Effect of Chloramphenicol on Light Dependent Development of Seedlings of Phaseolus vulgaris var. Black Valentine, With Particular Reference to Development of Photosynthetic Activity [1, 2, 3]

Maurice M. Margulies

Division of Radiation & Organisms, Smithsonian Institution, Washington, D.C.

Introduction

Seedlings of higher plants germinated in solutions of chloramphenicol (D-threo-N-dichloroacetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol) have their development inhibited. The most noticeable effect is the inhibition of the formation of normal green color of leaves (13, 16). Chloramphenicol is a specific inhibitor of protein synthesis in bacteria where it inhibits the incorporation into protein of amino acids attached to soluble ribonucleic acid (3). Chloramphenicol can also inhibit amino acid incorporation catalyzed by a cell-free system obtained from higher plants (14).

The work presented in this paper was undertaken to study light dependent chloroplast maturation, a process that is not yet fully understood. Chloramphenicol inhibition of this process would be one way of indicating participation of protein synthesis. An inhibitory effect of antibiotic on the light dependent development of photosynthetic activity of *Phaseolus vulgaris* var. Black Valentine has been found. In addition, a partial inhibition of chlorophyll synthesis was observed. Other light dependent responses of the plant were not affected.

Materials & Methods

► Growth of Plant Materials: *Phaseolus vulgaris* (L.) var. Black Valentine was used throughout. Etiolated plants were grown as described by Wolff and Price (20), except that seeds were not sterilized with hypochlorite. Normal green plants were grown in a greenhouse and were subirrigated with nutrient solution. Etiolated plants were used 6 days after planting, and primary leaves of the greenhouse grown plants were used 8 to 12 days after planting.

► Application of Chloramphenicol: Chloramphenicol was a gift of Parke, Davis and Co. Unless mentioned, solutions of antibiotic contained 4 mg/ml. Solutions were applied to leaves by either of two procedures which follow.

I. Leaves, which included the entire epicotyl, a short piece of hypocotyl, and one cotyledon (see fig 1) were placed in petri dishes which contained a disc of filter paper and chloramphenicol solution. For each square centimeter of petri dish area 1/10th ml of solution was used, so that contact with the cotyledon of each of the leaves was provided, but the leaves themselves were not submerged. This procedure was used when the effects of concentration of antibiotic were examined.

II. Intact plants were sprayed with a solution of chloramphenicol applied as a mist from an atomizer. The spray was directed at the hypocotyl hooks from above. For every flat of approximately 200 plants 50 ml of solution were used.

► Irradiation of Leaves & Plants: Leaves or intact plants were transferred to an irradiation chamber an hour after they had been treated with chloramphenicol. Irradiation was with light from white fluorescent lamps. The intensity of irradiation was about 1,000 $\mu w/cm^2$ and was measured with a thermopile. This corresponded to an intensity of illumination of about 1,000 ft-c. Leaves in petri dishes were in contact with test solution throughout the irradiation period. Unless mentioned, this period lasted for 24 hours. Except during white light irradiation periods, live plant material was handled in dim green light (19).

► Determination of Anthocyanin: The 2-cm portion of hypocotyl from between 1.5 and 3.5 cm below the point of attachment of the cotyledons was extracted and the anthocyanin content of the extract determined (16). Extractions were done in triplicate and ten 2-cm portions of hypocotyl were used for each extraction. Only relative values of anthocyanin are given. The anthocyanin content of hypocotyls of unirradiated plants was not determined since it was negligible (9).

► Determination & Separation of Chlorophylls: A sample containing only leaves was pressed between absorbent paper to remove adhering water. The sample was weighed and was then heated in boiling water for 30 seconds. Chlorophyll was extracted by grinding leaves with 80 % acetone in a Virtis-type

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homogenizer. Particulate material was removed by filtration and the chlorophyll content of the solution was determined according to Arnon (1). Each leaf sample weighed 0.5 to 2.0 g. Usually, chlorophyll content of a batch of leaves was determined by extracting three replicate samples.

The chlorophyll of chloroplast suspensions was extracted by mixing with 80 % acetone. Particulate material was removed by centrifugation and the chlorophyll content of the solution was determined as above.

