm, g) *Spirogyra*-total filaments length 16,4 mm h) *Spirogyra* — total filaments length 29.1 mm

3. METHODS

3.1. Dosage of light

The light source, a 1000 W projection lamp (model Osram E. 70), was connected to the mains through a slide rheostat, which permitted to maintain the voltage on the constant level 215 V ± 2 V. The details of the lightning arrangement can be seen from fig. 2. The light beam, made convergent by the lens S is filtered through the filter F and after reflection from the microscope mirror reaches the spot AB where the experimental chamber with the leaves or the algal filaments is placed. The filter, 10 cm

thick, was filled with a solution of ferrous ammonium sulphate in 2% H_2SO_4 prepared according to the prescriptions of W i t h r o w and P r i c e (1953). The filter transmits the visible light radiations almost without losses (400—700 m μ) but by absorbing the infrared radiations prevents the chamber with the plants from being overheated during illumination.

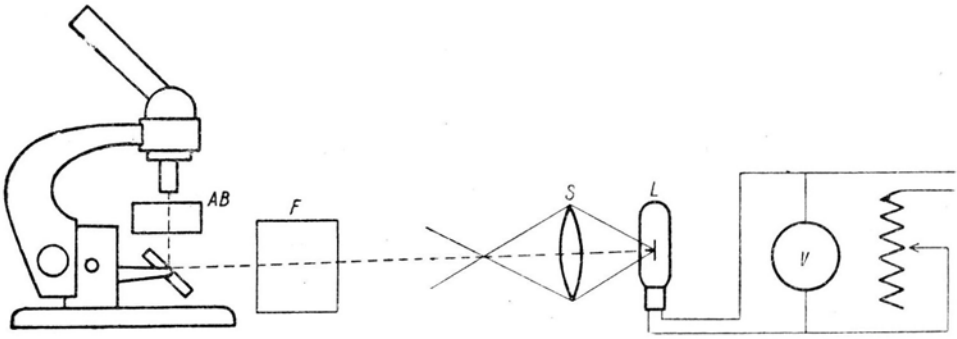

Fig. 2. Diagram illustrating illumination of objects during experiments. AB — box with reaction chambers, F — water filter, S — lens, L — projection lamp, V — voltmeter

The measurements of the light intensity were made with a photoelement (B. L a n g e) graduated in luxes and inserted exactly at the place normally occupied by the leaves or algal filaments.

The light intensity was modified by changing the distance between the light source and the microscope and, when very low light intensities were needed, by interposing matted glass plates.

3.2. *Measurements of chloroplast arrangements*

The construction of the plant chamber permitted the direct observation of the assimilating cells with a 100—150-fold magnification. In a previous paper (Z u r z y c k a and Z u r z y c k i, 1950), we have shown that it is possible to express numerically the differences occuring in the arrangement of chloroplasts. Thus in the case of *Funaria* and *Lemna* the number of chloroplasts in the epistrophe position was counted in ten cells, and the result expressed as the percent of the total number of chloroplasts contained (as far as possible) in the same 10 cells. A different procedure was applied in the case of *Mougeotia;* with the help of an Abbe drawing equipment the outlines of 5—10 cells and of their chloroplasts were drawn on paper, the corresponding areas planimetrically measured, and finally the areas of the chloroplasts were expressed as percentages of the cell areas.

3.3. *Measurements of photosynthesis*

In the present investigations the method used of the measurement of the assimilation rate ought to fulfil three conditions:

a) it has to be sensitive enough to enable the measurements of the gaseous metabolism in a leaf only several mm^2 in area, i. e. its sensivity had to be of the order 10^{-3} µl.

b) it must enable the illumination of the object with light of known and changeable intensity.

c) it must permit the observation of the assimilating cells with at least 100 fold magnification and simultaneously measurements of their photosynthesis intensity should be made possible.

Several microvolumetric methods were devised for measuring the gaseous exchanges with the accuracy here necessary (T o b i a s, 1943, G l i c k, 1946). Of these only two make possible the direct observation of the object experimented with (S t e f a n e l l i, 1937, T y l e r and B e r g, 1941) and neither of them is suitable for researches on photosynthesis.

The capillary microvolumeter described here is a modification of T o b i a s and G e r a r d's (1941) respirometer adapted to fulfil the above enumerated conditions.

3.3.1. T h e p r i n c i p l e o f t h e m e t h o d

The apparatus is a capillary respirometer of the differential type, i. e. consists of two chambers connected by a capillary tube. The displacements of a drop of liquid in the capillary indicate the relation changes of volume occuring in the two chambers. The two chambers form an airtight system without communication with the atmosphere (fig. 3).

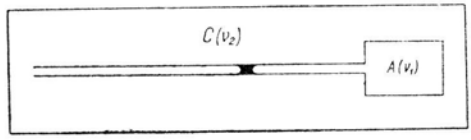

Fig. 3. Diagram illustrating capillary respirometer. C — compensation chamber of volume v_2, A — reaction chamber of volume v_1, index droplet in capillary

The leaf selected for the experiments is placed in chamber A together with a drop of Warburg's carbonate buffer. During photosynthesis or respiration carbon dioxyde is emitted or absorbed by the buffer, with the result that in the gaseous phase its concentration is maintained at a constant level and the observed volume changes in the gaseous phase are only due to the absorption or emission of oxygen by the plant.

According to Cunningham and Kirk (1940) following relation takes place between the change $\triangle v$ in volume v of chamber A and the displacement d of the indication drop in the capillary:

$$\Delta v = F \cdot d \cdot \frac{v_1 + v_2}{v_2} \cdot \frac{v_2}{v_2 - F d}, \tag{1}$$

where F is the effective cross-sectional area of the capillary, v_1 and v_2 the volumes of the reaction chamber and the compression chamber respectively. If $v_2 \geqslant v_1$, than the ratios $\frac{v_1 + v_2}{v_2}$ and $\frac{v_2}{v_2 - F d}$ are nearly equal to unity and therefore without commiting a significant error it can be assumed that

$$\Delta v = F \cdot d. \tag{2}$$

3.3.2. Description of the respirometer

In a brass plate 35 mm long, 25 mm wide and 2 mm thick two elliptical holes measuring 3 × 5 mm are made. At the bottom the holes are closed with a piece of glass fixed to the brass plate with shellac or aralgite. The bent and funel shaped ends of two capillaries are inserted in the immediate vicinity of the holes (fig. 4). From above the holes are closed by a glass plate set on a vaseline ring. The capacity of the two chambers A and B thus formed is approximatively 50 µl. Each chamber is connected by a capillary with the compression chamber. The function of chamber B consists in controlling temperature changes.

The capillaries are of the prismatic type, the cross section of their bore is slightly elliptic (capillaries with cylindrical bore have proved to be inconvenient). The cross sections of the bores ranged from 0,01 to 0,05 mm², however, the tubes most often used had a bore of 0,025 mm², and a length of approximatively 20 cm.

The brass plate together with the capillaries is placed in the compensation chamber C, the capacity of which is approximately 90 ml. It is made of sheet brass and is semicylindrical in shape. From the top it is fitted with a cemented glass plate G (fig. 5). The chamber can be closed by screwing down the rubber lined plate P and in this case the communication with the atmosphere is secured by the tap K. The compensation chamber is immersed in the metal box R filled with water supplied by a Höppler's ultrathermostat (during the experiments it appeared that a constant flow of water was not necessary as small changes of temperature did not influence the final measurements).

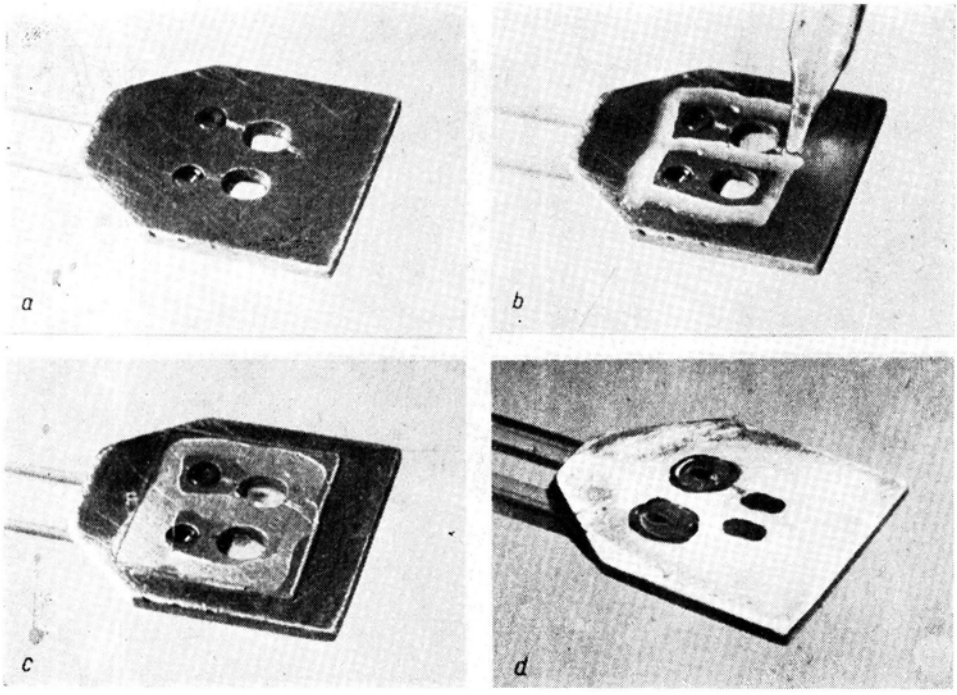

Fig. 4. Reaction chambers: a) the chambers are opened, b) smearing with vaseline, c) the chambers closed, d) another kind of reaction chambers smaller than those most often used

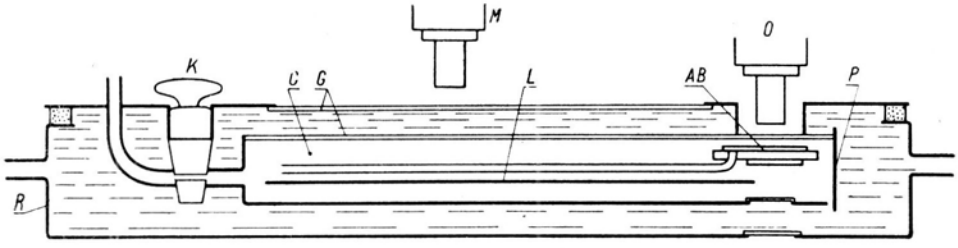

Fig. 5. Diagram illustrating box with compensation chamber. R — box with compensation chamber C, AB — plate with reaction chambers and capillaries, L — scale, K — tap joining compensation chamber with the atmosphere, G — glass panes for observing the scale with microscope M and the chambers with microscope O

The box is placed on the same stand as the Zeiss microscope (model LuWdE) from which the table has been removed, and the position occupied by the microscope is such as to permit the direct observation of the living leaf placed in the reaction chamber (fig. 6). Both reaction

chambers are illuminated with light which is reflected from the microscopic mirror, the condensor being removed, and penetrates to them through glass plates fitted into the bottoms of the compensation chamber and the water bath. 8x and 10x objectives were used for the observation of the material placed in the reaction chambers.

