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CHLOROPHYLL METABOLISM AND THE LEAF CONTENT IN SOME OTHER TETRAPYRROLE PIGMENTS

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ZELLER[43] wrote that "Willstätter has shown that, as astonishing as it may be, the chlorophyll content of the plants varies only within very small limits and that the ratio of the two components a and b is almost always the same, i.e. 3:1." He summarises in this sentence the general belief which developed after the publication of the remarkable book of Willstätter and Stoll.[44] This belief was to consider chlorophyll accumulation in the growing leaf as simply resulting from the continuous synthesis of new molecules adding to the former ones. One can easily prove that the process is in fact more complex.

1. CHLOROPHYLL RENEWAL AND ACCUMULATION IN THE GREEN LEAF

1. *Daily rhythms*

Data published by Bukatsch and Wendel[3, 39] point out the fact that in the course of a single day the amount of pigments may vary within a single leaf (or a series of leaves). Pigment accumulation is not a continuous process. One finds each day periods where the chlorophyll is accumulating and others in which it ceases to accumulate or tends to disappear more or less rapidly. Accumulating periods occur during the day; the others mostly during the night. These facts were confirmed in a series of various species[3,23,29,39,40].

Examples of diurnal variations observed in the pigment content are given in some papers of this book. The variations are not exactly of a strict endogenous type. Fig. 1 shows that, for *Fragaria vesca*, the position of the diurnal synthetic period changes with the applied daylength. The amplitude of the variations changes also in relation to daylength. The daily variations are very important in the young leaf. In the strawberry, the increase of the pigment content during the accumulation period may reach, in some cases, more than 40 per cent of the initial content at the beginning of the day. When the leaf becomes older, the amplitude of the diurnal variations decreases more or less strongly as may be seen in Fig. 2.

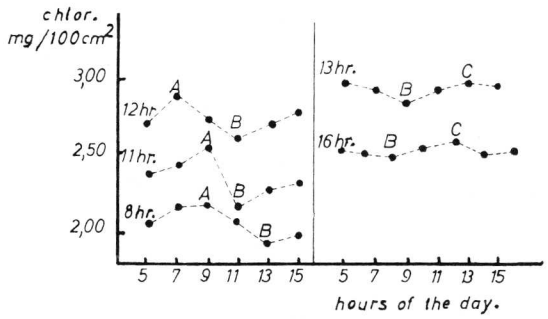

FIG. 1. Daily variations of the chlorophyll content in the nearly mature leaf number 16 of *Fragaria vesca* plants. In short-day, the chlorophyll content rises immediately from the beginning of the day (at 5 o'clock); in long-day, it rises 4 to 5 hr later. Ordinary temperature; natural day-light.

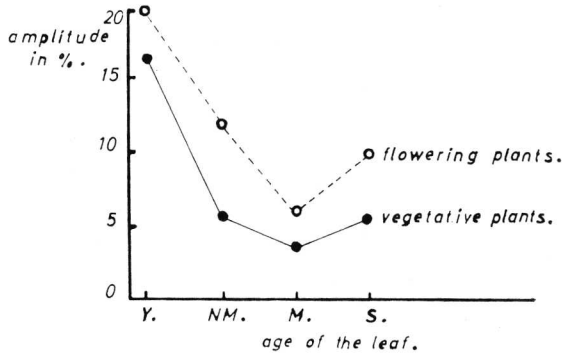

FIG. 2. Effect of the age of the leaf on the amplitude of the daily variations of the chlorophyll content (mean values of the amplitude observed in flowering and in vegetative plants). Ordinary temperature; natural day-light.

The diurnal rhythms have been interpreted as resulting from the existence of two continuous and contradictory processes of pigment accumulation and destruction in the leaf.[28] During the day, the synthesis should predominate; during the night, destruction should be more important. This concept involves the assumption that there is a continuous renewal of chlorophyll molecules in the leaf.

It is interesting to point out that the daily rhythms in pigment content do not occur in exactly the same way for the two chlorophylls a and b. As a result the ratio between the quantities of chlorophyll a and chlorophyll b varies in the course of a single day.[40] It seems that the lowest value of the ratio a and b is generally found in the middle of the day (cf. Bukatsch, this symposium). The different behaviour of the two chlorophylls a and b is also made evident in the study of the variation of the average a/b ratio in the ageing of the leaf (Fig. 3). In the strawberry plant, this ratio tends to decrease slowly from the young leaf to the old one (when grown in long-day). The same phenomenon can be observed in the leaf of *Kalanchoë blossfeldiana*.[34] It is remarkable that although the variations of the a/b ratio are evident, they are however restricted within relatively narrow limits.