Carotenoids were removed from petroleum ether solutions of leaf pigments by chromatography on sugar (17). The chlorophyll band was removed from the column and repeatedly extracted with ether, or the column was developed further to separate the chlorophylls, and the individual chlorophyll bands were removed and extracted. The ether solutions were evaporated to dryness in vacuo, and the residues dissolved in acetone. The petroleum ether solutions applied to the columns were prepared by repeatedly extracting 80 % acetone extracts of heat-killed leaf material. Acetone was removed from the petroleum ether by washing with water and the petroleum ether was dried with anhydrous sodium sulfate. Absorption spectra were measured with a Cary recording spectrophotometer.

► Measurement of Photosynthesis: Photosynthesis was determined by measuring the light dependent fixation of carbon dioxide-C^{14}. Leaf samples were exposed to carbon dioxide-C^{14} in Warburg flasks or specially constructed irradiation chambers similar to the one described by Aronoff (2). Each leaf sample weighed about 50 mg and consisted of leaf pieces from at least four different plants that had been treated identically.

When Warburg flasks were used as irradiation chambers, only relative rates of carbon dioxide fixation were determined. Each flask had a capacity of about 20 ml, two side arms, and a flat rectangular bottom. The sodium carbonate-C^{14} was prepared from barium carbonate-C^{14} obtained from Oak Ridge National Laboratory. Sodium carbonate-C^{14} (20–30 mc/mmole) (5–10 μc) was placed in a side arm. Carbon dioxide was generated by adding 0.1 ml 10 % (w:v) phosphoric acid through a venting plug. The flasks were then shaken for 10 minutes in the dark, followed by irradiation from below for 10 minutes. At the end of the irradiation period, 0.1 ml of 20 % KOH (w:v) was added to the second side arm through a venting plug. After 10 minutes the flasks were opened and the leaves killed in boiling 80 % ethanol. For samples exposed to carbon dioxide-C^{14} in the dark, the procedure was the same, except that leaves were not irradiated during the 30 minute incubation period. The gas in the flasks was air. The temperature of the water bath was 20 C. Light was provided by a pair of white fluorescent lamps for each line of manometers. The lamps were submerged in the water bath and yielded 1,000 ft-c at the level of the flasks.

