Daily changes in the concentration of chlorophyll in growing leaves

S. WIĘCKOWSKI

The question whether there are any periodical daily changes of the chlorophyll concentration in leaves has been a problem of discussions for some time. Such daily changes in fully developed and no longer growing leaves have been reported by some workers (Bukatsch 1939, 1940, Wendel 1940, 1957, Bavrina 1959), but have not been confirmed by the results obtained in other investigations (Seybold 1942, Więckowski 1957, Bauer 1958, and others).

Seybold and Falk (1959), who have analysed critically earlier experiments on the daily changes in the concentration of chlorophyll, have reached the conclusion that the concentration of the pigment in fully developed and no longer growing leaves undergoes no daily changes. On the other hand Seybold and Falk believe that daily changes in the chlorophyll concentration are possible in growing leaves. The question has become all the more interesting since the discovery that the growth of the leaf surface and the synthesis of chlorophyll are to some extent independent of each other (Więckowski 1959).

The present study had a double aim: a. to study the diurnal changes occurring in the chlorophyll concentration during the whole growth period of leaves, b. to discover the causes of these changes.

MATERIAL AND METHODS

The experiments were carried out on the primary leaves of the beans *Phaseolus vulgaris* var. *Bronowicka*. The plants were cultivated in a thermostat on Knop's nutrient solution in light or in darkness. The seeds were first soaked for 24 hours in well water at room temperature and then kept for the next 48 hours in darkness at $+24^{\circ}\text{C}$ on dishes lined with damp blotting paper for germination. To obtain a uniform material

for the experiments the best developed germinated seeds were picked. The selected plants were placed over the nutrient solution in a thermostat at temperatures ranging $+ 29^{\circ} \pm 0,5^{\circ}\text{C}$. The so prepared cultures were alternately exposed to light during 12 hours (8 a. m. to 8 p. m.) and kept in darkness for the next 12 hours (8 p. m. to 8 a. m.). Plants grown in continuous light were used as controls.

Fluorescent lamps were used as the source of light. The intensity of light, in the wave length range 400—700 $\text{m}\mu$, measured with a Kipp thermopile using WG1 and RG8 filters, was about 24 000 ergs./sq. cm. x sec. The area of leaf surface was measured planimetrically on prints of leaves made on sensitized paper.

Observations of the daily changes in the accumulation of chlorophyll were carried out, besides beans, also on growing leaves of *Platanus acerifolia* Willd. The plant used for these experiments grew in conditions approaching natural at the Botanical Garden of the Jagiellonian University.

For determinations of the chlorophyll level either whole leaves or discs 6 mm. in diameter cut out from a suitable point of the leaf blades were used.

The pigments were extracted in a mortar with acetone with an addition of glass and CaCO_3 . The acetone extracted was filtered and the pigments were transferred to diethyl ether in a separating funnel. The ether solution was dehydrated and made up to a constant volume. The concentration of the pigments in ether solution was determined with a Uvispek Hilger spectrophotometer for wave lengths of 660 $\text{m}\mu$ and 642,5 $\text{m}\mu$ using a silica prism. The absolute values were calculated from the formula deduced by Comar and Zscheile (1942).

All experimental procedures were more fully described in an earlier report (Więckowski 1959).

RESULTS

Daily changes of the chlorophyll concentration in growing leaves of beans

The characteristic changes of the chlorophyll concentration in growing leaves can be observed in plants grown in continuous light. During the first three days of growth the amount of chlorophyll in a unit of area increases but drops in the course of later growth.

In an earlier report (Więckowski 1959) it has been demonstrated that changes of this kind in the amount of chlorophyll are brought out by

the fact that the growth of the leaf area is to some extent independent of the synthesis of chlorophyll. At first, during the first three days after it comes out of the seed coat, the growth of a leaf is slower than in later stages. On the other hand, the synthesis of chlorophyll proceeds at

Fig. 1. Changes of the chlorophyll concentration in growing leaves of beans. Plants kept in continuous light. A a — chlorophyll a; A b — chlorophyll b; B — ratio of chlorophyll a to b

Time was counted from the moment the plants were exposed to light in the thermostat

a nearly uniform rate almost from the beginning of leaf growth. In the first phase the growth of leaves is slower than the synthesis of chlorophyll, whereas, in the next phase the situation is reversed, i.e. the rate at which the leaf area increases is greater than the rate with which chlorophyll is synthesized.