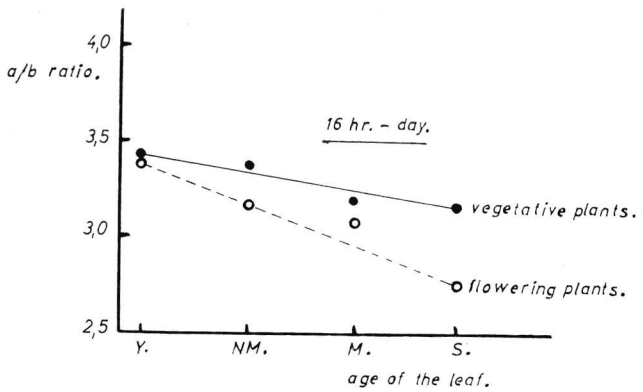

FIG. 3. Regular lowering of the $\left(\frac{\text{chlorophyll a}}{\text{chlorophyll b}}\right)$ ratio during the ageing of the leaves in long days.
Y = young leaf; N.M. = nearly mature leaf; M = mature leaf; S = senescent leaf.
Ordinary temperature; natural day-light.

2. *Rate of the renewal of the chlorophylls in the green leaf*

It is easy to incorporate *in vivo* radioactive elements (C^{14} in particular) into the chlorophyll molecules. Roux and Husson[26] were the first to obtain radioactive chlorophyll. Since that time, works making use of tracers have multiplied. On the whole, they show that the pigment renewal is rapid in the young leaf, and that it still exists in the mature leaf, although at a slower rate.

Aronoff[1] has published a measurement of the half-life time of the chlorophyll molecules (a+b). He fed Soja plants with $C^{14}O_2$ during one hour, and a week later he measured the decrease in the radioactivity of the chlorophylls. The calculation of the half-life time is based on the assumption that after a week the C^{14} is uniformly distributed among the various constituents of a single leaf and among the carbon atoms of the molecules. Under these conditions, as Solomon[36] has shown, one may use the following equation:

$$K = \frac{(\log Mo^* - \log M^*)\, 2{\cdot}3\, M}{t}$$

where K is the amount of pigment renewed per day and per unit of leaf surface or per weight, t the time in days, M the quantity of pigment per unit surface or weight, Mo^* the radioactivity present in the pigments at the beginning of the experiment and M^* the radioactivity found after the time t.

Aronoff measures in this way a half-life time for the chlorophyll (a+b) of about one day. It is possible that this result is more concerned with the renewal of phytol than that of the tetrapyrrole ring.

We have repeated the experiment of Aronoff with *Soja hispida*, using Δ-amino–laevulinic acid 4–C^{14} as precursor. The acid was introduced according to the method of Roux by feeding a petiole during 24 hr. Under these conditions, the maximum of C^{14} incorporation in the young leaves is obtained 2 days after the beginning of the feeding with the labelled precursor. After this time, the chlorophyll C^{14} disappears following curves that on a logarithmic scale are straight lines (Fig. 4). From such lines, it is possible to deduce that the renewal of the tetrapyrrole ring is characterized, for the two chlorophylls, by a half-life time of about 5 days.

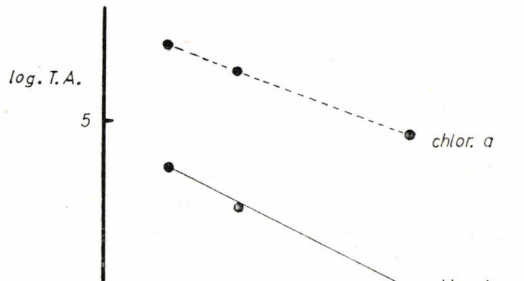

FIG. 4. Decrease of the labelling of the chlorophylls a and b in Soybean third leaves vs. time, 4-C^{14}–amino-laevulinic acid being fed during 24 hr at the beginning of the experiment. Log. T.A. = logarithm of the total activity (in counts/min) found in the chlorophylls contained in 1 g fresh weight of leaves. Temperature: 20°C constant; 8 hr-day; artificial white light; about 5000 lux.

The series of measures of Table 1 shows that, under various experimental conditions, the half-life time of the tetrapyrrole nucleus of chlorophyll a is higher than that of chlorophyll b. In 4, Table 1, the tetrapyrrole of chlorophyll b is renewed about three times faster than that of chlorophyll a.

TABLE 1. HALF-LIFE TIME OF THE CHLOROPHYLL a AND b MOLECULES IN THE YOUNG LEAVES OF *Soja hispida* (IN DAYS; RESULTS OBTAINED IN 4 DIFFERENT EXPERIMENTAL SERIES)

Series no.	Half-life time of chlorophyll a	Half-life time of chlorophyll b
1	5·8	4·3
2	5·0	5·5
3	6·6	4·0
4	6·0	1·7

Nevertheless, the main difference between the behaviour of the two chlorophylls a and b in the experiments done with the C^{14} labelled precursors lays in the fact, that in most cases, C^{14} incorporation in chlorophyll a is easier than in chlorophyll b. This is an argument for the assumption that chlorophyll b cannot be a precursor of chlorophyll a. On the contrary, the experiments of Godnev and Shlyk,[17] Wolwertz[42] and Michel-Wolwertz (this symposium) indicate that chlorophyll b can be formed from molecules of chlorophyll a. If we accept this last conclusion and if we compare it with the respective rates of renewal of the two chlorophylls and the relative intensity of their labelling, we are obliged to admit that the chlorophyll b molecules are not randomly formed from any of the chlorophyll a molecules. Chlorophyll b has to find its origin in a particular kind of chlorophyll a molecules which are synthesized at a higher rate than the average. We are obliged to assume the heterogeneity of chlorophyll a, a hypothesis which is consistent with the data published by Brown and French,[2] Krasnovsky,[21] and data of Michel-Wolwertz (this symposium).