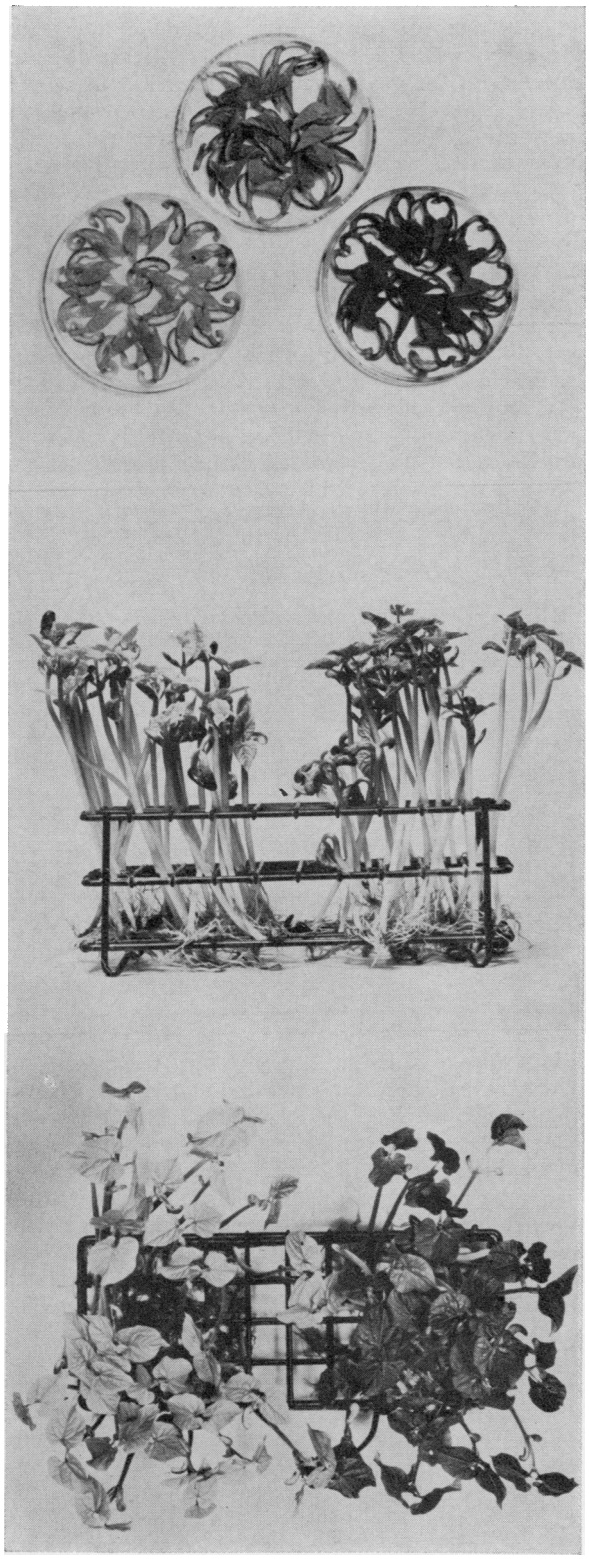
Irradiation chambers were used so that a large number of samples could be exposed to the same atmosphere containing carbon dioxide-C^{14} of known specific activity. Two test chambers were arranged in parallel with a gas generator and vacuum line. The chamber and gas generator were evacuated, and 250 μmoles of carbon dioxide-C^{14} of known specific activity were generated. The gas in the generator was flushed into the chambers with carbon dioxide free air till the pressure was nearly that of the atmosphere. The volume of the system which contained radioactive carbon dioxide was about a liter. One chamber was covered with aluminum foil and the other was illuminated for 10 minutes. Light, supplied from white fluorescent lamps, had an intensity of about 1,000 ft-c at the level of the chamber. The ambient air temperature was about 20 C. After illumination, the chambers were evacuated, flushed with carbon dioxide free air, and the radioactive carbon dioxide trapped in sodium hydroxide. Then the chambers were opened and the leaf samples dropped into boiling 80 % ethanol.

Leaves and ethanol extract were ground in a Tenbroeck homogenizer. Aliquot portions of the homogenate, corresponding to 0.2 to 1.0 mg of fresh leaf material were plated on 2.4 cm diameter planchettes, spread with additional ethanol as necessary, acidified with a drop of 0.1 N hydrochloric acid and dried at 90 to 100 C. Radioactivity was determined with a thin window gas flow detector operated as a Geiger counter.

Fixation per gram fresh weight for the total incubation period was calculated for illuminated and unilluminated leaf samples. The fixation dependent on the 10 minute illumination period is the difference between these two values, and was used to calculate the rate of fixation per unit chlorophyll. The chlorophyll content of leaves was estimated by analysis of samples other than those used to test for carbon dioxide fixation.

► Hill Reaction: Each 25 g of leaves was ground for 1 minute in a Waring blendor at line voltage with 200 ml of solution containing: 0.4 M sucrose; 0.05 M sodium phosphate; 0.01 M NaCl; 0.01 M Versene acid; adjusted to pH 7.0. The resulting homogenate was filtered through cheesecloth, and was then centrifuged at 250 to 500 $\times$ g for 2 minutes. The resulting supernatant suspension was centrifuged at 10,000 $\times$ g for 30 minutes. The pellet from this centrifugation was suspended in 2 to 5 ml of solution with the aid of a Tenbroeck homogenizer, and the suspension was filtered through glass wool. The solution used for suspending the pellet was the same as that used for grinding leaves, except that Versene was omitted.

The Hill reaction with quinone as oxidant was measured manometrically (18), and that with ferricyanide as oxidant was measured by observing the decrease in optical density at 400 mμ. Each 3.0 ml of reaction mixture contained 1.5 μmoles of ferricyanide and 2.0 ml of the buffer used for suspension



of the plastid particles. The millimolar extinction coefficient used for calculation of rates of ferricyanide reduction was 0.96 (A. T. Jagendorf, personal communication). Irradiation for the quinone reaction was with light from white fluorescent lamps which gave an intensity of 1,000 ft-c at the level of the manometer flasks. The light for the ferricyanide reaction was obtained from a 300 watt photoflood bulb and was filtered through 10 cm of 1 % (w:v) copper sulfate. The light intensity at the 3 ml cuvettes used as reaction vessels was 5,000 ft-c. The quinone Hill reaction was conducted at 20 C and the ferricyanide Hill reaction at about 25 C.