Figure 1 illustrates the changes in the concentration of chlorophyll a and b in a growing leaf. Plants grown in continuous light. The shape of

both these curves is similar. The ratio of chlorophyll *a* to chlorophyll *b* becomes stabilized during the first day after the leaves break out from the seed coat (fig. 1B).

The daily changes in the concentration of chlorophyll were observed on plants grown in alternate 12 hour periods of light and darkness. The first of these alternate periods was started at the moment the germinated seeds were placed over the nutrient solution i. e. about 72 hours after the seeds were placed in water for soaking. On the curves illustrating the

Fig. 2. Growth of the leaf area. Plants kept alternately 12 hours in light and 12 hours in darkness

results the successive light periods are marked as I, II, III, IV etc. The leaflets came out from the seed coat more or less in the third period and at this moment the observations were started and were continued till the end of the IXth period of light, when the growth of leaves was almost completed. The curve illustrating the growth of the leaf area in plants cultivated in alternate periods of light and darkness (fig. 2) has approximately the same shape as this curve for plants grown in continuous light. The phase of slow growth lasts for the first 2 or 3 days (periods II—IV); in the next phase (periods V—IX) leaves grow more rapidly.

Figure 3 illustrates the rhythm of the daily changes of the chlorophyll concentration during the whole time of leaf growth. The daily

oscillations of concentration are manifested both by chlorophyll *a* and chlorophyll *b*. The amount of chlorophyll in a unit of area increases when leaves are exposed to light and drops in plants kept in darkness. During the first hours of exposure to light the increase of chlorophyll *b* is greater than of chlorophyll *a*. The rate of the increase drops after several hours, and the drop is greater in the case of chlorophyll *b* than of chlorophyll *a*. These changes are essentially similar during all the light periods. In darkness the amount of chlorophyll in a unit of area decreases.

Fig. 3. Daily changes of the chlorophyll concentration in growing leaves of beans. Plants kept alternately 12 hours in light and 12 hours in darkness. A *a* — chlorophyll *a*; A *b* — chlorophyll *b*; B — ratio of chlorophyll *a* to *b*

The results presented on fig. 1, 2, 3 were obtained in experiments started and performed simultaneously

The ratio of chlorophyll *a* to chlorophyll *b* also manifests characteristic changes (fig. 3B). In darkness the value of the ratio increases, then during the first few hours of exposure to light it drops very rapidly and again increases near the end of the light period. These oscillations become less marked as leaves grow and after a few days they are hardly noticeable.

The daily increase in the amounts of chlorophyll *a* and *b* in a unit of area is much greater in the first phase of growth than later. E.g. during the IVth period of light the concentration of chlorophyll *a* increased from

Fig. 4. Points at which discs were cut out from the leaves of beans for chlorophyll determination

about 1,5 to about 3,2 mg. per sq. decimetre. At the same time the concentration of chlorophyll *b* rose from about 0,4 to about 1,4 mg. per sq. decimetre. In later stages the daily increases in the concentration of chlorophyll *a* and *b* are smaller. E.g. in the VIIth period of light the concentration of chlorophyll *a* rose from about 1,8 to about 2,4 mg/dcm² and of chlorophyll *b* from about 0,8 to 1,0 mg/dcm².

Since every point plotted on the graphs in fig. 3 corresponds to the results obtained by extracting chlorophyll from one leaf only, some discrepancies may be possible, although the selection of plants was very careful. To make sure that the increase of the chlorophyll concentration was of the same order as shown by the curves in fig. 3 additional determinations were carried out. For this purpose nine leaves were selected from which discs 6 mm. in diameter were cut out at three points of each leaf, once at 8 a. m., once at 2 p.m., and once at 8 p.m. Each sample consisted of three discs cut out from three leaves. The points on leaves where the discs were cut out are shown in fig. 4 and the results are summarized in Table 1.