3. *Factors affecting the accumulation of chlorophyll in the leaf*

The chlorophyll metabolism and the accumulation of these pigments in the leaf are affected by two kinds of factors: internal factors and external factors. The internal factors depend on the genetic properties of the species; under normal growth conditions, hemp, for example, accumulates chlorophyll in another way than does the strawberry plant. The heredity of the accumulation mechanisms stays probably, at least partially, in the chloroplasts themselves, as it seems to appear from the results of Renner[25] and Schötz.[27]

The external factors are acting differently according to the properties of the species and of the chloroplasts. Several are known from a long time. Former authors were concerned with the effects of the light intensity on the accumulation of the chlorophylls (shadow leaves and light leaves), or the effects of the temperature (in relation with the altitude, for instance). One has also studied the effects of various deficiencies (in iron, in magnesium, etc.) or the toxicity effects (copper, nickel). We will see further that a micronutrient like cobalt may have an influence on the pigment accumulation. Recently, some hormones, like giberellic acid, have also been shown to have an action on chlorophyll accumulation (Table 2).[24]

TABLE 2. ACTION OF GIBBERELLIN ON THE TOTAL CHLOROPHYLL CONTENT OF LEAF NO. 10 (ADULT STAGE) IN CELERY (IN MG/100 CM²)

Control	1·50
150 p.p.m. GB	1·04
300 p.p.m. GB	0·87
(control—150 p.p.m.)	$0{\cdot}46^{++}$
(control—300 p.p.m.)	$0{\cdot}63^{++}$

We have been especially concerned with the study of the control of the chlorophyll metabolism by daylength. This study is greatly facilitated by the use of conditioned rooms which allow to keep all conditions fixed, except those under study. Without these rooms, our knowledge of the effects of daylength on the green pigments accumulation would have progressed less easily.

We have pointed out above, that in the course of a single day the type of the variations of the pigment content depends on the applied daylength. In the strawberry plant, when the night is long—the day being short, from 8 to 12 hr—one notices an increase of the pigment content (expressed in cm² of leaf area) as soon as the day begins. The increase depends on the hour of the beginning of the day. If one suddenly begins the enlightenment 1 or 2 hr later, one correspondingly delays the increase of the content by 1 or 2 hr.

On the other hand, when the night is short (the day being long, from 12 to 16 hr), the pigment content does not increase at the beginning of the day. It only begins to increase 3 or 4 hr later. As a result of this behaviour, the total accumulation of the chlorophylls in the leaf, from day to day, also depends on the relative length of day and night (Fig. 5).

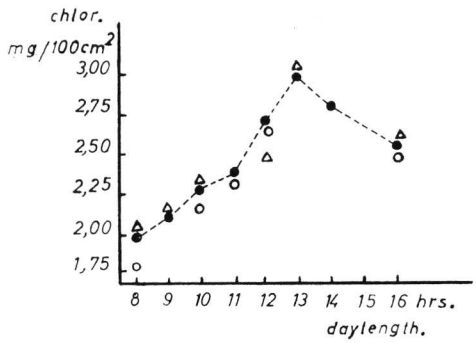

FIG. 5. The chlorophyll content of some adult leaves of *Fragaria vesca* in function of the applied daylength. Δ leaf no. 10 of vegetative plants; ● leaf no. 16 of vegetative plants; O a leaf of flowering plants. Ordinary temperature; natural day-light.

Many authors arrived at the same conclusion.[5, 6, 15, 16, 20, 29, 38, 41]

The photoperiodic nature of the control of the pigment accumulation in the leaf seems to be supported by a series of facts:

1. A first argument is that an interruption of the night by a period of light acts on the pigment accumulation. The fact that this action depends on the position of the light interruption during the night [8, 30, 34] suggests the photoperiodic nature of the control (Fig. 6).