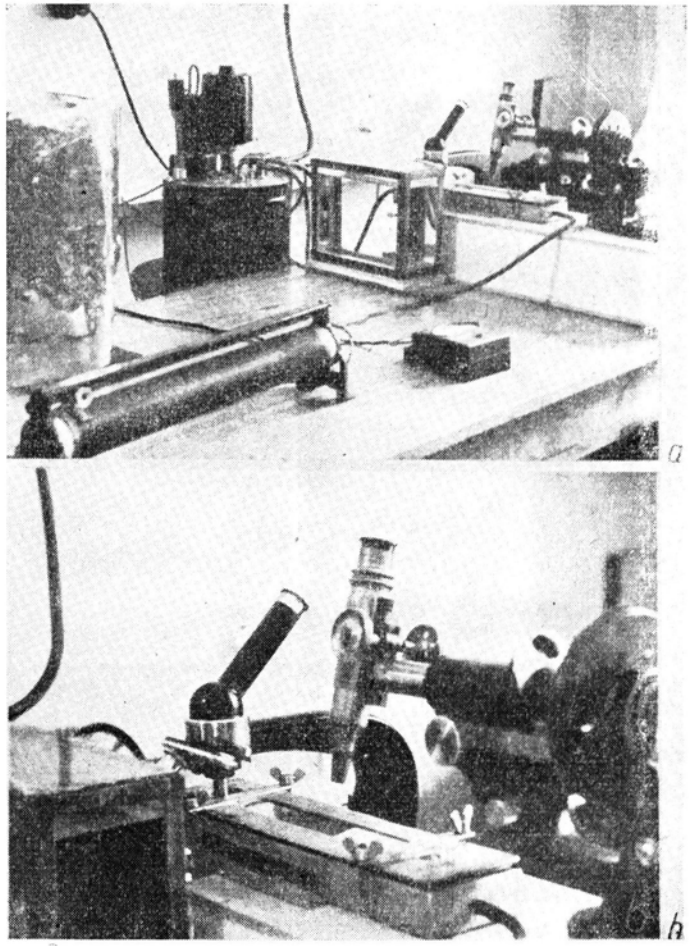

Fig. 6. a) The apparatus used for experiments, b) the box and the two microscopes

The position of the meniscus of the liquid in the capillary tube is defined with a microscope put in position according to M e t z n e r (1928), the magnifying power of the microscope fitted with an eyepiece scale being 30 times.

3.3.3. The experimental procedure

For cleaning, the capillaries are connected with their loose ends to a vacuum pump and in the funnel shaped ends drops of a convenient liquid are introduced. Successively they are cleaned with chromic mixture (for 5 mins), distilled water (for 15 mins) and alkohol (5—10 mins). Finally their ends are wrapped up in small cotton wool wads and the air filtered through cotton wool is passed for an hour through the capillaries.

A drop of kerosene formed the liquid indicator in the capillaries. The kerosene is purified according to Cunningham and Kirk (1940) by repeated shaking with concentrated H_2SO_4 and stored over NaOH. The introduction of the liquid into the capillaries is performed by dipping their loose ends into the kerosene which is forced into the bores by capillary attraction. When enough kerosene has been introduced into the tubes, a small ball of cotton wool is placed at its ends and the whole liquid is drawn out with the exception of a small drop forming a column of approximately 5 mm length. In this way the bore is covered with a very thin layer of kerosene, facilitating the displacement of the remaining liquid drop. The drop is pushed to the part of the capillary nearest the reaction chamber.

The borders of the reaction chambers are surrounded with white vaseline which is smeared in liquid state from a pipette (the metallic walls of the chambers are covered with a thin film of wax). With a micropipette 25 µl of Warburg's buffer no. 10, composed of 10 parts of 0.1 m Na_2CO_3 and 90 parts of 0.1 m $NaHCO_3$, are introduced into the bottom of the chambers.

Two leaves (or two buches of algal filaments) of more or less the same size are placed on a thick cover slide; the first leaf, after being killed by quick boiling, dries on the slide, and coated thinly with wax is destined for chamber B, while the second (the living), to be placed in chamber A, is covered with a drop of tap water. The cover slide after being turned over is put on the previously prepared vaseline borders, care being taken, that the two leaves occupy each the middle of the corresponding chambers. Thus the leaves are inside hanging drops and not in direct contact with the buffer solution from which they are separated by a thin layer of air (fig. 7).

The movement of the liquid indicators in the capillaries shows that the chambers are tightly closed. By applying a convenient pressure on the cover slide the kerosene drops can be manoeuvred to occupy a position in the middle of the capillaries side by side. Then the reaction chambers are placed in the compensation chamber and left for about 20 minutes for the adjustement of temperatures, care being taken to open the tap. Changes in the positions of the index droplet are measured under

low microscopic magnification. The field of visibility of the microscope is approximatively 5 mm in diameter. The instrument is adjusted with the macro- and mircometric screws so that its eyepiece scale coincides with the milimeter scales placed between the capillaries, then the position of the external meniscus of the kerosene drop is read with an exactitude of 0.05 mm.

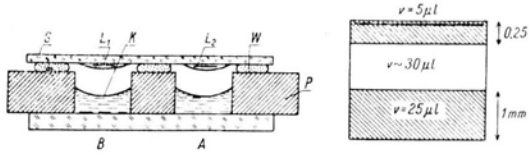

Fig. 7. a) Cross section diagram of reaction chambers AB. P — metal plate, K — carbonate buffer solution, G — cover glass W — vaseline ring, L_1 — dead leaf, L_2 — living leaf in water drop. b) Diagram of simplified arrangement of liquid drops in the chamber. Data obtained from this diagram are used to calculate the diffusion rate of gases

Before starting the experiments the value of coefficient I (see page 37) must be determined. For this purpose first the positions of the index droplet in the capillaries are noted, then the tap K is opened, the air in the compensation chamber is compressed or rarefied several times and each time the displacements of the both kerosene drops in the capillaries are read. The ratio $D_A : D_B$, where D_A and D_B are the displacements of the drops in the tubes A and B respectively, gives the value of I. In the calculations of the experimental results the mean of several determinations of I is used. The main experiments are not begun till after another 20 minutes.

The temperature during all experiments ranged from 19 to 22°.

3.3.4. Estimations of errors

I. The magnitude of the error introduced by the application of the simplified equation (1) depends upon $\triangle v$. However the amount of oxygen liberated in a single experiment never exceeds 0.5 µl, but even if it is assumed that $F \cdot d = 1$ µl, the value of $\triangle v$ calculated from equation (1) will be greater only by 0.000567 µl than the value calculated according to the simplified relation (2). It follows from these considerations that the simplification of equation (1) introduces a constant error which causes the readings to be 0.057% smaller than the change in volume which takes place in reality.

II. Precision of readings. Owing to the microscopic magnification it is possible to read the positions occupied by the menisci with an accuracy of the order of 0.01 mm. In practice, however, this accuracy is reduced to 0.05 mm by the errors resulting from the repeated

adjustements of the microscope to successive sections on the capillary scale. This in turn introduces an error in the determination of $\triangle v$ depending upon the area of the bores of the capillary tubes. This error ranges from 0.00053 µl when the area of the inside bore is 0.0106 mm² to 0.00183 µl when it is 0.0567 mm².

III. Errors caused by readings not being simultaneous in both capillaries. With some practice it takes 20—30 seconds to make one reading. Assuming intense production of oxygen (e.g. 100×10^{-3} µl per 10 mins), a difference of 0,5 minute between the readings in the two capillary tubes may cause an error as big as 5×10^{-3} µl. In most cases however, oxygen is liberated or consumed at a rate approximately 10 times slower. Moreover, the readings are alternately taken first in one capillary and then in the other and as a result this kind of error may be reduced almost completely.

IV. Precision of the capillary tubes. The capillary tubes were calibrated by measuring the changes in the length of a short column of mercury which was past through the tubes. In most capillary tubes used in this work the differences in the internal bore did not exceed 0.5%.

V. The film of kerosene, which coates the inside walls of a capillary tube, diminshes its inside bore and makes the area F smaller than that established with mercury, when the tube is still dry. The correction is calculated from the decrease in the length of a column of kerosene passed through a clean and dry capillary tube. This correction is always taken into account in establishing the working area of the inside bore of capillaries. When the capillaries are very thin the correction is 1.5 to 2.5% of the bore area, which is rather great value especially if it is compared with Cunningham's and Kirk's (1940) data perhaps it is due to the elliptic shape of cross sections used in this work.

VI. The resistance of kerosene column in the capillary. When the column of kerosene is being moved it offers a certain resistance and to overcome it, a difference of pressure on two sides of the drop is necessary. To establish exactly the force of this resistance an apparatus — the diagram of which is given in fig. 8a — is used. To a vessel 1.5 l in volume a capillary is joined and the kerosene drop in the tube can be observed with a microscope. A tube 2 mm² in cross section area is joined to the vessel and filled with mercury. The level of the mercury in the tube can be regulated by pressing a rubber reservoir. With this arrangement the pressure of the gas in the vessel can be changed in a known way, and the moment at which the kerosene drop in the capillary begins to move is observed with the microscope. Fig. 8b illustrates how the resistance depends on the length of the kerosene column for two cross sections areas of capillaries. The resistance is ex-

pressed in terms of the volume $\triangle v$ by which the volume of the reaction chamber ($v = 50\ \mu l$) must be changed to produce a movement of the kerosene drop. It appears from the graph that when the length of the kerosene column is approximately 5 mm, even in very thin capillaries

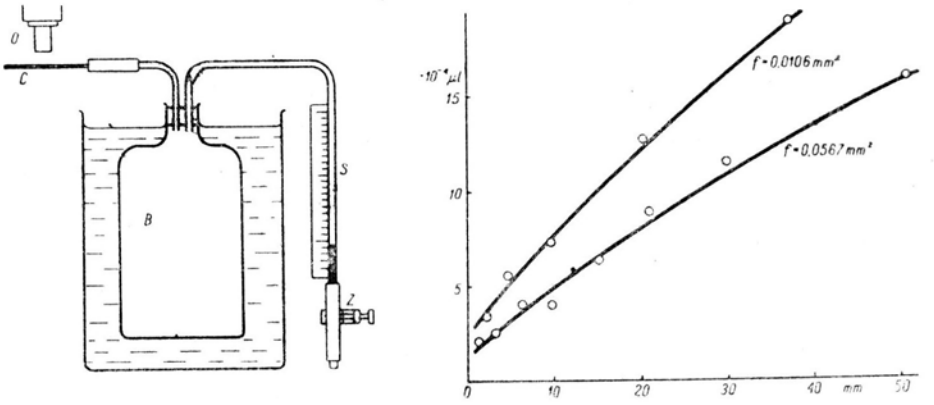

Fig. 8. a) Diagram illustrating apparatus for measuring resistance of kerosene drop in capillary. C — capillary, O — microscope objective, B — vessel in water bath, S — graduated glass tube, Z — mercury reservoir. b) Relation between reaction chamber volume increase (v — 50 µl) which causes movement of kerosene column (ordinates) and the length of kerosene column (abscissae) for two capillary diameters

a change in volume of 0.0005 µl causes the meniscus to move. It must be noted that the above estimation is made for very unfavorable conditions, in fact the volume of the gaseous phase in the reaction chamber is more than half smaller because of the volume of the liquids which are also in the chamber. Moreover, if the indicating drop does not change the direction of its movement, the difference of pressures on both sides of it is maintained on the very limit of static resistance and the slightest change in volume causes a movement of the meniscus.