The first part of the results characterize the fourth light period and the second part the fifth light period. The increases of chlorophyll concentration in the IVth period are much greater than in the Vth. If the concentration of chlorophyll at 8 a.m. of each day is assumed to be 100, then at 8 p.m. of the fourth day the concentration of chlorophyll *a* is 234 and of chlorophyll *b* 280, while the corresponding values for the 5th day are 127 and 122. This means that here also the changes in chlorophyll concentration are of the same order as shown in fig. 3. The ratio of chlorophyll *a* to chlorophyll *b* changes similarly.

Table 1

Changes of chlorophyll concentration in the same leaves of beans during exposure to light (mg/l)

Period of illumination**		8 ⁰⁰ a. m.			2 ⁰⁰ p. m.			8 ⁰⁰ p. m.		
		chloro- phyll <i>a</i>	pos. *	chloro- phyll <i>b</i>	chloro- phyll <i>a</i>	pos.	chloro- phyll <i>b</i>	chloro- phyll <i>a</i>	pos.	chloro- phyll <i>b</i>
IV	Sample 1	0,945	1	0,362	1,553	2	0,756	2,050	3	0,845
	Sample 2	0,877	2	0,349	1,572	3	0,672	2,103	1	0,839
	Sample 3	0,796	3	0,170	1,719	1	0,848	1,981	2	0,783
	Average	0,873		0,294	1,615		0,759	2,045		0,822
	rel. value	100,00		100,00	184,99		258,16	234,25		279,58
	Ratio <i>a/b</i>	2,97			2,13			2,49		
V	Sample 1	1,398	1	0,570	1,614	2	0,778	1,791	3	0,684
	Sample 2	1,472	2	0,661	1,528	3	0,619	1,817	1	0,695
	Sample 3	1,354	3	0,488	1,612	1	0,762	1,770	2	0,721
	Average	1,408		0,573	1,585		0,720	1,793		0,700
	rel. value	100,00		100,00	112,57		125,65	127,34		122,16
	Ratio <i>a/b</i>	2,46			2,20			2,56		

* The numbers indicate the position in leaf blade from which the samples was taken (c. f. fig. 4).

** C. f. fig. 2.

The drop of the chlorophyll concentration in darkness during the first phase of growth is smaller than in the later phase.

The shapes of curves for both chlorophyll *a* and *b* in fig. 3A are essentially similar to the curves in fig. 1A. During the first 2—3 days the amount of chlorophyll per unit of area increases and then, in spite of the daily oscillations, it decreases.

It will be demonstrated further on that the drop of the chlorophyll concentration in darkness is mainly associated with its „dilution“. The growth of leaves takes place during the first several hours in darkness (Więckowski 1959) and the amount of chlorophyll present in the leaf is then distributed over a larger surface. The irregular oscillations seem to be due to the irregular rate of the growth of the leaf surface. As has been pointed out above, in the first phase leaves grow more slowly than chlorophyll is synthesized, so that the increase of the amount of chlorophyll in light is large and „dilution“ in darkness small. In the second phase the growth of leaves is more intense and consequently the „dilution“ of chlorophyll in darkness is larger while the rise of the chlorophyll concentration per unit of area in light is smaller.

In plants acted upon by alternate periods of light and darkness the rate of the chlorophyll synthesis during the periods of light is higher than during the same time in leaves cultivated in continuous light.

The causes of the drop of chlorophyll concentration in darkness

In the next stage of experiments the behaviour of chlorophyll during the 12 hours of darkness was investigated. The experimental procedure was as follows. Before placing the plants in darkness a disc 6 mm. in diameter was cut out from a leaf which was not detached from the plant and a print of the leaf was made on sensitized paper. The disc was cut out at point 1 of the leaf (see fig. 4). After 12 hours of darkness the next disc was cut out from the same leaf at point 3 and another print of the leaf was made. Each sample consisted of three discs cut out from three leaves. The amount of chlorophyll in the samples so obtained was determined. On the prints the area of leaves was measured. In this way it was possible to compare the drop of chlorophyll per unit area with the total increase of the surface.

Such a procedure would be acceptable without any doubts if the growth of leaves was uniform over the whole surface of a leaf, but this was not the case. Actually some parts of a leaf grow more intensely than others and the distribution of the areas of intense growth changes in various stages of growth. On the whole growth is more intense in the marginal parts (Staszkiewicz, unpublished).