Results

Whether or not leaves are treated with chloramphenicol, the only visible difference after 24 hours of irradiation is in the pigmentation of the leaves (fig 1). The same is true for intact plants (fig 2). In both instances chloramphenicol-treated leaves are yellow-green, while untreated controls are deep green. In both treated and untreated intact plants, the leaves open and expand, hooks open, and hypocotyls become red with anthocyanin. Irradiation of intact plants for another 24 hours produces very marked and approximately equal expansion of leaves of treated and untreated plants (fig 3). The untreated plants, however, are somewhat taller. In addition, the leaves of untreated plants contain starch while those of chloramphenicol-treated plants do not. Starch was tested for with I_2-KI solution after extraction of leaves with boiling 80 % ethanol.

The lack of inhibitory action of chloramphenicol on hook opening has been confirmed using excised hooks (table I). The irradiated hooks were exposed to 400 $\mu w/cm^2$ of red light (600–700 mμ) for 10 minutes, and then incubated in the dark for 24 hours, at which time hook opening was measured (8). Measurement of the anthocyanin content of hypocotyls of chloramphenicol-treated and control plants showed no inhibitory action of antibiotic at the end of 24 hours of irradiation. However, a 25 % inhibition

Fig. 1 (*top*). The effect of chloramphenicol and light on leaf development. Reading from *left to right,* the petri dishes contain: (1) water, (2) 4 mg/ml chloramphenicol, and (3) water. Dish 1 had been kept in the dark for 24 hours, and dishes 2 and 3 exposed to light for 24 hours.

Fig. 2 (*center*). The effect of chloramphenicol on plant development in the light. The plants on the left had been treated with a solution of chloramphenicol (4 mg/ml) and those on the right were treated with water. Both groups of plants were then exposed to light for 24 hours.

Fig. 3 (*bottom*). The effect of chloramphenicol on plant development in the light. The plants on the left had been treated with a solution of chloramphenicol (4 mg/ml) and those on the right were treated with water. Both groups of plants were then exposed to light for 48 hours.

Table I

Effect of Chloramphenicol on Hook Opening

Treatment		Hook opening (degrees)
Red light	Chloramphenicol (1 mg/ml)	
−	−	−3±5*
+	−	41±20
−	+	3±7
+	+	51±13

* Standard deviation.

Table II

Effect of Chloramphenicol on Anthocyanin Content of Hypocotyls

Treatment		Anthocyanin/g fr wt (relative values)
Chloramphenicol (4 mg/ml)	Hr irradiated	
−	24	13±2*
−	48	17±3
+	24	13±2
+	48	13±1

* Standard deviation.

was observed at the end of 48 hours of irradiation (table II).

Leaves irradiated for 4 or 24 hours in contact with chloramphenicol solution did not have detectable photosynthetic activity, even though considerable quantities of chlorophyll had been formed (table III). When it was assumed that doubling of carbon dioxide fixation in the light over that in the dark could have been detected, it was evident that at the end of 4 hours of irradiation, the chlorophyll in chloramphenicol-treated leaves was one-fifth as effective as chlorophyll in control leaves. At the end of 24 hours, the chlorophyll from treated leaves was not more than a tenth as effective as chlorophyll in control leaves.

Table III

Effect of Chloramphenicol on Development of Photosynthesis

Treatment of etiolated leaves		Photosynthesis/g fr wt* (relative values)	Chlorophyll (mg/g fr wt)	Photosynthesis/chlorophyll
Chloramphenicol (4 mg/ml)	Hr irradiated			
−	0	−0.7	<0.005	...
−	4	13.	0.29	45
−	24	73.	1.4	52
+	0	0.2	<0.005	...
+	4	1.7	0.20	<10**
+	24	−2.1	0.54	< 4**

* Corrected for a dark fixation of about 2.
** It was assumed that a total fixation of 4 or greater could have been distinguished from the dark fixation of 2.