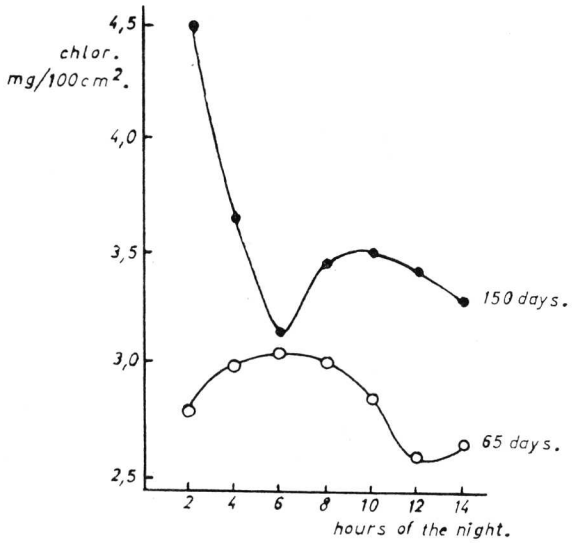

FIG. 6. Effect of the position of the light-break given during the night on the chlorophyll content of old leaves of *Kalanchoë blossfeldiana*, var. *Fleur de feu*. Interruption of the night by 15 min light. The effect is not the same when the plants are young (65 days) or old (150 days). Temperature: 20°C constant; artificial white light; about 5000 lux.

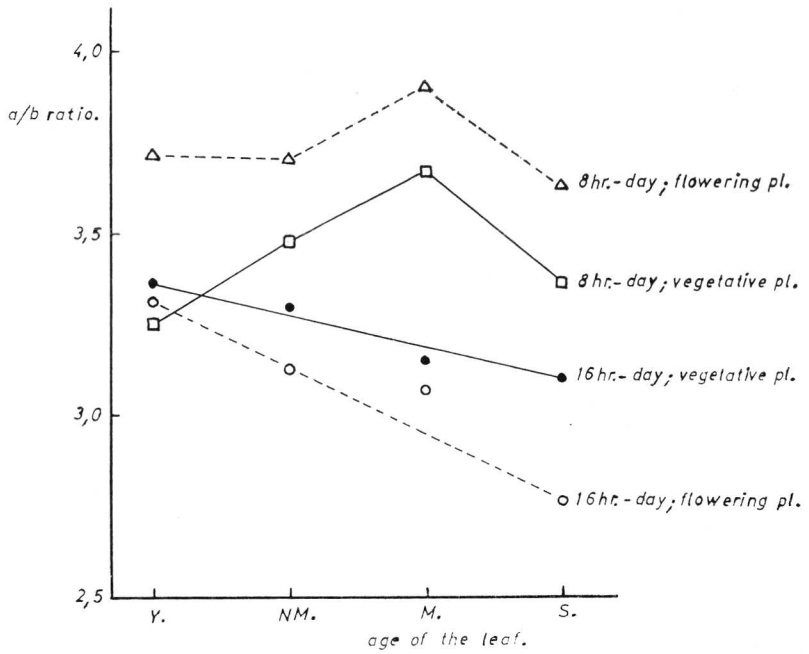

FIG. 7. Dependence of the values of the a/b ratio on the applied daylength. In *Fragaria vesca*, the ratios are higher in short day than in long day. Ordinary temperature; natural day-light; Y = young leaf; N.M. = nearly mature leaf; M = mature leaf; S = senescent leaf.

2. A second argument may be taken from Withrow and Withrow.[41] These authors show that the increase of the daylength by periods of weak light intensity modifies the pigment content of the leaves of tomato plants.
3. A third argument arises from the fact that the ratio between the two pigments a and b, in the course of the ageing of the leaf depends also for certain species, on the daylength[7, 29, 38] (Fig. 7).

One of the most remarkable effects of daylength on the pigment content is obtained when transferring suddenly a plant from one daylength to another which is sufficiently different. This is the "transfer-effect" described for the first time by Cheuvart[6] in hemp. If one allows Indian hemp plants to grow in short days, they remain short (10–15 cm) and begin immediately to flower. Their small leaves accumulate little pigments (from 2·5 to 2 mg/100 cm^2). When transferring these hemp plants at the time of flowering (the 40th day after the sowing) from short-day to long-day conditions, they begin again to grow; the newly formed leaves are very big (Fig. 8) and they accumulate a quantity of pigments which in the 6th–8th leaf may have a concentration in excess of 3·5 mg/100 cm^2. The plants grown from seeds in 16 hr-day do not accumulate more than 3 mg/100 cm^2 in their leaves (Fig. 9). It is to be noticed that the increase of content of pigment in the transfer from 8 to 16 hr also occurs in the leaves which are already adult at the time of the transfer (2nd, 3rd, 4th leaf).