Thus it becomes necessary to compare the rate of growth at points 1 and 3 (fig. 4) to the rate of growth of the whole leaf. For this purpose the following measurements were made. Directly before placing the plants in darkness and after cutting out at point 1 the disc of area x , a print of the whole leaf was made on sensitized paper. The area v of the whole leaf was measured from the print. After 12 hours of darkness a print of the same leaf was again made and the new area z was measured. During the 12 hours of darkness the area of the hole at point 1 increased from x to y . In order to diminish the error in the measurements of

the areas of the holes in the leaves, the print of the holes were magnified approx. 85 times with the use of an epidiascope.

Let us assume that the area of the part of the leaf blade at point 1 increased in the same degree as the area of the hole at this point.

If the growth of the leaf were uniform over the whole of its surface, then

$$y : x = z : v.$$

Actually the average values obtained for eight leaves in the first phase of growth were

$$y : x = 1,46 \text{ and } z : v = 1,50.$$

It is thus apparent that the increase of the area of the whole leaf is greater than of the area at points 1. However, since these differences have no significant influence on the results here reported no corrections were taken into account, all the more so as the area of the hole probably increased slightly less than would be the increase of the corresponding leaf surface if no opening was cut out. The possible error arising in this connection is thus further reduced.

Moreover, the concentration of chlorophyll at points 1 and 3 in samples taken simultaneously had to be compared. The results are listed in Table 2. As is to be seen, in spite of individual differences (the leaves are not always symmetrical) the average for three leaves, calculated from five determinations manifests an insignificant variability for both chlorophyll *a* and chlorophyll *b*.

Table 2

Concentration of chlorophyll in various parts of leaves of beans in the first phase of growth (mg/l)

	Pos. 1			Pos. 2		
	chloro- phyll <i>a</i>	chloro- phyll <i>b</i>	chlorophyll <i>a+b</i>	chloro- phyll <i>a</i>	chloro- phyll <i>b</i>	chlorophyll <i>a+b</i>
V 5 ¹⁵ p.m.	2,094	0,961	3,055	2,074	0,856	2,930
III 5 ³⁰ p.m.	1,227	0,469	1,696	1,282	0,512	1,794
IV 0 ⁴⁵ p.m.	1,185	0,528	1,713	1,206	0,573	1,779
IV 5 ²⁰ p.m.	1,619	0,672	2,291	1,650	0,689	2,339
IV 6 ⁰⁰ p.m.	2,047	0,850	2,897	1,968	0,831	2,799
Average	1,634	0,696	2,330	1,636	0,692	2,328
rel. value	100,00	100,00	100,00	100,12	99,43	99,91

The drop of the chlorophyll concentration and the increase of leaf area after 12 hours of darkness are compared in Table 3. If it was only the effect of the „dilution“ of chlorophyll, the relationship apparent in this table would be inversely proportional. Indeed, the existing relation-

Table 3

The decrease of the amount of chlorophyll compared to the increase of the leaf area in beans during 12 hours of darkness.
Leaves in the first phase of growth. Chlorophyll in mg/litre, leaf area in sq. centimetres

1	Before darkness			After 12 h of darkness			Leaves area		Ratio of chlorophyll before dark./after dark.			Ratio of leaves area after dark/before dark.
	chloro- phyll a	chloro- phyll b	chloro- phyll a+b	chloro- phyll a	chloro- phyll b	chloro- phyll a+b	before darkness	after darkness	chloro- phyll a	chloro- phyll b	chloro- phyll a+b	
	2	3	4	5	6	7	8	9	10	11	12	13
III/IV	1,433	0,654	2,087	0,997	0,397	1,394	5,86	8,99	1,44	1,63	1,50	1,53
III/IV	1,369	0,552	1,921	0,944	0,273	1,217	9,67	16,09	1,45	2,04	1,58	1,66
III/IV	1,550	0,696	2,246	0,892	0,247	1,139	9,68	17,63	1,74	2,80	1,97	1,82
IV/V	1,862	0,807	2,669	1,395	0,631	2,026	29,26	38,98	1,33	1,29	1,32	1,33
IV/V	1,924	0,844	2,768	1,388	0,530	1,918	30,14	42,22	1,39	1,58	1,44	1,40
III/IV	1,600	0,686	2,282	1,211	0,512	1,723	10,93	16,62	1,32	1,35	1,33	1,52
III/IV	1,586	0,716	2,302	1,175	0,505	1,680	12,09	18,35	1,35	1,41	1,37	1,52
Average	1,618	0,708	2,326	1,143	0,442	1,585	15,38	22,70	1,416	1,602	1,468	1,476
Relative value									100,00	100,00	100,00	100,54 92,13 104,24