VII. T e m p e r a t u r e c h a n g e s. In the closed system a general change in temperature causes no movement of the drop in the capillary tube, however, such a movement is caused by differences in temperature between a) the reaction chambers and the compensation chamber, or b) the two reaction chambers. A change of temperature of $\triangle t$ causes in the reaction chambers:

1) a change in volumne of the gaseous phase $\triangle v_g = v_g \cdot \alpha \cdot \triangle t$ where v_g is the volume of the gaseous phase, α — the expansion coefficient of the gas;

2) a change in volume of the liquid phase: $\triangle v_c = v_c \cdot \beta \cdot \triangle t$ where v_c is the volume of the liquid phase, β — the expansion coefficient of liquid;

3) a change in the solubility of the gaseous phase $\triangle v'_g = v_c \cdot \gamma \cdot \triangle t$ where γ is the change in solubility coefficient per 1^0;

4) a change in vapour pressure of the liquid $\triangle p = \delta \cdot \triangle t$ where δ is the change of vapour pressure per 1^0.

a) The case a, i. e. when a difference in temperature arises between reaction and compensation chambers, is quite common, mainly when the reaction chambers are illuminated with bright light. However, if the two reaction chambers are heated evenly, the changes as in points 2,3 and 4 are in both chambers the same because v_c in both chambers is the same. On the other hand differences in the displacement of the indicator drops may arise if v_g is different in one of the chambers. To eliminate these differences the absolute values of v_{gA} and v_{gB} need not be known, it is sufficient to know the ratio $\frac{V_{gA}}{V_{gB}} = I$. This ratio is easily obtained from measurements of displacements of indicator drops at known air pressures in the compensation chamber, which was already described. By multiplying the displacement of the meniscus in chamber B by I a value is obtained, which is exactly comparable with the meniscus displacement in the capillary A. The displacements of the indicators in the capillaries caused by heating the still empty chambers are given in Table I.

Table 1

Meniscus movements caused by heating of chambers. 25 µl of buffer solution are placed in chambers A and B. I = 1.351, f of capillary = 0.050 mm²

Time in minutes	V_A scale units	V_B scale units	V_B I scale units	$V_A - V_B$ I	
				scale units	10^{-3} µl
0	0	0	0	0	0
5	— 31	— 18	— 24	— 7	— 3,5
10	— 63	— 52	— 70	+ 7	+ 3.5
15	— 101	— 80	— 108	— 7	— 3,5
20	— 140	— 100	— 131	— 9	— 4,5
25	— 175	— 135	— 183	+ 8	+ 4.0
30	— 223	— 158	— 214	— 9	— 4,5
40	— 311	— 232	— 312	+ 1	+ 0,25
50	— 383	— 278	— 375	— 8	— 4,0
60	— 423	— 312	— 422	— 1	— 0,5

When the coefficient I, which in this case is 1.351, is included the data obtained show, that in neither chamber gas is produced and that the differences in displacement of the drops in the capillaries are caused by the heating of chambers with different v_g.

b) A difference in temperature between the two reaction chambers would cause errors which could not be checked. As precautions have been

taken to ensure against such a possibility it can be assumed that this difference is not considerable. The precautions mentioned are: 1) the two chambers are close together in an easily heat conducting medium, 2) both chambers are illuminated in the same way, 3) a dead leaf approximately of the same area and the same absorption is placed in the control reaction chamber B.

To control whether the two chambers are evenly heated when illuminated, they are first illuminated for some time with strong light, which is then turned of and the chambers are left in darkness. If the temperature in both chambers, changes evenly, the measurements after the light is turned of, when plotted in a graph, give a straight line. If, however, the temperature in one of the two chambers (A or B) is higher than in the other, the curve plotted for darkness is irregular. Such a control is absolutely necessary mainly when strong light is used for experiments, and only those experimental results can be accepted, which are checked for the even heating of the two chambers.

VIII. Changes in carbon dioxide concentration. The air which is in equilibrium with the no. 10 buffer has at 20° 0.45% of CO_2 (assuming that the amount of CO_2 in solution is 140.10^{-6} mol/liter) which means that this concentration is approximately 15 times greater than in the atmosphere. If it is assumed that the maximum consumption of carbon dioxide in the course of one experiment is 1 µl, then the carbon dioxide concentration in the 25 µl of the buffer falls from $140 \cdot 10^{-6}$ to $112 \cdot 12^{-6}$ mol/liter, i. e. by approximately 20%. Whe the carbon dioxide concentration is at this fairly high level, a difference of 20% in the concentration (a decrease from 0.45% to 0.36%) can have no important effect on the course of photosynthesis.

The change in the CO_2 concentration changes the amount of this gas dissolved in the liquid phase, and this in turn can influence the volume of CO_2 in the gaseous phase. The decrease in the amount of CO_2 in the gaseous phase depends on $\triangle v$ and when $\triangle v$ is in maximum and equales 1 µl — i. e. when the concentration falls by 20% — the decrease in the amount is equal to $v_c \cdot \alpha_{co2} \cdot \triangle p_{co2} = 25 \cdot 0.878 \cdot 0.0009 = 0.0198 \approx 0.02$ µl, where v_c is the volume of the liquid in µl, α_{co2} the coefficient of CO_2 solubility, and $\triangle p_{co2}$ the percentage fall of CO_2 concentration in the gaseous phase. The change in the amount of CO_2 dissolved in the liquid phase gives a constant error of 2%, by which the results obtained exceed the actual process.

IX. Changes in O_2 concentration. The same reasoning as the one just made when applied to the concentration of oxygen (p_{o2} — the initial concentration is 0,2, and the maximum change of volume 1 µl) shows that the amount of oxygen changes by 17%. The

decrease of the amount of oxygen in the gaseous phase corresponding to this drop of concentration is $v_c \cdot \alpha_{o2} \cdot \triangle p_{o2} = 25 \cdot 0.0309 \cdot 0.034 = 0.0315$ µl. This also gives a constant error of 3.1% by which the results obtained are smaller than the actual process.

X. Rapidity of diffusion. As the reaction chambers of the respirometer are not shaken, it is possible that gases are not diffused quickly enough to attain, in the intervals between two measurements, an equilibrium between the amount of gas which is assimilated or given out by a leaf and the corresponding change in volume of the gaseous phase. To solve this question Linderstrøm Lang's (1942) theory of non stationary states is used. For the calculations simplified shapes of the liquid in the chamber are assumed as shown in fig. 7b. It is necessary to discuse three points:

a) the rapidity of carbon dioxide diffusion between the gaseous phase and the leaf which can be assumed to be an infinitely thin element at the bottom of a drop.

b) the rapidity of oxygen diffusion in the same conditions,

c) the rapidity with which carbon dioxide is absorbed or given out by the drop of buffer.

The ratio of the true molecular pressure, in the gaseous phase of the gas in question, to the pressure which would be exerted if equilibrium were reached instantly is defined by the following equations:

a) $$\frac{P_t}{P_m} = 1 - \frac{1}{6} \frac{3 v_g + v_c \alpha}{\alpha v_c + v_g} \cdot \frac{b^2}{D t} \sum_k \frac{\alpha_k}{q} \left[1 - \exp\left(-\beta_k^2 \frac{\pi^2}{4 b^2} D t \right) \right] \quad (3)$$

(deduced from Linderstrøm Lang's (1942) equation 71)

b) $$\frac{P_t}{P_m} = 1 - \frac{1}{2} \frac{b^2}{D t} \left[1 - \exp\left(-\frac{\pi^2}{4 b^2} D t \right) \right] \quad (4)$$

(if $\alpha = 0$ then from (3))

c) $$\frac{P_t}{P_m} = 1 - \frac{1}{3} \frac{v_g}{v_c \alpha + v_g} \frac{b^2}{D t} \sum_k \frac{\alpha_k}{q} \left[1 - \exp\left(-\beta_k^2 \frac{\pi^2}{4 b^2} D t \right) \right] \quad (5)$$

Linderstrøm Lang's (1943) equation 3).

where: P_t — true molecular pressure of the gas in the gaseous phase during time t,
P_m — molecular pressure of gas in the gaseous phase which would be exerted if equilibrium were reached instantly,
V_g — volume of gaseous phase in ml,
V_c — volume of liquid phase in ml,
b_1, b_2 — depth of liquid layer in cm,
α — solubility coefficient of gas in liquid,
D — diffusion constant in cm/hour,
t — time in hours,

q — amount of gas produced or absorbed by each volume unit of liquid phase per time unit,

α_k β_k — coefficients defined by L i n d e r s t r ø m L a n g's (1942) equations 53, 60 and 70, and equations 4 and 5 (L i n d e r s t r ø m Lang 1943).

Fig. 9 shows with what rapidity equilibrium is reached in three instances detailed above (points a, b, and c).

From the curve it appears that equilibrium between the leaf and the gaseous phase is reached fairly quickly, but the establishing of balance between the gaseous phase and the buffer lasts much longer. The graph

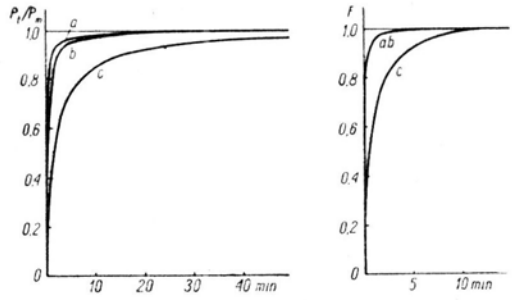

Fig. 9. P_t/P_m and F plotted against time for CO_2 diffusion from leaf to gaseous phase (a), for O_2 diffusion from leaf to gaseous phase (b) and for CO_2 absorption in buffer (c)

in fig. 9a is valid when it becomes necessary to establish the absolute amount of gas produced or absorbed from the beginning of an experiment till time t. In the present work there is no such necessity, though it is important to establish after what time the change per time unit in the amount of gas in the gaseous phase equals the amount of this gas produced or absorbed per time unit by the plant material experimented with. This relation is shown in fig. 9b and is obtained from equation

$$F = \frac{(v_g + v_l \alpha) \frac{d P_t}{d t}}{q \cdot v_l} \qquad 6)$$

where F — is the ratio of the changes per time unit in the amount of the gas in the gaseous phase (including the correction for solubility in liquid) to the production or absorption of the gas by the experimental plant.

