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Effect of Chloramphenicol on Chlorophyll Synthesis of Bean Leaves[1,2]

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Summary. Chloramphenicol has been found to inhibit the decrease in lag phase of chlorophyll accumulation in bean leaves that is brought about by a brief illumination followed by a prolonged dark period. The effectiveness of chloramphenicol depends on time of application. It is more effective when applied at the beginning of the dark period than at the end.

When etiolated leaves of flowering plants are placed in the light, protochlorophyllide is rapidly converted to chlorophyllide (10, 14). By the usual spectrophotometric techniques chlorophyllide is not distinguishable from chlorophyll a. This period of rapid chlorophyll formation is followed by a period of a few hours in which chlorophyll does not accumulate or accumulates at a slow rate. This rate gradually increases to a maximum which is maintained for a prolonged period (9, 11,13). The time course of chlorophyll accumulation depends on the age of the plants. In bean plants less than 5 days old, a linear, but low rate of chlorophyll synthesis is observed following the initial conversion of protochlorophyllide to chlorophyllide (11). The period between the initial conversion of protochlorophyllide to chlorophyllide, and the maximal rate of chlorophyll accumulation has been termed the lag period. The lag period can be shortened or abolished by treating plants with a short light period followed by a period of incubation in darkness (9, 12). This reaction is controlled by light absorbed by phytochrome (8). The lag phase in formation of chlorophyll is suggestive of enzymatic adaptation. Such an interpretation suggests that brief illumination followed by a period of prolonged darkness eliminates the lag by resulting in synthesis of enzymes necessary for chlorophyll formation. This possibility was investigated by studying the effect of chloramphenicol, applied before and after brief treatment with light and prolonged incubation in the dark, on chlorophyll formation on subsequent illumination. Chloramphenicol has been reported to inhibit partially chlorophyll formation during illumination of etiolated plants (5), and to partially inhibit chloroplast protein synthesis (6). It has been found that chloramphenicol completely inhibits stimulation by light of subsequent chlorophyll accumulation when the antibiotic is applied early in the dark period between the 2 illuminations, but only partially inhibits when applied late in the dark period.

Materials and Methods

Black Valentine bean plants (*Phaseolus vulgaris* L.) were grown in the dark for 6 days, and leaves with a cotyledon and piece of hypocotyl attached were treated with chloramphenicol (5). Chlorophyll was determined from absorption of acetone extracts of leaves (7). Leaves were placed in the dark for 1 hour, illuminated for 10 minutes, replaced in the dark for 20 to 22 hours, reilluminated for 2 to 8 hours, and chlorophyll formed, measured. Leaves were transferred to chloramphenicol solution at various times starting with the beginning of the first dark period. In some experiments the first dark period was omitted, and the ability to synthesize chlorophyll at the beginning of the experiment was also measured, by placing leaves in the light for 2 hours, instead of 10 minutes, at which time pigments were extracted. Illumination was with white fluorescent light at an intensity of 1000 ft-c and was carried out at 25°.

Results

When leaves are illuminated for a 10 minute period, then incubated in the dark for 20 hours, followed by a second illumination period, the rate of chlorophyll accumulation is a function of time of application of chloramphenicol (fig 1). Chloramphenicol applied 1 hour before the 10 minute illumination period, at the end of the 10 minute illumination period, or 1 hour after return to darkness, resulted in leaves that form chlorophyll at essentially the same rate during the second, and

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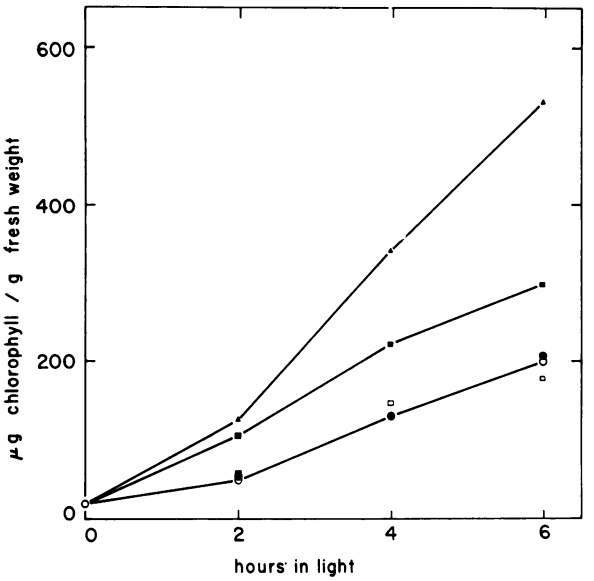

FIG. 1. Effect of time of addition of chloramphenicol on rate of chlorophyll formation. One hour before 10 minute illumination (○); at end of 10 minute illumination (●); 1 hour after end of 10 minute illumination (□); 19 hours after end of 10 minute illumination, 1 hour before start of extended illumination (■); no addition of chloramphenicol (△).