ship is inversely proportional when the total drop of chlorophyll ($a + b$) is referred to the increase of the leaf surface (compare columns 12 and 13 in Table 3), but when the decrease of chlorophyll a and the decrease of chlorophyll b are referred separately to the increase of the leaf surface no such relationship is apparent. In darkness new amounts of chlorophyll a are synthesized and some chlorophyll b is decomposed (compare columns 10 and 11 with column 13, Table 3). It follows, therefore, that the drop of the chlorophyll concentration in leaves kept in darkness is brought out by two factors: 1) chlorophyll is „diluted“ and 2) actual changes occur in the amount of the pigments. The data assembled in Table 3 indicate that any amount of newly formed chlorophyll a corresponds to an equal amount of decomposed chlorophyll b . Possibly under prevailing conditions chlorophyll b is transformed to chlorophyll a .

The results just reported were obtained on leaves in the first phase of growth. Similar experiments were carried out also on leaves in the second phase of growth. The concentration of chlorophyll a and b in these leaves at point 1 and 3 when samples are taken simultaneously, is shown in Table 4.

The average differences in the concentration of both kinds of chlorophyll do not exceed one per cent, but the individual differences between determinations are much greater than between the same measurements carried out on leaves in the first phase of growth.

Table 4

Concentration of chlorophyll in various parts of leaves of beans in the later phase of growth (mg/l)

	Pos. 1			Pos. 3		
	chlorophyll a	chlorophyll b	chlorophyll $a + b$	chlorophyll a	chlorophyll b	chlorophyll $a + b$
VII 6 ⁴⁵ p.m.	1,569	0,516	2,085	1,526	0,484	2,010
VIII 9 ⁰⁰ a.m.	1,423	0,464	1,887	1,451	0,489	1,940
VIII 6 ⁴⁵ p.m.	1,532	0,499	2,031	1,562	0,518	2,080
V 7 ³⁰ p.m.	2,346	0,829	3,175	2,158	0,714	2,872
VI 7 ³⁰ p.m.	1,918	0,680	2,598	1,843	0,621	2,464
VII 9 ⁴⁰ a.m.	1,674	0,500	2,174	1,864	0,668	2,532
Average	1,744	0,581	2,325	1,734	0,582	2,316
Relative value	100,00	100,00	100,00	99,45	100,17	99,63

When the drop in the amount of chlorophyll per unit of area is compared with the total increase of leaf area after 12 hours of darkness (Table 5) the relationship obtained for chlorophyll a and b is inversely

Table 5

The decrease of the amount of chlorophyll compared to the increase of the leaf area in beans during 12 hours of darkness. Leaves in the later phase of growth. Chlorophyll in mg/litre, leaf area in sq. centimetres

[illegible]

proportional. No other changes, except for the synthesis of chlorophyll are distinguishable, though, probably even in this later phase of growth chlorophyll *a* is synthesized and chlorophyll *b* decomposed in darkness, but in the course of plant development the process becomes much less intense. The changes brought out by the process become so small that they cannot be detected by the techniques here described.

Daily changes in the concentration of chlorophyll in growing leaves
of *Platanus acerifolia*

At this point the question arose in what way the results here reported can apply to changes actually occurring in nature? Some facts have been explained by other workers and these will be mentioned further on in

Fig. 5. Points at which discs were cut out from leaves of *Platanus acerifolia* for chlorophyll determinations

the discussion. Let us consider as an example the changes in the concentration of chlorophyll in growing leaves of *Platanus acerifolia*.

For determination of the concentration of chlorophyll 30 leaves were selected from which discs 6 mm. in diameter were cut out at three points of each leaf, once at 6 a.m., once at 12, and once at 6⁴⁵ p.m. Each sample consisted of ten discs cut out from the leaves. The points at which leaf samples were cut out are shown in fig. 5 and the results are presented in Table 6.