From fig. 9b it appears that in the case of the slowest process, i. e. the absorption of CO_2 by the buffer, after 10 mins F nearly, and after 15 mins equals one. It follows that after 10—15 mins the rapidity of diffusion can cause no error.

XI. Evaporation of the water drop. During the experiments the leaf is placed in a water drop which 1) prevents it to be in direct contact with the buffer, as the pH of the buffer is very high and in some cases may be harmful to the leaf and 2) makes insignificant the ratio of K : Na ions. However, the shortcoming of the water drop is, that in one closed chamber there are two drops with different osmotic pressures and as a result the water drop evaporates and the vapour condensates in the buffer drop. In short, experiment lasting 2 to 4 hours, the evaporation of the water drop, has no practical importance, but when the experiment lasts more than 5 hours the water drop decreases considerably and it is necessary to open the chamber and supply the leaf with some water.

When all the possibilities of errors are considered they can be divided into two groups:

1. Constant errors which cause a constant difference between the readings made and the true changes in the volume of carbon dioxide. These are:

The simplification of equation (1)	— 0.057%
Changes in CO_2 concentration	+ 2.0%
Changes in O_2 concentration	— 3.1%

These errors make a difference only when the absolute assimilation rate is being calculated.

2. Accidental errors:

	capillary caliber 0. 01 mm²	capillary caliber 0.05 mm²
Resistance of the kerosene column	0.0005 µl	0.0002 µl
Precision of the capillary	0.00005 µl	0.00025 µl
Error caused by readings not being simultaneous	0.005 — 0.0005 µl	
Temperature changes and lack of precision in determining I	~ 0.005 µl	

Taking everything into account the precision of the method described here can be estimated to be 10^{-3} µl, i. e. the same as when the Cartesian diver method is used. However, if the experiments are to be succesful, many difficulties must be overcome and much time and patience is needed.

3.3.5. Recording of experiments

In Table II the record made during the experiment on epistrophe-apostrophe reaction is given as an example of other records taken in the course of this work (see also fig. 23).

Table 2

Date of experiment Nov. 8th, 1953, Lemna triulca L20 Leaf area 7,4 mm². Area of the internal cross section of capillary f — 0.0257 mm², I — 1.06. Temperature 21°. At 8.30 a. m. 200 luxes illumination begins, at 9.15 a. m. leaf is placed in the dark (O luxes)

Time	Time from begining of darkness	Number of chloroplasts in epistrophe in the 10 cells	Position of menis-cus in capillary A mm	ΔV_A mm / 100	Position of menis-cus in capillary B mm	ΔV_B mm / 100	$\Delta V_B \cdot I$ mm / 100	$\Delta V_A - \Delta V_B \cdot I$ mm/100	$\Delta V_A - \Delta V_B \cdot I$ 10^{-3} µl
9.15	0	82	61.50	0	61.05	0	0	0	0
9.20	5		61.10	— 40	60.95	— 10	— 11	— 29	— 7,5
9.25	10	56	60.70	— 80	60.80	— 25	— 26	— 54	— 14
9.30	15	—	60.60	— 90	60.90	— 15	— 16	— 74	— 19
9.35	20	43	60.50	— 100	61.10	+ 5	+ 5	— 105	— 27
9.40	25	—	60.15	— 135	61.10	+ 5	+ 5	— 140	— 36
9.45	30	28	59.80	— 170	60.95	— 10	— 11	— 159	— 41
9.50	35	—	59.40	— 210	60.80	— 25	— 26	— 184	— 48
9.55	40	22	59.00	— 250	60.70	— 35	— 37	— 213	— 55

4. RESULTS

4.1. Time changes of photosynthesis and phototactic chloroplast movements.

In this part of the work the plant material used for experiments is kept for some time (20—60 mins) in constant light conditions and then the intensity of light is changed. After the change of intensity the behaviour of chloroplasts is watched carefully and their arrangements are noted every 5 or 10 minutes. At the same time changes in the volume of oxygen in the gaseous phase are noted. In figs. 10—23 changes in chloroplast arrangements are represented by the upper curves and the production or absorption of oxygen is shown in the lower ones. The curves are typical examples chosen from many repetitions.

4.1.1. Low light intensity — darkness

Lemna (fig. 10). A change to darkness from low light intensity causes in the cells of *Lemna* leaves a transition of chloroplasts to the side cell walls (apostrophe). After some 30 mins equilibrium is reached and the chloroplast arrangement then is usually incomplete apostrophe which remains unchanged for a long time. Oxygen is assimilated at an unchanging rate all the time the leaf is in darkness. This indicates that the mo-

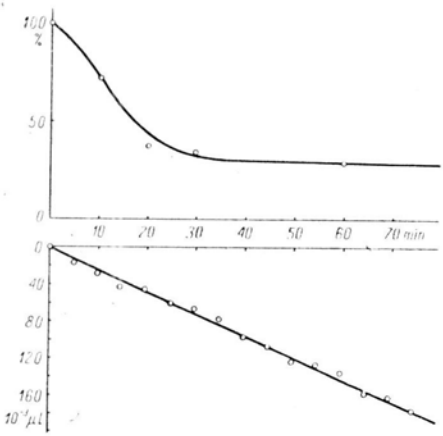

Fig. 10. *Lemna* L. 21. Time changes of chloroplast arrangements and gaseous metabolism. Upper curve — changes in percentage of epistrophe, lower curve — oxygen absorption in 10^{-3} µl. Abscissae — time in minutes, light intensity 200→0 luxes, leaf area 7.06 mm², respiration rate — 22.9 . 10^{-3}µl/10 mins

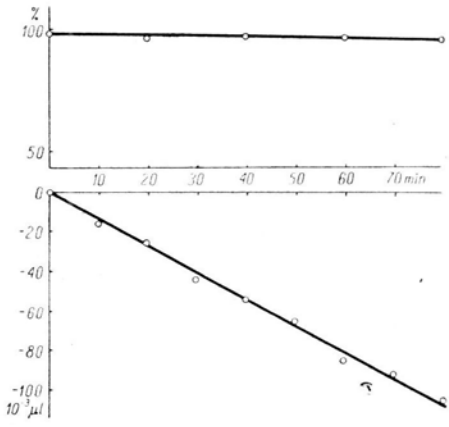

Fig. 11. *Mougeotia* M7. Total length of filaments approx. 82 mm. Light intensity 430→0 luxes. Respiration — 13.7 . . 10^{-3}µl/10 mins. Descriptions in figs 11 to 23 as in fig. 10

vement of chloroplasts to apostrophe is not related with any measurable change in the rate of respiration. Similar results are obtained in the case of *Funaria* leaves.

Mougeotia (fig. 11). When the cells of this alga are darkened the chloroplasts do not move and remain in the same position, which they had in low light intensity. Here also the rate of respiration does not change from the moment the darkness begins.

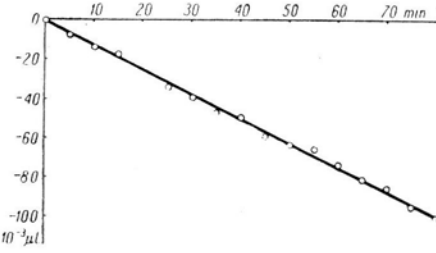

Fig. 12. *Spirogyra* S8. 16 fragments with total length 30.4 mm. Light intensity 200→0 luxes. Respiration — 12.9 . 10^{-3} µl/10 mins

Spirogyra. The chloroplasts of this plant have no phototactic movement capability and in this case also the rate of oxygen absorption does not change in darkness (fig. 12).

4.1.2. Darkness — low light intensity

Lemna. When after an interval of darkness, which causes apostrophe, the plant is illuminated with light of low intensity, the chloroplasts move

to the bottom and upper cell walls, and after 15—30 mins full epistrophe is reached. The light used (200 luxes) causes a low assimilation (below the compensation point), but in all cases the consumption of oxygen is at first markedly higher than later, when it falls to reach a constant level after 30 mins. E. g. in experiment no. 11 (fig. 13) the absorption of oxygen

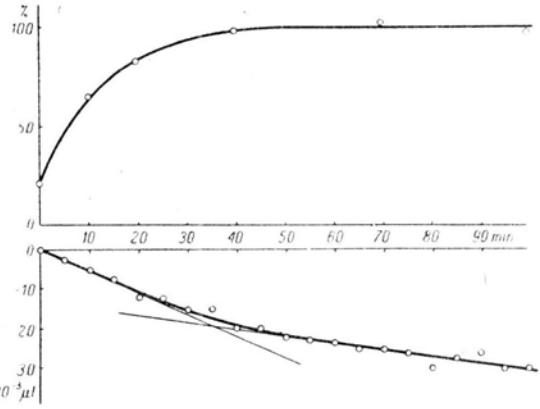

Fig. 13. *Lemna* L11. Leaf area 3.9 mm². Intensity of light 0→200 luxes. Initial assimilation — $5.7 \cdot 10^{-3}$ µl/10 mins, at the end — $1.6 \cdot 10^{-3}$ µl/10 mins

is at first — 5, $7 \cdot 10^{-3}$ µl during 10 mins, and later it settles at the level of — 1, $6 \cdot 10^{-3}$ µl/10 mins. This indicates that at first just after the light is turned on, when most chloroplasts are still on the side walls, assimilation is slack, but when the chloroplasts move to epistrophe its rate increases. The time when changes in assimilation rate take place corresponds fairly well with the time of chloroplast arrangement changes.

Funaria (fig. 14). In this case the results are similar to these of *Lemna*. Here also the time necessary to attain a constant rate of assimilation corresponds approximately to the time in which full epistrophe is reached.