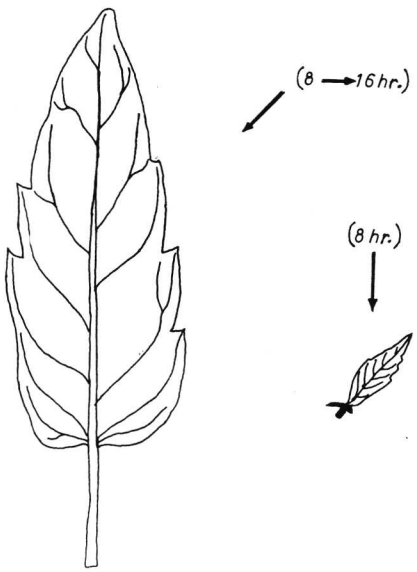

FIG. 8. Comparison of the surface of the full expanded 6th leaf of hemp plants cultivated in 8 hr-day, with that of same full expanded leaf after the transfer of the plants from 8 to 16 hr-day. The transfer occurs after 40 days of culture in short-day (redrawn after C. Cheuvart, Thesis, Liège, 1954).

The "transfer-effect" has been described in *Fragaria vesca*[29], in *Kalanchoë blossfeldiana*[34], in *Soja hispida*, var. *Capitole*[33] and in *Sinapis alba*[35].

The experiment with soybean, is particularly interesting. The plants were first grown from seeds in two groups; one in 8 hr-day and the other in 16 hr-day at 20°C constant under artificial light of Phytor lamps (*ca.* 5000 lux). The plants grow until the formation of the

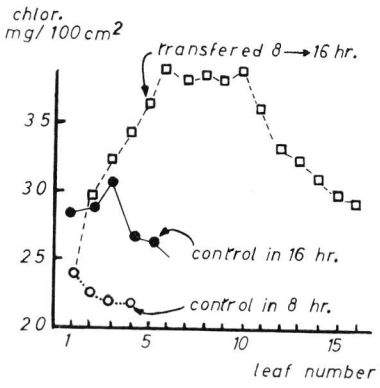

FIG. 9. Effect of the transfer from 8 to 16 hr-day on the accumulation of chlorophyll in the leaves of hemp. Transfer 40 days after sowing. Temperature: 20°C constant; artificial white light; about 5000 lux[6].

3rd leaf. At that time, one removes the cotyledons and all the leaves (including the auxiliary buds which begin to grow). The terminal bud is also taken off, and the third very young leaf is left. One then transfers in 16 hr-day a series of plants grown in 8 hr-day, and inversely, one transfers in 8 hr-day a series of plants grown in 16 hr-day.

The accumulation of the green pigments in the third leaf of the four series is given in Fig. 10. One observes that, in the series, which is transferred from 8 hr to 16 hr-day, the accumulation of the pigment is strongly activated, whereas it is moved down in the series which is transferred from 16 hr to 8 hr-day. One should notice that the leaf areas are practically not modified by the transfer. It is strictly an effect of the transfer from one daylength to another in the pigment accumulation. In fact, the labelling of the pigments by C^{14} allows one to show that, in this experiment, the pigment metabolism is different in the four series.[33]

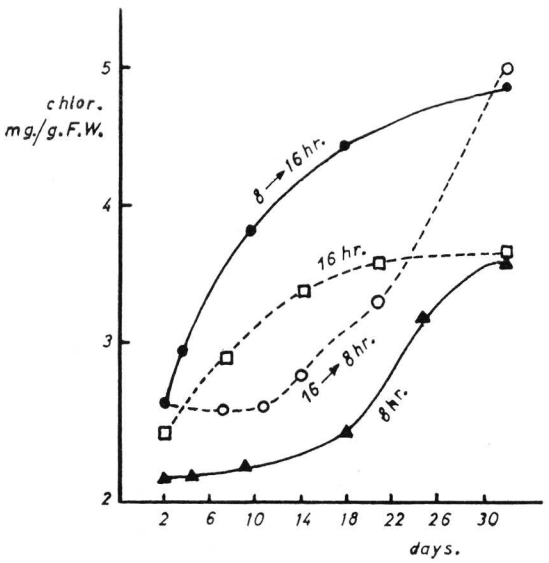

FIG. 10. Effect of the transfer from 8 to 16 hr-day on the accumulation of chlorophyll in the third leaf of soybean plants (explanations in the text). In abscissa days after the transfer.

II. RELATION BETWEEN CHLOROPHYLL ACCUMULATION AND THAT OF TOTAL HAEMATINE IN THE GREEN LEAF

1. *Relation between the chlorophyll and the haematine content of the leaf*

The metabolism of the tetrapyrrole pigments containing magnesium, and the metabolism of those containing iron (of the haematine type) are not independent in the green leaf.