prolonged illumination. When chloramphenicol is applied 19 hours after the 10 minute illumination (an hour before the second illumination), the rate of chlorophyll formation is greater than when applied earlier. But, chlorophyll formation is less than if chloramphenicol is not used at all. A difference in chlorophyll content between leaves without chloramphenicol, and leaves to which chloramphenicol was applied an hour before the second illumination, is just detectable at the end of 2 hours illumination. This difference increases with time, and is quite clear at the end of 4 hours illumination. These results suggest that some substance, which is necessary for maximum chlorophyll accumulation, is synthesized by the leaves between 1 and 19 hours of the dark incubation. To determine whether or not this effect of chloramphenicol was due to changes in the ability of the leaf to synthesize chlorophyll independent of the 10 minute illumination, leaves were tested for ability to synthesize chlorophyll during a 2 hour illumination, as a function both of time of application of chloramphenicol, and presence or absence of a 10 minute preillumination. When the 10 minute illumination is omitted, the amount of chlorophyll formed in 2 hour illumination is the same whether chloramphenicol is applied at the beginning or the end of the 22 hour dark period (fig 2). When leaves have been given a 10 minute illumination, as before, the effect of chloramphenicol is much greater when applied at the beginning, rather than the end, of the dark period. This shows that the difference due to time of application is not based on limited penetration, nor changes in synthetic ability independent of preillumination. In the absence of chloramphenicol, the ability of leaves to synthesize chlorophyll is equal or slightly lowered after incubation in the dark for 21 hours. In the experiment recorded in figure 2, ability to synthesize chlorophyll was lower after 21 hours incubation in the dark. Essentially the same results presented in figures 1 and 2 are obtained when values of chlorophyll are calculated per leaf rather than per g fresh weight.

Discussion

Chloramphenicol inhibits formation of some component required for chlorophyll accumulation during illumination of leaves. The component synthesized is probably protein, since chloramphenicol is an inhibitor of protein synthesis (5). Formation of this protein occurs in the dark as a result of brief illumination of leaves. Even in leaves incubated in chloramphenicol overnight,

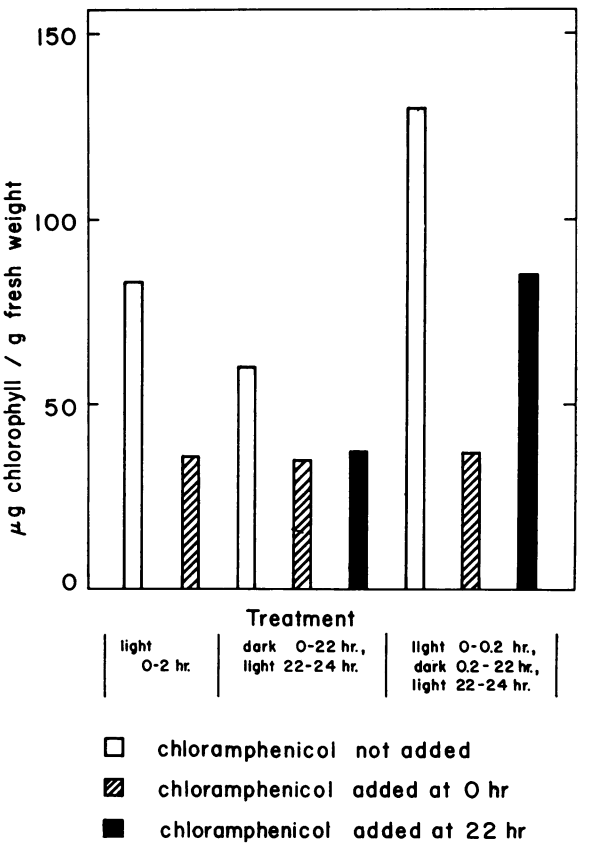

FIG. 2. Effect of time of addition of chloramphenicol to leaves kept in the dark, and to leaves given a 10 minute illumination on chlorophyll synthesis in a subsequent 2 hour illumination period.

chlorophyll formation continues during at least 6 hours of illumination. These results appear to contradict a report that chloramphenicol, applied during greening, stops chlorophyll formation almost immediately (2). Chlorophyll accumulation is inhibited by other inhibitors of protein synthesis, and by inhibitors of RNA synthesis (1). It has not yet been demonstrated that nucleic acid needed for chlorophyll accumulation can be formed in the dark as a result of brief illumination. It is suggested, however, by the observation that actinomycin d inhibits chlorophyll formation in bean leaves only when applied early in the greening process (4). These results are in accord with a mechanism in which absorption of light by phytochrome produces an active gene, resulting in formation of a new messenger RNA and formation of a new protein.

The requirement for protein synthesis for maximal rates of chlorophyll accumulation could be due to the need for synthesis of enzymes required for synthesis of chlorophyll (3). The effect of actinomycin d on chlorophyll formation in bean argues for this interpretation. However, leaves grown in the dark are capable of accumulating more protochlorophyllide than normal when supplied with δ-aminolevulinic acid (11). Thus, if enzymatic synthesis is limiting, it is at steps leading to formation of δ-aminolevulinic acid. It has been suggested that formation of stoichiometric amounts of protein is needed, rather than formation of catalytic amounts (3, 5). Such a view envisions protochlorophyllide attached to a protein, needed for its conversion to chlorophyll, and/or, for incorporation of chlorophyll into lamellae. The continued inhibition of chlorophyll formation by actidione during greening of *Euglena* supports this interpretation (3). At present, it cannot be decided whether protein synthesis is required for formation of enzymes required for chlorophyll synthesis, or for synthesis in stoichiometric amounts, of a protein needed for incorporation and stabilization of chlorophyll in the lamellae.

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