Mougeotia. In the case of this alga the relation between assimilation and the chloroplast arrangement is extremly well visible (fig. 15). When the cells are illuminated with light of low intensity and then placed in darkness for 30 mins, the chloroplasts do not change their arrangement and remain all the time in epistrophe. When the weak light is turned on, again all the chloroplasts are and remain without change in the flat position. From the moment the illumination begins, assimilation reaches a constant level and remains unchanged for about 2 hours. If the same alga cells are first illuminated with strong light and then left in darkness for 30 mins, the chloroplasts will move to the parastrophe position and remain in this position in darkness (S t a h l 1880, S e n n 1908). If

afterwards these cells are illuminated with light of low intensity, the chloroplasts will turn round to a flat position and will remain so far about 1 hour. The assimilation rate, which previously in the same alga

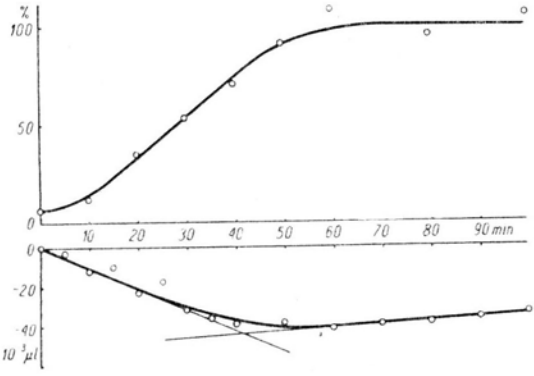

Fig. 14. *Funaria* F14. Two leaves with total area 1.01 mm². Light intensity 0→430 luxes. Assimilation at the beginning — $10 . 10^{-3}$µl/10 mins, at the end + $1{,}4 . 10^{-3}$µl/10 mins

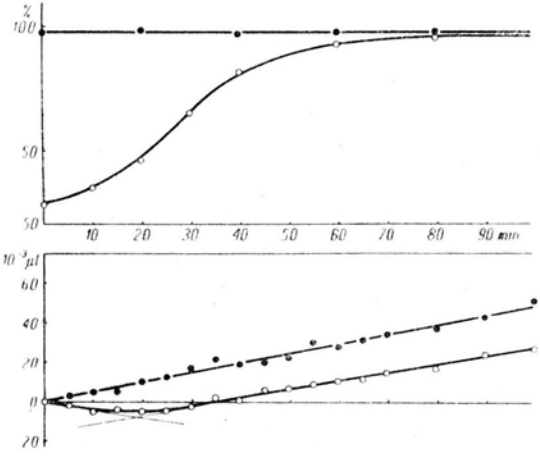

Fig. 15. *Mougeotia* M3. Total length of filaments approximately 53 mm. Light 0→430 luxes —.— chloroplasts in epistrophe, —.— before placing in the dark the alga is illuminated with bright light (parastrophe). Assimilation at the beginning — $3.1 . 10^{-3}$µl/10 mins, at the end + $5 . 10^{-3}$µl/10

cells reached a constant value immediately when weak light was turned on, now is for some time much lower (below compensation point), then gradually begins to increase and finally reaches the same constant value as in the first part of this experiment.

Spirogyra. When after being kept in darkness for 30 mins the *Spirogyra* filaments are illuminated assimilation begins immediately at a constant rate (fig. 16).

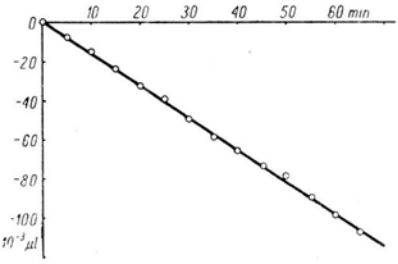

Fig. 16. *Spirogyra* S7. Total length of 35 fragments 98 mm. Light 0 → 200 luxes. Assimilation — 10.7 . 10^{-3} µl/10 mins

4.1.3. Low light intensity — strong light

Lemna. The change of light intensity from 200 to 8300 luxes causes a quick movement of chloroplasts to the side walls. Full parastrophe is reached after some 20 mins. The rate of photosynthesis, which in so

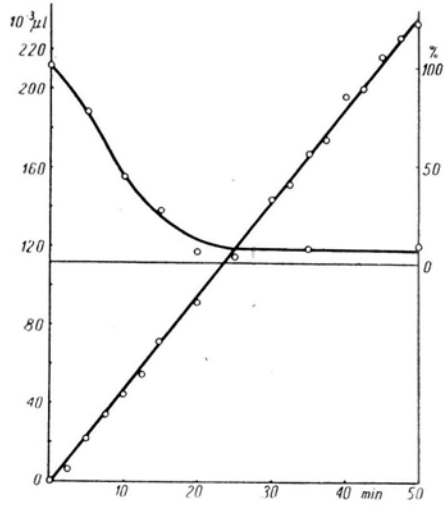

Fig. 17. *Lemna* L8. Leaf area 3.8 mm². Light 200 → 8250 luxes. Assimilation — 48 . 10^{-3}µl/10 mins

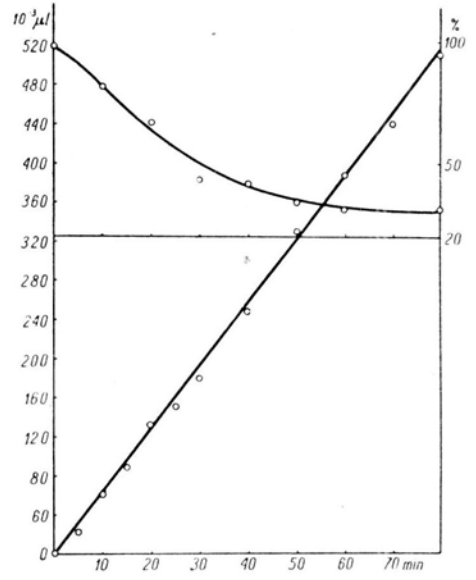

Fig. 18. *Mougeotia* M15. Total length of filaments approximately 96 mm. Light 430 → 12500 luxes.. Assimilation + 68 . 10^{-3}µl/10 mins

strong light is fairly high, does not change during all the time the strong light acts. In spite of repeated measurements it was not possible to find any difference in the rate of photosynthesis between the time when most chloroplasts were still in the flat arrangement and the time when all of them were already in parastrophe. An example of this kind of experiments is given in fig. 17. It appears that the rush of chloroplasts to parastrophe does not influence in any way the rate of assimilation.

The behaviour of *Mougeotia* is much the same (fig 18). Strong light causes a turn of chloroplasts sideways and this process usually lasts 60 mins. The assimilation rate is constant from the movement when intense illumination begins.

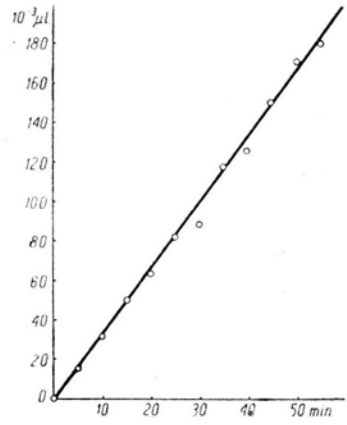

Fig. 19. *Spirogyra* S10 15 fragments with total length 36.4 mm. Light 200 → 8250 luxes. Assimilation + $32.7 \cdot 10^{-3}$ µl/10 mins

In *Spirogyra* the assimilation rate is also constant; all the time illumination with strong light lasts (fig. 19).

4.1.4. Strong light — low light intensity

In this kind of experiments it is necessary to observe carefully whether both reaction chambers are heated evenly, otherwise the equalization of temperature differences, which could arise during illumination with strong light, may cause a considerable source of error.

Lemna. From critically chosen experimental results it appears that there is a relation between the chloroplast arrangement and assimilation rate (fig. 20). After about 30 mins the chloroplasts return to epistrophe from the parastrophe arrangement. The assimilation slow at first, quickens after some time and reaches a constant value after about 30 mins.

Mougeotia (fig. 21). In strong light the chloroplasts are in a side position and move to a flat one in weak light. This process lasts approximately 50 mins. Assimilation slow at first quickens after some time. From the experiment from which curve 21 is plotted it appears that assimilation reaches a constant value before the chloroplast arrangement is fully epistrophe in all cells.

Spirogyra. At the same moment as strong light is changed to light of a low intensity, the rate of photosynthesis in this alga reaches a constant value (fig. 22).

The course of four successive phototactic reactions taking place without any interruptions in a *Lemna* leaf is illustrated by fig 23. The curves demonstrating the course of the gaseous metabolism illustrate once more the processes already described.

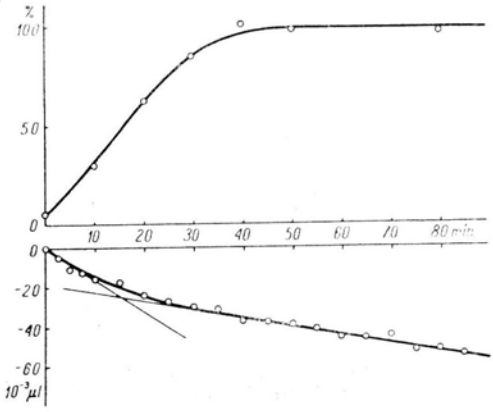

Fig. 20. *Lemna* L15. Leaf area 2.76 mm². Light 12500 → 200 luxes. Assimilation at the beginning — 16 . 10-³ µl/10 mins, at the end — 4.2 . 10⁻³µl/10 mins

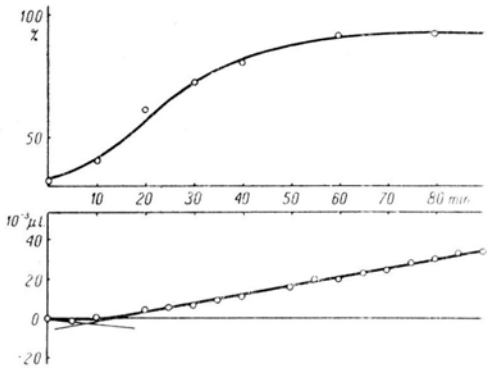

Fig. 21. *Mougeotia* M12. Total length of filaments approximately 185 mm. Light 12500 → 430 luxes. Assimilation at the beginnig — 0.5 . 10⁻³µl/10 mins, at the end — 4.7 . 10-³µl/10 mins

On the whole it can be said that while in *Spirogyra*, the control plant, in which the chloroplast arrangement cannot change, assimilation rate reaches a constant value immediately when new light conditions prevail, in those plants in which the chloroplasts have a capability for phototactic

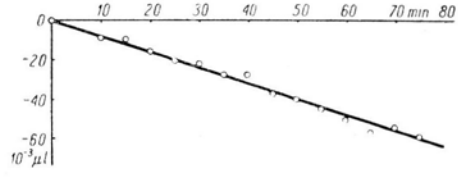

Fig. 22. *Spirogyra* S10. 15 fragments of total length 36.4 mm. Light 8250 → 200 luxes. Assimilation — 8 . 10-³ µl/ 10 mins

movements, photosynthesis can be influenced by the chloroplast arrangement. However, this influence appears only when light intensity is low, while in strong light the rate of assimilation is constant and does not depend on the chloroplast arrangement.