The effectiveness of chlorophyll of control leaves was the same at 4 and at 24 hours.

The rates of carbon dioxide fixation were the same for etiolated leaves that had been irradiated for 24 hours or for greenhouse grown leaves, 100 to 150 μmoles/mg chlorophyll/hour (150–200 μmoles/g fr wt/hr). The rates for chloramphenicol-treated leaves were 2 to 10 μmoles/mg chlorophyll/hour.

Although chloramphenicol results in an inhibition of development of photosynthetic activity when it is present during the period of chlorophyll accumulation, it has no effect on photosynthesis when chlorophyll is already formed (table IV). Leaves were irradiated for 24 hours without chloramphenicol and were then returned to the dark. Some of them were transferred to dishes containing chloramphenicol. The remainder were left in water. At the end of 4 hours, both antibiotic-treated and control leaves were tested for the ability to fix carbon dioxide in the light. No significant difference was noted.

Table IV

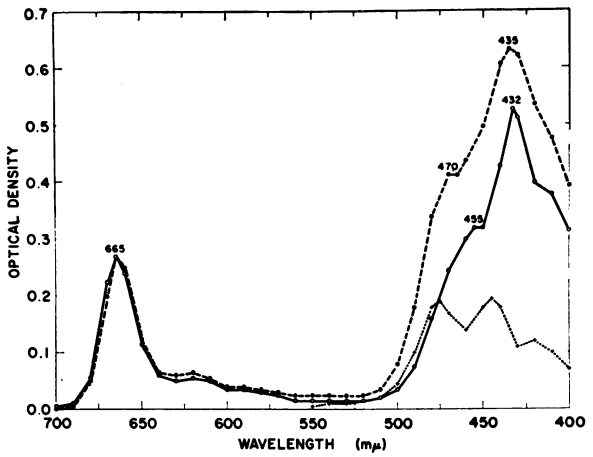
Effect of Chloramphenicol on Photosynthesis of Leaves Greened by 24 Hours of Irradiation

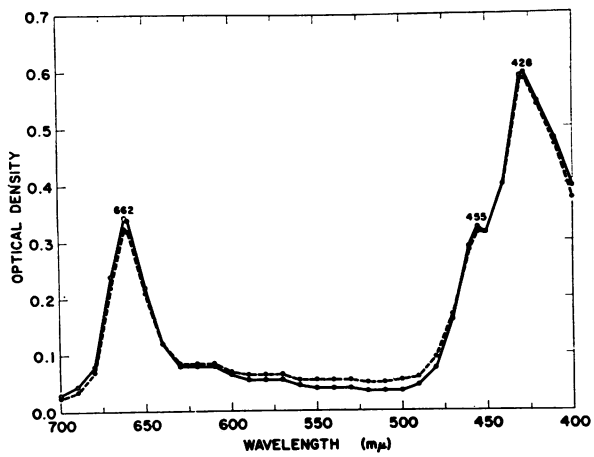
Experiment	Chloramphenicol (4mg/ml)	Photosynthesis/g fr wt* (relative values)	Chlorophyll (mg/g fr wt)	Photosynthesis/chlorophyll
1	−	37	1.5	25
	+	29	1.3	22
2	−	16	1.5	11
	+	14	1.3	11

* In Experiment 1, corrected for a dark fixation of 2; in Experiment 2, for a dark fixation of 1.

The green pigments of both treated and untreated leaves appear to be the same. Thus, the absence of photosynthetic activity is not due to a lack of chlorophylls. Absorption spectra of 80 % acetone extracts from both sources can be superimposed from 550 to 700 mμ, but not from 400 to 500 mμ (fig 4). In this region, extracts from chloramphenicol-treated plants have the greater relative absorption. The difference spectrum shows absorption maxima at 475, 445, 420 mμ. These data show that the ratios of chlorophyll a to chlorophyll b in extracts of treated and untreated plants are approximately equal. The ratio of chlorophylls to carotenoids, however, is lower in treated than untreated plants. The green pigments are readily transferred from 80 % acetone to petroleum ether. This eliminates the possibility that the pigments are chlorophyllides (20).