Table 6

Changes of chlorophyll concentration in growing leaves of *Platanus acerifolia* in day time. May 16, 1959 (mg/l)

	6 ⁰⁰ a.m.			12 ⁰⁰			6 ⁴⁵ p.m.		
	chloro- phyll a	pos.	chloro- phyll b	chloro- phyll a	pos.	chloro- phyll b	chloro- phyll a	pos.	chloro- phyll b
Sample 1	2,382	1	0,945	2,460	2	1,083	2,706	3	1,042
Sample 2	2,438	3	0,882	2,472	1	0,966	2,612	2	1,002
Sample 3	2,019	2	0,700	2,446	3	1,039	2,694	1	1,142
Average	2,280		0,842	2,458		1,029	2,671		1,062
Relative value	100,00		100,00	107,85		122,22	117,15		126,13
Ratio a/b	2,71			2,39			2,52		

The data assembled in Table 6 led to the following conclusions. In day time the concentration of chlorophyll *a* and chlorophyll *b* rises. In the early hours of the day the increase of the concentration of chlorophyll *b* is greater than of chlorophyll *a* and at the same time the ratio of chlorophyll *a* to *b* decreases. In the afternoon the rate at which chlorophyll *b* is synthesized decreases while the rate of the synthesis of chlorophyll *a* remains almost unchanged. Simultaneously the ratio of chlorophyll *a* to *b* rises again. In the morning the value of this ratio is 2,71, at noon it drops to 2,39 and in the evening it rises again to 2,52 (at 6⁴⁵ p.m.). This means that the changes of the chlorophyll concentration in leaves of *Platanus acerifolia* are very similar to the changes taking place in leaves of beans. Presumably changes occurring during the night are also similar to the changes in leaves of beans kept in darkness. Obviously this last statement will be correct only when the conditions are suitable, e.g. a drop of temperature may inhibit the processes causing changes in the level of chlorophyll.

DISCUSSION

The data here reported indicate that the concentration of chlorophyll increases in light and drops in darkness. These results are in agreement with those reported by Bauer (1958) for plants growing in natural conditions. Bauer observed a rise of the chlorophyll concentration beginning in the early morning and continuing until late in the evening. At night the concentration dropped. Similar changes were recently observed by Mitrakos (1960) in growing cotyledons of *Perilla ocy-moides*. The plants were cultivated in controlled conditions.

Sironval (1953) also investigated the daily changes of the chlorophyll concentration in growing leaves. However, the daily rhythm in his experiments was somewhat different. According to Sironval the concentration of chlorophyll increases from the early morning until the early afternoon and then it begins to drop. The drop is continued through the night. The drop of the chlorophyll concentration in the afternoon observed by Sironval is difficult to explain and may be brought out by some external factor exerting an influence at some special time of the day. On the other hand, the smaller daily oscillations of the chlorophyll concentration in the later phase of leaf growth than in the early phase observed by this author are conformable with Sironval's results.

It is difficult to discuss the reports of other workers (e.g. Giubbiet and Bazanova 1955), because they do not mention exactly at what stage of growth were the leaves in their experiments. It is thus impossible to decide whether the results refer to growing or fully developed leaves. If there are any oscillations in mature leaves they must be brought out by factor other than in growing leaves.

Very characteristic is the daily change in the ratio of chlorophyll *a* to *b*. As has already been mentioned the value of this ratio increases during the night, whereas during the first hours of light it drops rapidly to reach the minimum after several hours and then rises again. In the course of leaf development the observed oscillations rapidly decrease, so much so, that after several days they can no longer be detected with the applied experimental techniques. These changes were observed both in leaves of beans and of *Platanus*.

