We can, however, enlarge our initial observation that basic proteins suppress root growth to say that the ability to suppress root growth seems to be a property held in common by polycations. It is significant that lysylglycine, at the same weight concentration as polylysine, and at a much higher molar concentration is not inhibitory. That polycations, weight for weight, are more strongly adsorbed to surfaces than are low molecular weight substances of similar functional group composition (McLaren 1954) is doubtless not without significance.

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Effect of Intermittent and Continuous Light on Chlorophyll Formation in Etiolated Plants

By

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Abstract

Short impulses of white light induce continuous synthesis of chlorophyll *a* and *b* in etiolated barley leaves. No lag phase is observed, and the rate of chlorophyll *a* accumulation is much higher than that of chlorophyll *b*, so that the a/b ratio is very high (12 to 20). The chlorophyll accumulation reaches a plateau at about 70 flashes after which the rate of their formation decreases appreciably.

When the etiolated plants, after exposure to about 80 to 100 flashes, are transferred to continuous light, one can observe that the rate of formation of chlorophyll *a* and *b* increases during the first hour, and after that becomes still more rapid. At the same time the a/b ratio falls and it reaches a value of about 3 normally found in green leaves.

Introduction

It is known that when etiolated plants are exposed to continuous light a lag phase of about two hours occurs in the formation of chlorophyll, after the initial protochlorophyllide has been transformed to chlorophyllide (Virgin 1955). This lag phase is abolished when prior to continuous white irradiation the etiolated plants are exposed for a short time to white or red light, followed by a three to five hours' dark period (Withrow *et al.* 1956, Virgin 1957 and 1958, Mitrakos 1961).

Recently, Madsen (1963) has shown that the lag phase in the formation of chlorophyll does not appear when etiolated plants are exposed to intermittent light (1 ms flashes alternating with 15 min dark periods), and that

a continuous accumulation of the chlorophyll takes place. The experiments were restricted only to 20 flashes, and the measurements were based on the absorption spectra of intact etiolated barley leaves.

It was of interest, therefore, to see 1) whether the accumulation of chlorophyll really proceeds without lag phase in intermittent light; 2) what happens when the etiolated plants are exposed to intermittent light for more than 20 flashes; and 3) what is the effect of continuous light on the chlorophyll formation in (etiolated) plants previously exposed to intermittent light for variable periods of time.

Materials and Methods

Seven day old etiolated barley plants grown at 20° were used. An electronic flash connected with a timer provided the intermittent illumination (1 ms flashes alternating with 15 min dark periods). General Electric power grove tubes (white light fluorescent) were used for continuous illumination (2000–3000 lux).

The extraction of chlorophyll was achieved by grinding one gram of leaves in a mortar with pure acetone (two times 3 ml), followed by extraction with 80 % acetone to a final volume of 30 ml. The extract was filtered twice and its absorption was measured in a Beckman DU spectrophotometer. The chlorophyll concentration was calculated according to McKinney (1941).

Results and Discussion

Figure 1 shows the formation of chlorophyll *a* and *b* in etiolated barley plants after exposure to continuous light. A two hour lag phase can be seen as well as a slow drop of the a/b ratio.

Figure 2 shows the variations of the chlorophyll *a* and *b* content, and of the a/b ratio during the exposure of etiolated barley plants to intermittent light. The biosynthesis of chlorophyll started from the beginning of the flash treatment. No lag phase was observed. The accumulation of chlorophyll *a* reached a plateau at approximately 70 flashes, after which the rate of its

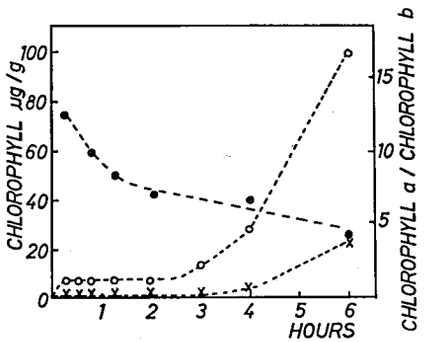

Figure 1. *Time curve for the formation of chlorophyll a and chlorophyll b in etiolated barley leaves during continuous illumination.* Chlorophyll is expressed in µg/g fresh weight. (○) Chlorophyll *a*; (×) Chlorophyll *b*; (●) *a/b* ratio.

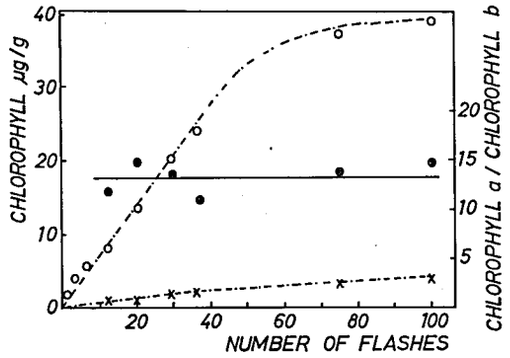

Figure 2. *Effect of intermittent light on the accumulation of chlorophyll a and b in etiolated barley leaves.* Chlorophyll is expressed in µg/g fresh weight. The conditions are given in the text. Symbols see Figure 1.

synthesis decreased appreciably. The rate of chlorophyll *a* accumulation was much higher than that of chlorophyll *b*. A high a/b ratio (12 to 20) was obtained. It is not possible to say whether chlorophyll *b* was present from the beginning of the light treatment, since the presence of protochlorophyllide and protochlorophyll renders any clear-cut decision difficult on this point.