When measuring the total quantity of the haematine in the leaf, one finds that the content is high in the young leaf and decreases during the ageing of the leaf. Fig. 11 shows this decrease in *Perilla nankinensis.*

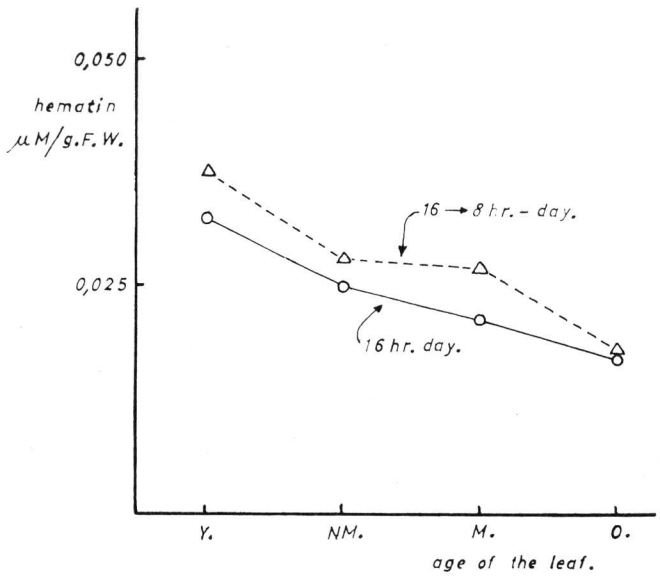

FIG. 11. Decrease of the haematine content of the leaves during their ageing in *Perilla nankinensis.* Temperature: 20°C constant; Artificial white light; about 5000 lux; Y = young leaf; N.M. = nearly mature leaf; M = mature leaf; O = old leaf.

The decrease of the total haematine content corresponds to changes of the activity of some haematinic enzymes. In *Fragaria vesca,* one observes that under normal conditions of culture in the field, the catalase is more active in young leaves (with less chlorophyll), than in old leaves (with more pigments). In old leaves one finds only 50 to 70 per cent of the activity in young leaves. The contrary occurs for peroxidase. In this case, the activity is lower in the young leaf than in the old leaf. The activity of the young leaf reaches 20 to 40 per cent of that of the aged leaf.[4] The activities of the two enzymes vary in an inverse manner as if they rearrange themselves during the growth of the leaf.[32] Dekock *et al.*[11] reach an analogous conclusion in experiments which are however different. Generally, the behaviour of the principal haematine enzyme systems in the course of ageing of the leaf is complex. But chemical methods,[19] are available to show the rather constant decrease of the total haematine content during the ageing of the leaf. At the end of the leaf growth, the haematine content reaches a minimum value when the chlorophyll reaches a maximum value; the leaf is adult.

Hill and Scarisbrick[19] and Davenport and Hill[10] have published several measurements according to which in the adult leaf a kind of equilibrium exists between the content in some heme compounds and the chlorophyll content. The measurements performed on total haematine by Davenport,[9] Sironval,[31] and Dekock *et al.*[11] indicate that in the adult leaf one encounters about 60 molecules of chlorophyll per molecule of haematine. The above authors rely on various experiments; Davenport varied the chlorophyll and haematine concentrations in inducing some deficiences in micronutrients; Dekock *et al.* used different levels of Fe, K and P: Sironval made use of the action of daylength applied to various species.

The regression curve which our measurements established between the total chlorophyll and total haematine in using the action of daylength is given in Fig. 12. It is represented by the equation; $1000\,y = 16{\cdot}28\,x - 0{\cdot}85$, where y and x are the haematine and chlorophyll contents respectively (expressed in μmoles/g fresh weight). Dekock *et al.*[11] find almost the same equation; $1000\,y = 17{\cdot}05\,x - 1{\cdot}84$. This proves that one may consider as a general law that in the adult leaf the molecular ratio chlorophyll/haematine is about 60.

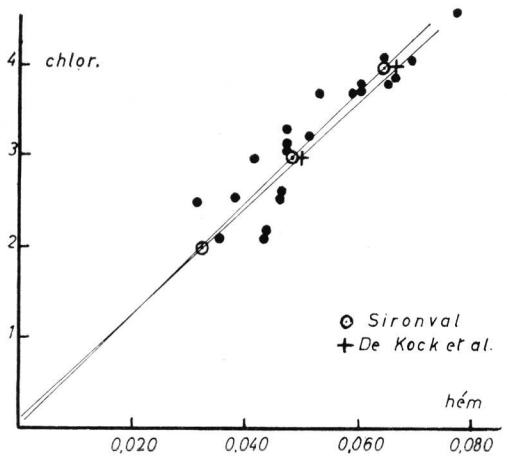

FIG. 12. Regression lines between total haematine and total chlorophyll in adult leaves, as calculated by Dekock *et al.*[11] and Sironval. ● = Values obtained by Sironval in different species and in different daylengths and temperatures, but in natural day-light. Chlorophyll and haematine are given in μM/g fresh weight.

Two conclusions follow:

1. The two types of pigments are not independent; there exists a relation between the metabolism of chlorophyll and haematine.

2. The leaf contains a regulatory system which allows it to adjust the quantities of haematine and chlorophyll so that a definite ratio is maintained.

There are few measurements on the total chlorophyll to total haematine ratio in isolated chloroplasts. Two preliminary measurements which we have done have yield ratios of between 80 and 90 molecules of chlorophyll to one of haematine. This indicates that the chloroplasts alone contain the greatest part on the haematine present in the leaf blade. It is therefore probable that in normal chloroplasts of adult leaves, the chlorophyll/haematine ratio is maintained at a constant value as it is for the entire leaf.