Changes in the value of the ratio of chlorophyll *a* to chlorophyll *b* were observed also by Seybold and Egle (1938), Bauer (1958), Mitrakos (1960). Since the value of the ratio of chlorophyll *a* to *b* rises markedly during the night, the decrease of the chlorophyll concentration during the night cannot be explained merely by the „dilution“ of the pigments. If the changes consisted only in the „dilution“ of chlorophylls the ratio of chlorophyll *a* to *b* would not be altered. In view of the differences in the value of the ratio it seems that in darkness changes take place in the composition of the pigments. Indeed, it has been demonstrated that in darkness some amounts of chlorophyll *a* are synthesized, though the synthesis is much less intense than in light, while chlorophyll *b* breaks down. It has been demonstrated, moreover, that, the amount of synthesized chlorophyll *a* corresponds to the amount of decomposed chlorophyll *b*. The supposition, therefore, arises that in darkness chlorophyll *b* is transformed to chlorophyll *a*. The

possibility of a transformation of chlorophyll *b* to chlorophyll *a* is pointed out by several authors (see Rabinowitch 1945, page 554). The capacity for the transformation of chlorophyll *b* to chlorophyll *a* seems to be greatest in very young leaves and decreases in the course of development. Probably at the same time irreversible changes take place in leaves which make chlorophyll *b* more stable.

The above explanation seems the most plausible, though it is not impossible that chlorophyll *a* is synthesized by an enzyme, as is suggested by some workers (see: Röbbellen 1956, Virgin 1960), and that simultaneously chlorophyll *b* is decomposed being considered as a more labile substance than chlorophyll *a* (Mittrakos 1960).

The fact that in the experiments here reported the amount of chlorophyll *a* synthesized in darkness equalled the amount of decomposed chlorophyll *b* may be accidental. E.g. Seybold and Egle (1938) found that in darkness the increase of the level of chlorophyll *a* was greater than the decrease in the level of chlorophyll *b*. The final solution of this problem is, however, impossible on the ground of the now available data.

Are the „dilutions“ of chlorophyll as well as the decomposition of chlorophyll *b* and the synthesis of chlorophyll *a* in darkness the only factors responsible for the daily periodic changes in the concentration of chlorophyll in growing leaves? The answer seems to be that they are the main though not the only causes. Probably an important part in the oscillations is played by an inner rhythm in the ability to synthesize chlorophyll (Mittrakos 1959). There may be some other as yet unidentified factors affecting the daily rhythm which might also cause the drop of the chlorophyll concentration during the afternoon observed by many workers (Sironval 1953, Mittrakos et al. 1957); such factors may influence directly not the synthesis of chlorophyll but the growth of plant organs.

Finally, it seems relevant to point out that when alternate periods of light and darkness are applied the overall rate of chlorophyll synthesis is greater than in leaves of plants kept in continuous light. The results obtained by Rudolf (1934), Virgin (1955) and other, seem to indicate that, apart from the inner rhythm, this difference in the rate of the synthesis of chlorophyll is caused by the accumulation of a precursor of chlorophyll in darkness.

SUMMARY

The daily oscillations of the chlorophyll concentration in growing leaves of beans, throughout the time of growth, were investigated. The

plants were kept alternately for 12 hours in light and 12 hours in darkness.

It has been demonstrated that changes in the concentration of chlorophyll take place all the time that the area of leaves is growing. Immediately after plants are exposed to light the concentration of chlorophyll in leaves per unit of area begins to increase. In the first hours after exposure the amount of chlorophyll *b* increases more rapidly than the amount of chlorophyll *a*. Later the rate at which the amount of chlorophyll *b* increases is markedly reduced. During darkness the concentration of chlorophyll in leaves decreases.

In the first phase of leaf growth (the first 2—3 days) the concentration of chlorophyll increases more in day time than it decreases during the night. In the second phase (from about the third day till growth is terminated) the situation is reversed.

The decrease in the concentration of chlorophyll per unit of area of the leaf in darkness is caused mainly by the „dilution“ of chlorophyll, since leaves grow in darkness. Besides the „dilution“ of chlorophyll in darkness a certain amount of chlorophyll *a* is then synthesized and some chlorophyll *b* is decomposed. Consequently the value of the ratio of chlorophyll *a* to chlorophyll *b* increases. As leaves become older the rise in the value of the ratio of chlorophyll *a* to *b* in darkness becomes smaller and after several days it is even difficult to detect.

Changes of the chlorophyll concentration were also observed in *Platanus acerifolia* growing in almost natural conditions and it has been found that the nature of these changes is similar to the changes observed in beans.