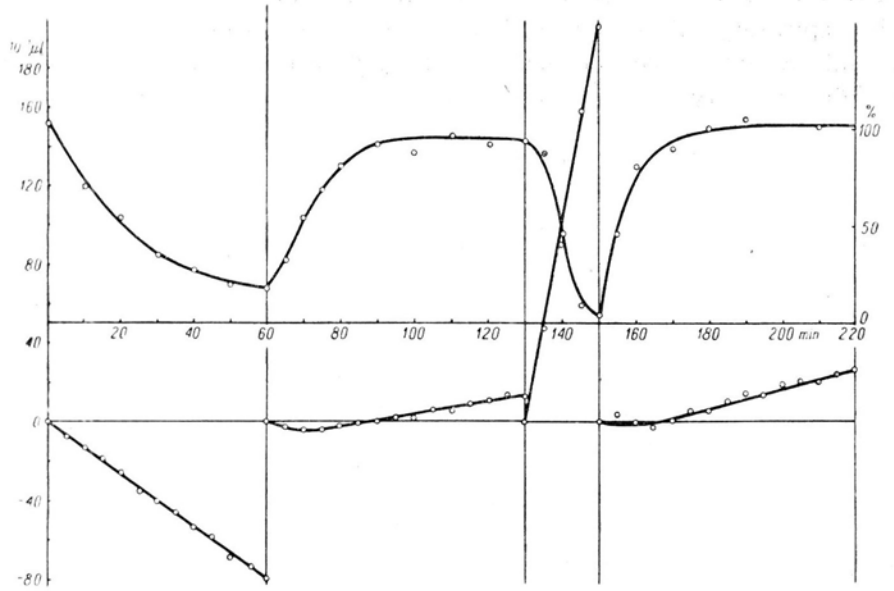

Fig. 23. *Lemna* L20. Leaf area 7.4 mm². Four successive phototactic reactions caused by changes in light intensity 200→0→200→12500→200 luxes

4.2. *Influence of light intensity on the chloroplast arrangement and assimilation after a state of balance is reached*

The method used here is the simple method of finding the light intensity — photosynthesis curve. After measuring the rate of respiration (20 mins) the object is illuminated during 30—40 mins and simultaneously the rate of photosynthesis is measured, then again measurements of respiration are made and so on by turns (U m b r e i t, B u r r i s and S t a u f f e r, 1948). If during illumination chloroplast movements take place, the time of illumination is prolonged till a state of balance in the chloroplast arrangement and photosynthesis is reached. In some cases, if the respiratory rate remains unchanged, measurements of respiration are made not after each interval of illumination, but after two successive intervals according to the pattern: respiration measurement — light intensity I, — light intensity II, — respiration measurement.

Lemna. The now obtained relation between the chloroplast arrangement and light intensity is in full accordance with the results published previously (Z u r z y c k a and Z u r z y c k i, 1953, Z u r z y c k i, 1953). The respiratory rate of the leaf in the dark is $3—5 \cdot 10^{-4}$ µl/min/mm², and the maximum assimilation in strong light is $13—20 \cdot 10^{-4}$ µl/min/mm² The ratio of respiration to photosynthesis (1 : 3 — 1 : 6) is relatively high,

probably because the leaves used for experiments were fairly young and their respiratory rate was remarkable. The compensation point is between 200 and 400 luxes. The curves recording chloroplast arrangements and the light intensity — photosynthesis curve — correspond well (fig. 24).

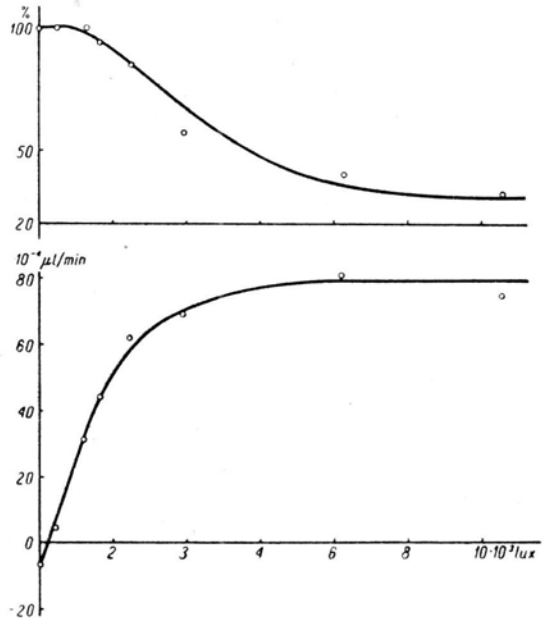

Fig. 24. *Lemna* L19. Light intensity — chloroplast arrangements (above) and light intensity — assimilation curves after equilibrium is reached. Leaf area 3.52 mm²

Epistrophe is maintained in the growing light intensity to the same point to which the rate of photosynthesis increases linearly. The constant maximum assimilation rate is reached at a moment which corresponds more or less to the moment at which full parastrophe is reached.

Funaria is less sensitive to light (Z u r z y c k i, 1953). The chloroplasts do not begin to move to parastrophe until light intensity is more than 4000 luxes. The linear increase of assimilation lasts until light intensity is approximately 3000 luxes. The constant maximum rate of photosynthesis is not reached until light intensity is 20 000 luxes, in this light intensity also a complete parastrophe is attained (fig. 25 and 26). Respiration rate is $0.5—1.3 \cdot 10^{-4}$ µl/min/mm², assimilation maximum is $17—26 \cdot 10^{-4}$ µl/min/mm², the ratio of respiration to maximum assimilation ranges from 1 : 16 to 1 : 22, the compensation point is approximately 300 luxes.

Mougeotia. The sensivity to light of this plant proved now to be different from what was found in the course of a previous work (Z u r z y c-

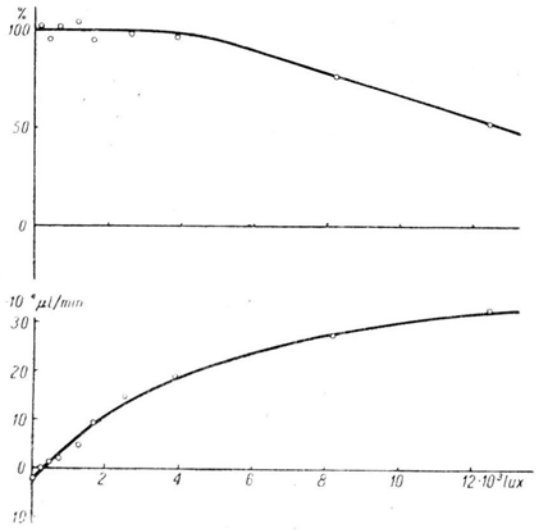

Fig. 25. *Funaria* F4. Total area of two leaves 1.49 mm². Descriptions of figs 25—28 as in fig. 24

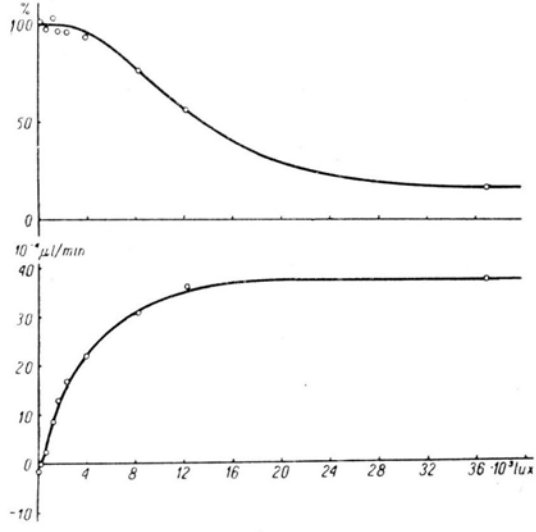

Fig. 26. *Funaria* F8. Total area of two leaves 1 08 mm²

k i, 1953). This difference may be caused 1) by the difference in the species of the plants used for experiments or by their different ecology, and 2) by the lesser sensivity of chloroplasts to light in autumn when the previous experiments were carried out. The latter reason seems to be more probable and such a drop in sensivity has been observed in *Lemna*

(Zurzycka and Zurzycki, 1953). When light intensity is 1500 luxes, the chloroplasts begin to move to parastrophe. Complete parastrophe is reached at 8000 luxes. The course of assimilation cor-

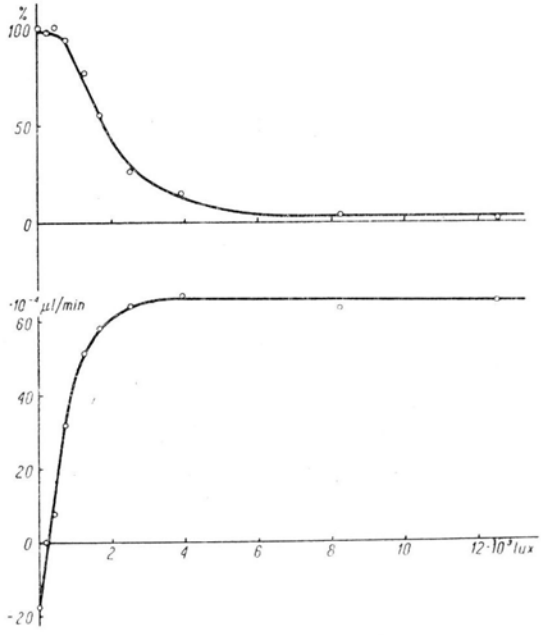

Fig. 27 *Mougeatia* Mb. Total length of filaments approximately 95 mm

respond to the curve illustrating the chloroplast arrangements. The increase of assimilation is linear to approximately 1500 luxes and its constant maximum value is reached at approximately 8000 luxes (fig. 27).

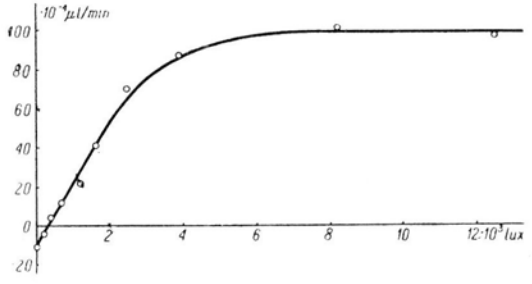

Fig. 28. *Spinogyra* S4. 28 fragments with total length 90.9.

Assimilation rate is $4—7 \cdot 10^{-4}$ µl/min/100 mm, maximum assimilation is $70—110 \cdot 10^{-4}$ µl/min/100 mm, and the ratio respiration: assimilation ranges from 1 : 12 — 1 : 17.

Spirogyra. The assimilation curve ressembles on the whole to the corresponding curves already described. The increase of assimilation is linear to approximately 2500 luxes and maximum is attained at approximately 7000 luxes. Respiration rate is $8.8 \cdot 10^{-4}$ µl/min/100 mm, assimilation maximum is $110 \cdot 10^{-4}$ µl/min/100 mm, and the ratio respiration: maximum assimilation is 1 : 13.

4.3. *Quantitative relation between chloroplast arrangements and photosynthesis*

In most plants in which phototactic chloroplast movements take place, the change to parastrophe is accompanied not only by the decrease in the directly illuminated chloroplast area, but also by their movement to the side cell walls which are less illuminated than the remaining parts of the cell. This is also the case in *Funaria* and *Lemna.* Only in *Mougeotia,* the chloroplast of which turns round inside the cell, the change of light absorption is probably proportional to the change of chloroplast area. For this reason this alga was used for experiments on the quantitative relation between assimilation and chloroplast arrangements.