It seems to be true that the quantities of cytochrome f and cytochrome b_6 (b_3+b_6 ?) of the chloroplasts of the adult leaf are also in more or less definite ratio with the total chlorophyll content. Table 3 compares the results of Davenport and Hill,[10] and Lundegårdh,[22] to those we obtained using the methods of Hill. As one may see, it seems that one finds quite regularly one molecule of cytochromes f for 200 to 400 molecules of total chlorophyll (a+b). Taking account of the inherent difficulties of the measurement (loss of cytochromes during the preparation of the chloroplasts ?), the agreement between the results is quite good.

TABLE 3. MOLECULAR RATIOS BETWEEN TOTAL CHLOROPHYLL AND CYTOCHROMES IN CHLOROPLASTS PREPARED FROM ADULT LEAVES

	Molecular ratio: $\left[\frac{\text{total chlorophyll}}{\text{cytochrome f}}\right]$	Molecular ratio: $\left[\frac{\text{total chlorophyll}}{\text{cytochrome } b_6}\right]$
Davenport and Hill[10]	380 (parsley) 430 (elder)	— —
Lundegårdh[22]	400–500 (spinach)	$\frac{\text{Chl}}{b_3+b_6} = 100$ $\frac{\text{Chl}}{b_3} = 200$ } (spinach)
Sironval	380; 200 (spinach)	97 (spinach)

Lundegårdh[22] says that this situation is "of some interest in view of the ideas of 'reaction centres' operating between chlorophyll and the energy converting mechanisms."[13,14]

The extraordinary constancy found in the leaf for the chlorophyll/haematine ratio supports this idea. It is, for example, possible that the ratio of 60 molecules of chlorophyll to one of haematine, corresponds to the organization of the leaf and of the chloroplast permitting the optimal functioning of the photosynthetic apparatus (when the other conditions are adequate).

2. *Effects of cobalt on the accumulations of haematine and chlorophyll*

Hallsworth *et al.*[18] have shown that a low concentration of cobalt increases the growth of clover plants, the weight of their nodules and the nitrogen fixation. In the presence of cobalt the increase of the weight of the nodules corresponds to an increase in the quantity of haemoglobin they contain. Delwiche *et al.*[12] have confirmed on *Medicago sativa* that, in the presence of an adequate rhizobium strain, the addition of cobalt increases the nitrogen fixation.

In studying the action of cobalt and daylength on the nodule formation of soybean, we have effectively found that in the presence of cobalt the weight of the nodules increases (10 to 40 per cent per plant). The quantity of haemoglobin contained in the nodules per plant is equally increased (Table 4). A considerable increase in the weight of the nodules in the presence of cobalt has also been obtained in the lupin.

TABLE 4. EFFECT OF COBALT ON ROOT NODULES FORMATION IN SOYBEAN AND LUPIN (IN LONG DAYS; COBALT GIVEN AT THE CONCENTRATION OF 5μM/l.)

		With cobalt	Without cobalt	Difference (+Co)−(−Co)
Soybean	Fresh weight of nodules per plant (mg)	40·5	30·4	+10·1
	haemoglobin in the nodules per plant ($\mu M \times 10^{-3}$)	7·30	6·21	+1·09
Lupin	Fresh weight of nodules per plant (mg)	24·0	15·3	+8·7

One might think that the increase of the quantity of haemoglobin contained in the nodules in the presence of cobalt corresponds to a net supplementary synthesis of haematine by the plant. This is not true. We have established that the increase of the haemoglobin content of the nodules (per plant) is accompanied by a decrease of the total haematine content of the leaves (per plant). In the lupin the leaf haematine content is lowered from 15 to 30 per cent. In the bean the diminution in the second leaf is about 10 per cent. There is a kind of "displacement" of haematine from the leaves (or certain leaves) to the nodules under the effect of cobalt, as if the plant was limited in its capacity of synthesis.

One observes that the decrease of the haematine content of the leaves is accompanied by a decrease of the chlorophyll content. We shall briefly consider soybean plants cultivated in 8 hr day (16 hr of night), in one case, with cobalt and in the other, without. The presence of cobalt increases the quantity of haematine in the nodules by $1{\cdot}18 \times 10^{-3}$ μmole per plant. The quantity of haematine contained in the second leaf alone is lowered by $0{\cdot}14 \times 10^{-3}$ μmole; at the same time, the second leaf loses 37×10^{-3} μmoles of chlorophyll. The chlorophyll/haematine ratio remains practically constant: 63 in the absence of cobalt and 57 in its presence. The same phenomenom is duplicated with 16 hr-day in soybean and is found equally well in lupin. However, when one does not observe a decrease in the haematine content in the presence of cobalt (as is the case, for instance, in the first leaf of soybean) one no longer observes a decrease in the chlorophyll content (Table 5).