The author is sincerely grateful to Professor F. Górski and Doctor J. Zurycki for their most valuable advice in the course of this investigation.

Laboratory of Plant Physiology
Polish Academy of Sciences, Cracow

(Entered: 10.3.1960)

REFERENCES

- Bauer A., 1958, Die Konstanz des Chlorophyllgehaltes bei Laubblättern im Laufe eines Tages, *Planta* 51: 84—98.
Bavrina T. V., 1959, Sutochnaja dinamika w sodierzani chlorofilla w listiach rastieni, *Fizjologia Rast.*, 6: 213—216.
Bukatsch F., 1939 Tagesschwankungen des Blattgrüngehaltes alpinen Pflanzen *Zeitschr. f. d. gesamte Naturwissenschaft*, 5: 263—272.
Bukatsch F., 1940, Tagesschwankungen des Blattgrüngehaltes alpinen Pflanzen II, *Zeitschr. f. d. gesamte Naturwissenschaft* 6: 197—198.

- Comar C. L. and F. P. Zscheile, 1942, Analysis of plant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method, *Plant Physiol.* 17: 198—209.
- Gjubbeniet F. R. i N. V. Bazanowa, 1955, Sutocznaja dinamika w sodzierzenie chlorofilla w listiach kartiofiela, *Dokł. Akad. Nauk SSSR* 105: 586—587.
- Mitrakos K., 1959, Tagesperiodische Schwankungen der Fähigkeit zur Chlorophyllbildung, *Planta* 52: 583—586.
- Mitrakos K., 1960, Kurzperiodische Schwankungen im Chlorophyllgehalt von Kotyledonen verschiedener Entwicklungsstadien, *Planta* 54: 365—373.
- Mitrakos K., E. Bünning, F. Eberhardt, 1957, Endogen-tagesperiodische Schwankungen der Chloroplasten Farbstoffe, *Z. f. Naturforschung* 12b: 813—814.
- Rabinowith E., 1945, *Photosynthesis and Related Processes*, vol. I Interscience Publishers, INC New York.
- Röbbelen G., 1956, Über die Protochlorophyllreduktion in einer Mutante von *Arabidopsis thaliana* (L.) Heynh., *Planta* 47: 532—546.
- Rudolph H., 1934, Über die Einwirkung des farbigen Lichtes auf die Entstehung der Chloroplastenfarbstoffe, *Planta* 21: 104—155.
- Seybold A., 1942, Weist der Chlorophyllgehalt der Blätter Tagesschwankungen auf?, *Bot. Archiv* 43: 71—77.
- Seybold A. and K. Egle, 1938, Lichtfeld und Blattfarbstoffe II, *Planta* 28: 87—123.
- Seybold A. und H. Falk, 1959, Die Heidelberger Chlorophyllbestimmungen -- Eine Überprüfung, *Planta* 53: 339—375.
- Sironval C., 1953, A propos du métabolisme de la chlorophylle dans la feuille (ses rapports avec la floraison), *Bull. Soc. Ray Bot. Belg.* 85: 285—295.
- Staszkievicz J., Badania biometryczne nad wzrostem liścia *Ipomea purpurea* (Unpublished).
- Virgin H. I., 1955, Protochlorophyll formation and greening in etiolated barley leaves, *Physiol. Plant.* 8: 630—642.
- Virgin H. J., 1960, Pigment transformations in leaves of wheat after irradiation, *Physiol. Plant.* 13: 155—164.
- Wendel K., 1940, Tagesschwankungen des Blattgrüngehaltes ebener Standorte, *Zeitschr. f. d. gesamte Naturwissenschaft* 6: 327—329.
- Wendel K., 1957, Über die Veränderlichkeit des Chlorophyllgehaltes einiger Pflanzen im Ablauf eines Tages, *Protoplasma* 48: 382—397.
- Więckowski S., 1957, Badania nad dziennymi zmianami w stężeniu chlorofilu w liściach roślin stanowisk nizinnych i górskich, *Acta Soc. Bot. Pol.* 26: 657—673.
- Więckowski S., 1959, The relation between the growth of leaves and the synthesis of chlorophyll, *Acta Biol. Crac.* 2: 1—11.