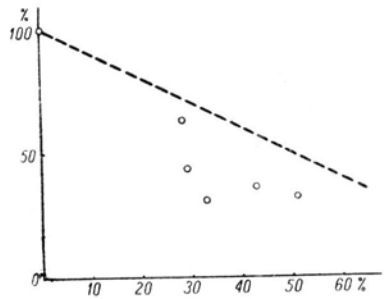

Fig. 29. *Mougeotia.* Dependence of initial assimilation expressed as percentage of final assimilation (ordinates) on initial chloroplast arrangement. The broken line represents the dependence of light absorption on chloroplast arrangements under the assumption, that all incident light is absorbed by the chloroplast

The technique used is as follows: first the complete or partial lateral arrangement of chloroplasts is induced with very strong light, then after measuring the respiration rate in darkness, light intensity is reduced to weak light and assimilation is registered for 10—15 mins and then again after 30 mins, when the assimilation rate reaches a constant level. After this the rate of respiration is measured once again. The results obtained are illustrated by a curve (fig. 29), in which the initial assimilation (ex-

pressed as a percentage of full assimilation) is plotted against the percentage decrease of assimilation area.

The results obtained are so scattered, that it is impossible to make any conclusions of a quantitative nature. However, it is remarkable that the drop of assimilation, when compared to the decrease of chloroplast area, is considerable. In graph 29 the broken line represents the changes of light absorption (ordinate) plotted against changes of chloroplast arrangements (abscissa); in plotting this curve it is assumed that all the light is absorbed by the chloroplast ($E = \infty$) (c. f. Zurzycki, 1953). It is found experimentally that assimilation always decreases more than one might judge from changes of absorption. This phenomenon, although it needs further confirmation on more plant material, seems to indicate that chloroplast arrangements determine the wave-length of light absorbed.

5. DISCUSSION

In the literature of the subject suppositions are often made that changes in chloroplast arrangements, and especially their flight to parastrophe, are connected with changes of assimilation(e. g. M o n t f o r t and N e y d e l, 1928). Experimental results here described indicate, that such a relation does exists, but only within a restricted range of light intensities, i. e. only in light of low intensity. The relation between the rate of photosynthesis and the chloroplast arrangement is confirmed a) by the transient inhibition of assimilation during the first few minutes of illumination with light of low intensity, when chloroplasts are not yet fully in the epistrophe arrangement, and b) by the fact that epistrophe is attained at the moment when assimilation reaches a constant level. The lack of influence of chloroplast arrangements on photosynthesis in strong light is explained by the light intensity — photosynthesis curves. The light intensities, which cause parastrophe, lie within the range of light intensities, which have no further influence on photosynthesis (the part of the curve parallel to the x — axis). The movement of chloroplasts from a flat to a lateral arrangement is always connected with a change of light absorption, but such a change influence the rate of photosynthesis only in low light intensities, when light is the limiting factor. In strong light the displacement of chloroplasts has no such influence. If therefore the biological significance of phototactic movements leading to epistrophe (weak light) can be understood as a tendency to absorb as much light as possible and thus increase the rate of photosynthesis, the lack of influence on photosynthesis of phototactic movements leading to parastrophe does not explain the significance of these movements. It seems

possible that a decrease of light absorption limits a harmful activity of strong light.

Many experiments were carried out on the drift of photosynthesis immediately after illumination begins. Osterhout and Haas (1918) were the first to observe, that when the thalli of *Ulva* were illuminated, after being previously kept in the dark, assimilation did not begin immediately at its full rate, but for some time (for 2 hours approximately) the rate gradually increased. Two years later Warburg (1920) found, that when a suspension of *Chlorella* cells was illuminated with strong light, the rate of photosynthesis increased gradually during an induction period lasting approximately two minutes. Tsi Tung Li (1928) using the buble method and working on such water plants as *Elodea, Myriophyllum, Potamogeton* etc. found, that after a change in light intensity, approximately two minutes had to elapse before assimilation rate reached a constant level. A transfer to weak light caused a characteristic transient inhibition or even an interruption of photosynthesis, a transfer to bright light inhibition. In his *Fontinalis* investigation Harder (1930, 1933) demonstrated a transient induction period, lasting usually 1 hour and often several hours. He also found, that the induction period and the later course of photosynthesis depended on the intensity of illumination and on the intensity of light to which the plant had been adapted previously. Neydel (1930) reported, that in *Trichomanes* and *Cladophora* assimilation rate reached a constant level after approximately two hours. Van der Paauw (1932) found that in *Hormidium* photosynthesis was inhibited for 1—2 minutes, and the duration of the induction period was clearly dependent on temperature. In some cases, e. g. when the leaf was kept in darkness for a long time, the induction was of a different kind and lasted 20—45 minutes. A similar inhibition lasting 50 mins was found in *Mnium* by Briggs (1933). Emerson and Green (1934) experimenting on photosynthesis of the red alga *Gigartina* demonstrated that assimilation did not begin immediately with a constant rate, and did not reach it till after 10—20 mins. A similar activation period was observed by Bukatsch (1935) in *Spirogyra*. This activation period lasts 1 hour and depends on temperature, the duration of the previous period of darkness, etc. In contradiction of the earlier results Gessner (1937) reports that in numerous water plants an induction period appears only in exceptional cases, e. g. when the plant is long kept in darkness, and that in most cases the maximum value of assimilation is attained already in the first measurements (with the method he used, measurements can be made every 20 minutes). Gessner questions most earlier results because of the insufficient precision of their technique. Smith (1938), investigating assimilation in *Cabomba*, demonstrated a transient induction period of 2—4 mins, both in

strong and weak light. Detailed investigations on the induction period were made by Steeman Nielsen (1942, 1949). He ascertained in *Fucus* a period of activation lasting 10—30 minutes after transfer from the dark to weak light, as well as after transfer from weak to strong light. After transfer from strong to weak light induction appeared only in low temperatures (it may be that in higher temperatures the induction was so short lasting, that it could not be registered). A similar phenomenon was observed in *Cladophora*, though the change from strong to weak light was not accompanied by the drop in assimilation. During the last few years special laboratory techniques are used more and more frequently and these make possible very frequent and even continuous measurements of photosynthesis rate. This enables to observe very accurately the changes in the rate of this process during the first minutes after illumination begins. Using the new technique Blinks and Skow (1938), Mc Allister and Myers (1940), Aufdemgarten (1939, a and b), van der Veen (1949) and Frank (1951) ascertained, that in numerous algae and in higher plants in the course of 1—5 minutes the rate of photosynthesis undergoes several complicated changes before it is established at a constant level. Using manometric technique it is found that in *Chlorella* the rate of photosynthesis in strong light is established at a constant level after 2—5 minutes (Emerson and Lewis, 1939, 1941, 1943, Schwartz, 1952), on the other hand in light of low intensity no transient induction period is detected with the manometric method (Warburg, 1948).

It can be assumed as a general rule, that if the method used permits a continuous measurement of photosynthesis, than independently of the material used the change in photosynthesis rate appear from 30 secs till 2 or most 5 minutes after the plant is illuminated. On the other hand longer induction periods are seldom observed, and if they are ascertained, they are mostly caused by some external factors. It seems probable, that the short induction periods are caused by disturbances in the chemical equilibrium between the initial and intermediate products of photosynthesis, by the reactivation and inactivation of some enzymes etc. (van der Veen, 1949). However, it does not seem probable that these factors would cause changes in the rate of photosynthesis, which would last half an hour or even several hours. It is likely that the long induction periods are caused by other factors than those just mentioned. One of these factors may be — besides the inactivation of chlorophyll (Steeman Nielsen, 1949) — the phototactic movements of chloroplasts.

Steeman Nielsen's (1942) paper describes an investigation on the influence of external conditions on the induction phenomenon. The behaviour of chloroplasts under the influence of external conditions has not been studied in *Fucus*, but if the well known chloroplast movements

of *Lemna* are compared to the inhibition process in *Fucus*, a close similarity between the two processes can be observed in many instances. The following similarities can be here mentioned: 1) the activation of photosynthesis after a transfer from the dark to light of low intensity which corresponds in time to the apostrophe — epistrophe reaction, 2) the drop of assimilation after a transfer from strong to weak light which corresponds to the parastrophe — epistrophe reaction, 3) the lack of a drop in assimilation after a transfer from 2300 to 1200 luxes (Steeman N i e l s e n, 1942, fig. 25) corresponding to the persistance of epistrophe within this range of light intensities, and 4) changes in the drop of assimilation when the plant is transfered to weak light, after being illuminated first with strong light and then kept in the dark during varying time intervals (Steeman N i e l s e n, 1942, fig. 27) and the analogical changes in chloroplast arrangements under similar conditions (Z u r z y c k a and Z u r z y c k i, 1953, fig. 3, curve 1a). On the other hand the following facts testify against the influence of chloroplast arrangements on photosynthesis: 1) the high value of the drop of assimilation, 2) the same rate in assimilation drop increase after a transfer to darkness from 2300 luxes and from 23 000 luxes, 3) the induction periods which occur also in plants the chloroplasts of which have no ability for phototactic movements (e. g. *Cladophora*).

It is impossible to explain all the observed long lasting induction periods by the changes in chloroplast arrangements, but phototactic movements can be among the factors influencing induction. Even if in the case of *Fucus* studied by Steeman N i e l s e n chloroplast movements had no important effect, the numerous analogies which occur between inhibition and phototactic movements must be accounted for. To decide finally whether in the case of *Fucus* the two processes are synchronized, a microscope control of chloroplast arrangements would be necessary (a simple measurement of light absorption in the thallus is definitely insufficient to exclude the possibility of chloroplast movements).

The shape of light intensity — photosynthesis curves is typical in all the experiments, i. e. the curve can be divided into three sections: 1) at first the increase of photosynthesis rate is linear and proportional to the increase of light intensity, 2) the increase in photosynthesis rate becomes slower (the bow shaped part of the curve), and finally 3) the further increase of light intensity has no influence on photosynthesis. In no instance a drop of assimilation was observed (Steeman N i e l s e n, 1949), probably because the light intensities used were not sufficiently high. G ó r s k i (1953) interprets the curve in the following way: part 1) of the curve, i. e. the part which runs along a straight line, corresponds to the condition in which light is the only limiting factor in the whole of the illuminated material; in section 2), i. e. the bow shaped section

of the curve, light is the limiting factor in a part only of the material and in the remaining parts light is arleady in excess; in section 3) of the curve light ceases to be the limiting factor in the whole of the plant material. When the assimilation layer is thin the bow shaped part of the curve (section 2) is comparatively short, as is the case in the unicellular layer of the alga *Hormidium* (van der H o n e r t, 1930). The somewhat greater length of this section of the curve in the case of *Funaria* can be explained by the greater concentration of chlorophyll in the chloroplasts of this species.