The experiment shows quite clearly the necessary relation between the total haematine and the total chlorophyll content of the leaf. When we supply cobalt, we reduce the quantity of haematine in the leaf and at the same time the quantity of chlorophyll. There is no doubt that the leaf possesses a regulatory system which modifies the content of haematine in accordance to that of chlorophyll or inversely.

3. *Effects of artificial light and of photoperiodic induction on the relation between chlorophyll and haematine*

We have established the points of Fig. 12 in applying diverse photoperiods to a series of plants: thus, different concentrations of chlorophyll have been obtained to which correspond different concentrations of haematine in the molecular ratio chlorophyll/haematine = 60. We have always found this ratio when the plants are cultivated in ordinary daylight, for short or long photoperiods and when they are not photoperiodically sensitive.

TABLE 5. EFFECT OF COBALT ON THE RELATIVE CONTENT IN TOTAL CHLOROPHYLL AND HAEMATINE IN ADULT LEAVES OF SOYBEAN AND LUPIN

		Chlorophyll		Haematine	
		With cobalt	Without cobalt	With cobalt	Without cobalt
Soybean, in long-day	leaf no. 1	100	100	100	100
Soybean, in long-day	leaf no. 2	91	100	87	100
Soybean, in short-day	leaf no. 1	99	100	107	100
Soybean, in short-day	leaf no. 2	81	100	90	100
Lupin, All the leaves (the young leaves included), in long-day		82	100	70	100
Lupin, All the leaves (the young leaves included), in short-day		96	100	89	100

It seems that in artificial light, for example under Phytor lamps (about 5000 lux), the ratio of chlorophyll to haematine is usually a bit higher than 60. It is between 60 and 90 in the adult leaf.

One can encounter higher ratios in certain photoperiodically sensitive species, when they are cultivated in unfavourable conditions for flowering. We have found, for example, in adult leaves of *Sinapis alba* cultivated in short days, ratios which are greater than 100 molecules of chlorophyll to one of haematine. When one induces flowering in *Sinapis* (in growing them in long days), the ratio is sensibly less and in artificial light lies between 60 and 80. Thus, in this case, the ratio decreases with increasing the length of the day which is favourable for flowering.

Such a behaviour seems to be common to photoperiodically sensitive plants which have been studied so far; in non-inductive conditions the leaves contain less haematine and the chlorophyll/haematine ratio is higher than in inductive conditions.[33] One might think that the induction of flowering is favoured by the existence of a certain equilibrium between chlorophyll and haematine in the leaves and that this equilibrium is susceptible to regulation by some external conditions, especially daylength in the case of plants which are photoperiodically sensitive. This might be explained by admitting that an adequate functioning of the leaf is necessary for flower induction and that this functioning is obtained for some precise relations between different constituents of the photosynthetic apparatus.

On the whole, the facts may be classified in two apparently contradictory categories:

(a) On one hand, the molecules of chlorophyll in the leaf are renewed at a certain rate; it is possible that for one or another form of these molecules the mean half-life time is short. One can provoke by environmental changes rapid modifications of the pigment content of the leaves, as well for chlorophyll as for haematine.

(b) On the other hand, despite their renewal, the pigments appear in the adult leaf in relatively fixed proportion. The ratio $\left(\frac{\text{quantity of chlorophyll a}}{\text{quantity of chlorophyll b}}\right)$ presents slight variations, and the molecular ratio $\left(\frac{\text{quantity of total chlorophyll}}{\text{quantity of total haematine}}\right)$ is constant in the neighbourhood of 60.

This corresponds to the fact that the metabolism of tetrapyrrole pigments is regulated by hereditary properties working in conjunction with environmental conditions in such a manner as to realize a certain composition in the adult leaf. This composition is in all probability the best possible for the physiological functions of the leaf in a given set of environmental conditions.

It is probable that the hereditary factors which tend to maintain the equilibrium between the diverse components are, at least in some measure, localized in the chloroplasts. Thus the constant ratio we have established between chlorophyll and haematine has one singular consequence. It is that in the normal leaf, the quantity of haematine must tend to zero as the quantity of chlorophyll becomes small. Indeed, in the experiments of Dekock *et al.* only very small amounts of haematine were obtained when the chlorophyll content was sharply diminished. But in variegated leaves, the white zones may still possess appreciable quantities of haematine for practically no chlorophyll. Perhaps, in this case one meets with new heriditary properties of the chloroplasts (plastome)[37].

The deeper study of the metabolism of the pigments will permit us to penetrate into the systems of control and to understand the relations between the functions of leaves and the evolution of the pigment composition of the chloroplasts. Up to the present, it is certain that, in this respect, the proteins, in particular the chloroplastic proteins, play a predominant role since the metabolism of the chlorophylls not only depends on the activity of several enzymes but also on photochemical reactions in which the properties of the pigment-protein complexes are probably essential features.

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