Figure 3 gives the results of a representative set of experiments, in which etiolated barley plants were exposed to 74 flashes, and then transferred to continuous light. As one can see, the rate of formation of chlorophyll *a* and chlorophyll *b* increased during the first hour of continuous illumination, and after that it became still more rapid. A sort of "lag phase" was observed. During this "lag phase" period the a/b ratio fell to 1/3rd of the original, and after 4 to 5 hours it reached a value of about 3, normally found in green leaves.

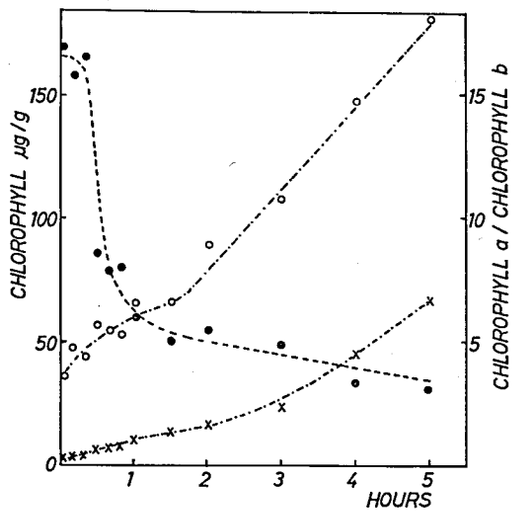

Figure 3. *Time curve for the formation of chlorophyll a and b in etiolated barley leaves, previously exposed to intermittent light* (74 flashes), *during continuous illumination.* The conditions are given in the text. Chlorophyll is expressed in µg/g fresh weight. Symbols see Figure 1.

The results of all the experiments distinctively point to the existence of two different types of chlorophyll accumulation: one functioning under the intermittent light, and forming essentially chlorophyll *a* (Type 1), and another functioning under continuous illumination and forming chlorophyll *a* and *b* in normal proportions (Type 2).

Since no lag phase was ever observed under the flashes we assume that etiolated plants contain essentially parts of the biosynthetic pathways for the type 1 accumulation of chlorophyll in an active form. The biosynthetic pathways corresponding to the type 2 accumulation of chlorophyll are able to function or are formed only under continuous illumination. The type 2 system synthesizes both chlorophyll *a* and chlorophyll *b* at a high rate; it seems to be the complete system. The formation of the complete system requires about 2 hours of continuous light. It is not yet possible to decide whether the two systems consist of two independent pathways in chlorophyll biosynthesis, or whether Type 1 is part of Type 2. However, the possibility of distinguishing the two systems is very useful in studying problems related to chlorophyll biosynthesis.

It is known that the red–far-red system is implicated in chlorophyll metabolism (Withrow *et al.* 1956, Virgin 1957 and 1958, Mitrakos 1961), and particularly, the lag phase stage of chlorophyll biosynthesis in etiolated plants exposed to light has been considered to involve phytochrome mediation. However the results of this study clearly show that exposure of etiolated plants to flashes does not abolish the lag phase. This may be due to the short duration of the flash exposure, since it has been shown (Mitrakos 1961) that the lag phase disappearance depends on the time of preillumination with white or red light, the highest effect being observed at 240 seconds. Before any conclusions can be reached on this point further studies have to be made.

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Sterile Germination Requirements of Seeds of Some Water Plants

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Abstract

A sterile germination study with seeds of some common phanerogamic water plants showed almost 100 per cent germination for seeds of *Alisma plantago-aquatica, Baldellia ranunculoides* and *Nymphaea alba*. Seeds of *Potamogeton lucens* could be germinated to about 40 per cent, seeds of *Polygonum amphibium* germinated sporadically while those of *Cladium mariscus* could not be germinated at all.

Freshly harvested seeds of Alisma and Baldellia showed an ability to germinate at both 20°C and 35°C. A stratification period of one month at +4°C gave germination of all species tested, with the exception of Cladium. Potamogeton germinated in light only, the other species both in light and darkness. Treatment times for surface sterilization in disinfectants are given.

Introduction

Attempts to obtain axenic cultures of phanerogamic water plants from surface sterilized seeds have often failed because of the special germination requirements of these seeds. They require low oxygen tension and low redox potentials for germination (see *e.g.* Carr 1961, Forsberg 1965) and for sterile germination, methods, other than those required for the germination of seeds of terrestrial plants are necessary. Axenic plants can be obtained from surface sterilized propagules after mechanical seed coat treatment (Crocker 1907, Allsopp 1952). A new and simple method for the sterile germination of *Chara*-oospores and seeds of *Najas marina* has recently been presented (Forsberg 1965). The following sterile germination study was made with seeds of some common water plants to obtain a basic knowledge of the sterile germination requirements to enable further controlled studies of higher water plants.