There is a pronounced correlation between the changes of chloroplast arrangements and the changes influenced by light intensity in the drift of photosynthesis. The facts which demonstrate this correlation are: 1) the flight of chloroplasts from epistrophe begins in the same light intensity in which photosynthesis rate ceases to be proportional to light, i. e. when light ceases to be the limiting factor in a part of the investigated plant cells, and 2) full parastrophe is reached when light is in excess in the whole of the investigated object. From what precedes it seems probable, that the only chloroplasts, which move from epistrophe to parastrophe are those, in which light is no longer the limiting factor. The complete lack of influence on assimilation drift of the partial displacement of chloroplasts to parastrophe during the epistrophe — parastrophe reaction in medium light intensity (i. e. in that light intensity in which the light is not yet in excess), seems to confirm such a supposition.

SUMMARY

1. A microtechnique based on a capillary tube respirometer for photosynthesis measurements was elaborated. This technique makes possible measurements of gaseous metabolism in leaves several mm^2 in area and in alga filaments several mm long. An analysis of possible errors indicates that the accuracy of the method is of the order 10^{-3} µl. In the respirometed used during the experiments the plant material can be illuminated with varying light intensities and can be magnified 150 x (for observation).

2. Simultaneous measurements of changes in chloroplast arrangements and photosynthesis drift disclose a relation between these two processes, but in weak light only. In low light intensities the increase of assimilation area (apostrophe — epistrophe and parastrophe — epistrophe reactions) is related to an increase of photosynthesis rate. Both respiration in the dark and the assimilation in strong light have a constant rate dependent of simultaneous changes in chloroplast arrangements. The two processes: assimilation and the epistrophe — parastrophe reac-

tion are independent of each other, because changes in light absorption caused by changes of chloroplast arrangements in strong light (light is then in excess) cannot influence photosynthesis, which is opposite to what takes place in low light intensities, when light is the limiting factor.

3. The shape of light intensity — photosynthesis curve shows some relation with chloroplast arrangements. As long as photosynthesis rate increases proportionally to light intensity chloroplasts are in epistrophe. Full parastrophe is reached in that light intensity, in which a further increase of intensity has no influence on photosynthesis.

The author wishes to express his most sincere gratitude to Prof. Dr F. Górski for the most valuable help and kind criticism he offered him in the course of this work.

Laboratory of Plant Physiology
Jagiellonian University
Kraków

(Received 12.VII.1954)

LITERATURE

Alister Mc E. D. and Meyer, 1940. Time course of photosynthesis and fluorescence. „Science" 92, 241 — 243.

Aufdemgarten H., 1939. Zur Kenntnis der sogenannten Induktionsvorgänge bei der Kohlensäureassimilation, „Planta" 29, 643 — 678.

Aufdemgarten H., 1939. Weitere Untersuchungen mit dem Gaswechselschreiber über die Kohlensäureassimilation, „Planta" 30, 343 — 352.

Blinks L. R. and R. K. Skow, 1938, The time course of photosynthesis as shown by a rapid electrode method for oxygen, „Proc. Nat. Acad. Sc." 24, 420 — 427.

Briggs E. G., 1933. Experimental researches on vegetable respiration XXI. Induction phases in photosynthesis and their bearing on the mechanism of the process, „Proc. Roy. Soc. B.", 113.

Brilliant W. A., 1949. Fotosintiez kak prociess iźniediejatielnosti rastienja, Akad. Nauk, Moskwa.

Bukatsch F., 1935. Beiträge zur Kenntnis der Kohlensäureassimilation durch Süsswasseralgen, „Jahrb. wiss. Bot.", 81, 419 — 447.

Cunningham B. and Kirk P. L., 1940, A new form of differential microrespirometer, „J. Gen. Physiol', 24, 135 — 149.

Cunningham B. and Kirk P. L., 1942. The oxygen consumption of single cells of *Paramecium caudatum* as measured by a capillary respirometer, „J. Cell. Comp. Physiol.", 20, 119 — 134.

Emerson R. and L. Green, 1934. Manometric measurements of photosynthesis in the marine alga *Gigartina*, „J. Gen. Physiol.", 17, 817 — 842.

Emerson A. and Lewis C. M., 1939. Factors influencing the efficiency of photosynthesis „Am. J. Bot.", 26, 808 — 822.

Emerson R. and Lewis C. M., 1939. Factors influencing the efficieny of photosynthesis „Am. J. Bot.", 26, 808 — 822.

Emerson R. and Lewis C. M. 1941. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis, „Am. J. Bot.", 28, 789 — 804.

Emerson R. and Lewis C. M., 1943. The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light, „Am. J. Bot.", 30, 165 — 178.

Frank J., 1951. The physical background of photosynthesis, „Symp. of the Soc. for Exp. Biol.", 5, 160 — 175.

Gerard R. W. and Hartline H. K., 1934. Respiration due to natural nerve impulses. A method for measuring respiration, „J. Cell. Comp. Physiol.", 4, 141 — 160.

Gessner F., 1937. Untersuchungen über Assimilation und Atmung submerser Wasserpflanzen, „Jahrb. Wiss. Bot.", *85*, 267 — 328.

Gjubbenet E. R., 1951. Rastienje i chłorofill, Akad. Nauk, Moskwa.

Glick D., 1949. Techniques of histo-and cytochemistry, Interscience Publ. Inc., New York.

Górski F., 1953. Contribution to the theory of limiting factors, „Acta Soc. Bot. Pol.", 22, 1 — 31.

Harder R., 1930. Über die Assimilation der Kohlensäure bei konstanten Aussenbedingungen I, „Planta" *11*, 263 — 293.

Harder R., 1933. Über die Assimilation der Kohlensäure bei konstanten Aussenbedingungen II, „Planta" 20, 699 — 733.

Honert van den, T. H., 1930. Carbon dioxide assimilation and limiting factors, „Rec. trav. bot. neerl.", 27, 149 — 286.

Linderstrøm Lang K., 1942. Approximate solution of certain diffusion problems in liquid systems, „Compt. rend. Lab. Carlsberg Ser. Chim.", 24, 249 — 280.

Linderstrøm Lang K., 1943. On the theory of the cartesian diver microrespirometer „Compt. rend. Lab. Carlsberg. Ser. Chim.", 24, 333 — 398.

Li Tsi Tung, 1929. The immediate effect of change of light on the rate of photosynthesis, „Ann. of Bot." 43, 587 — 601.

Metzner P., 1928. Über ein „Horizontal-Vertikal Mikroskop", „Zeitschr. f. wiss. Mikroskopie" 49, 1 — 10.

Montfort C. und Neydel K., 1928. Zur Beurteilung der „Inaktivierung" und des „Zeitfaktors" der Lichtwirkung bei der Assimilation stomatafreier Schattenfarne, „Jahrb. wiss. Bot.", 68, 801 — 843.

Moyse A., 1952. La photosynthèse I, „L'année Biologique", 28, 217 — 293.

Moyse A., 1953. La photosynthèse II, „L'année Biologique" 29, 166 — 244.

Neydel K., 1930. Vergleichende Studien über die Wirkung des Lichtes und ansteigender Temperatur auf die CO_2 Assimilation bei verschiedener Lichteinstellung, „Biochem. Z.", 228, 451 — 486.

Osterhout W. J. V. and Haas A. R. O., 1918. On the dynamics of photosynthesis, „J. Gen. Physiol." 1, 1 — 22.

Paauw van der F., 1932. The indirect action of external factors on photosynthesis, „Rec. trav. bot. nèerl." 29, 497 — 620.

Pratt R., 1943. Studies on *Chlorella vulgaris* VIII. Influence on photosynthesis of prolonged exposure to sodium bicarbonate and potassium bicarbonate, „Am. J. Bot.", 30, 626 — 629.

Schanderl H. und Kaempfert W., 1933. Über die Strahlungsdurchlässigkeit von Blättern und Blattgeweben, „Planta" 13, 700 — 750.

Schwartz M., 1952. Quantum efficiency, light intermittency and induction effect in photosynthesis, Theses, Doct. Phil. Univ. of Wisconsin.

Senn G. 1908. Die Gestalts- und Lagerveränderung der Pflanzenchromatophoren, Engelmann, Leipzig.

Smith E. L., 1938. The induction period in photosynthesis, „J. Gen. Physiol." 21, 151 — 163.

Stahl W., 1880. Über den Einfluss von Richtung und Stärke der Beleuchtung auf die einige Bewegungsercheinungen in Pflanzenreiche, „Bot. Zeit." 38, 297.

Steemann Nielsen E., 1942. Der Mechanismus der Photosynthese, „Dansk Bot. Ark." 11, 1 — 95.

Steemann Nielsen E., 1949. A reversible inactivation of chlorophyll *in vivo*, „Physiol. Plant." *2*, 247 — 265.

Stefanelli A., 1937. A new form of micro-respirometer with a note on the effect of cleavage on the respiration of the egg of Rana, „J. Exp. Biol." 14, 171 — 177.

Tobias J. M., 1943. Microrespiration techniques, „Physiol. Revs." 23, 51 — 75.

Tobias J. M., and Gerard R. W., 1941. An improved capillary microrespirometer, „Proc. Soc. Exp. Biol. Med." 47, 531 — 533.

Tyler A. and Berg W., 1941. A new type of microrespirometer, „Science" 94, 397 — 398.

Umbreit W. W., Burris R. H. and Staufer J. F., 1948. Manometric techniques and related methods for the study of tissue matabolism, Burgess Pub. Co., Minneapolis.

Veen van der R., 1949. Induction phenomena in photosynthesis I, „Physiol. Plant." 2, 217 — 234.

Veen van der R., 1949. Induction phenomena in photosynthesis II, „Physiol. Plant." 2, 287 — 296.

Voerkel H., 1934. Untersuchungen über die Phototaxis der Chloroplasten, „Planta" 21, 156 — 205.

Warburg O., 1919. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen, „Biochem. Z." 100, 230 — 262.

Warburg O., 1920. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen 11, „Biochem. Z." 103, 188 — 211.

Warburg O., 1948. Assimilatory quotient and photochemical yield, „Am. J. Bot." 35, 194 — 204.

Withrow R. B. and Price L., 1953. Filters for the isolation of narrow regions in the visible and near visible spectrum, „Plant Physiol." 28, 105 — 114.

Zurzycka A. and Zurzycki J., 1950. The influence of temperature on phototactic movements of chloroplasts, „Acta Soc. Bot. Pol." 20, 665 — 680.

Zurzycka A. i Zurzycki J., 1953. Badania nad ruchami fototaktycznymi chloroplastów, „Acta Soc. Bot. Pol." 22, 667 — 678.

Zurzycki J., 1953. Arrangement of chloroplasts and light absorption in plant cell., „Acta Soc. Bot. Pol." 22, 299 — 320.

Zurzycki J. and Zurzycka A., 1952. Investigation onto phototactic movements of chloroplasts in *Selaginella Martensii* Spring, „Bull. Acad. Sc. Polon.", Sér. B, 235 — 251.