When carotenoids are removed from the extracts by chromatography on sugar, the absorption spectra become nearly superimposable from 400 to 700 mμ (fig 5). This confirms that the mixture of green pigments in extracts from treated and control leaves





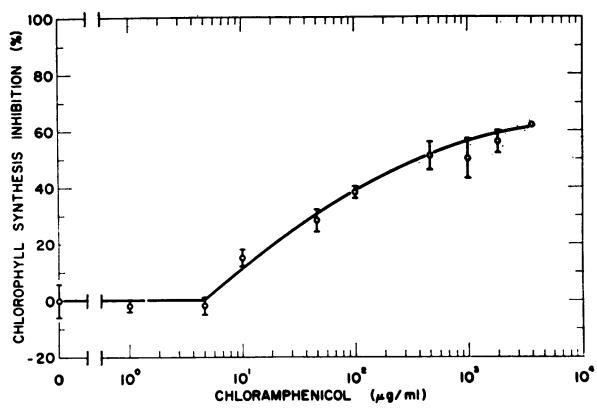


Fig. 4 (*top*). Absorption spectra in 80 % acetone of extracts of leaves treated with 4 mg/ml chloramphenicol (*closed circles*) and control leaves treated with water (*open circles*). The difference between the two is represented by the remaining curve (*crosses*).

is the same. If chromatograms of extracts from chloramphenicol-treated or untreated leaves are developed further, two main bands are found. One of these has absorption characteristics of chlorophyll a. The major absorption peak in the blue was at 430 mμ, and the minor at 412 mμ. The major peak in the red was at 663 mμ and the minor at 617 mμ. The ratio of the heights of the major red maximum to that of the major blue maximum was 0.78 for the pigment from untreated leaves and 0.82 for that from treated leaves. These values are in good agreement with those of Harris and Zscheile (7). The pigment contained in the second band, probably chlorophyll b, had peaks at 432, 457, 617, and at 645 mμ in the case of material from untreated leaves, and at 650 mμ for material from chloramphenicol-treated leaves. The ratio of heights of the major red maximum to the major blue maximum (645 mμ band/457 mμ band) was 0.35 for the pigment from untreated leaves, and 0.41 for that from chloramphenicol-treated leaves. This green pigment from chloramphenicol-treated leaves was contaminated with chlorophyll a as indicated by a shoulder in the absorption spectrum at 663 mμ. The location of the minor red peak from material obtained both from treated and untreated leaves differs from that of chlorophyll b (7).

Table V

Effect of Light Intensity & Chloramphenicol on Chlorophyll Synthesis

Light intensity (ft-c)*	Chlorophyll content of leaves (mg/g fr wt)		% Inhibition
	Chloramphenicol (4 mg/ml)		
	Absent	Present	
940	0.901±0.025**	0.239±0.024	74
740	0.896±0.021	0.262±0.007	71
520	0.846±0.075	0.254±0.046	70
360	0.854±0.029	0.310±0.023	64
180	0.854±0.046	0.297±0.008	65
125	0.840±0.088	0.274±0.010	69

* Irradiation period lasted 16 hours.
** Standard deviation.

The results of a typical experiment testing the effect of chloramphenicol concentration on chlorophyll accumulation are presented in figure 6. Leaves were irradiated for 18 hours. No inhibition was observed at 5 μg/ml, but a significant 15 % inhibition at 10

Fig. 5 (*center*). Absorption spectra in acetone of extracts of leaves treated with 4 mg/ml chloramphenicol (*closed circles*) and control leaves treated with water (*open circles*) after removal of carotenoids.

Fig. 6 (*bottom*). Effect of chloramphenicol concentration on chlorophyll synthesis. The concentration of chlorophyll in leaves without chloramphenicol was 1.08 mg/g fresh weight.

Table VI

Effect of Chloramphenicol Concentration on Ability of Chlorophyll to Catalyze Photosynthesis

Chloramphenicol μg/ml	Photosynthesis/chlorophyll* % of control		
	Expt. Series 1	Expt. Series 2	
		Expt. 1	Expt. 2
0	100	100±15	100±10
4	95±23	...	...
5	...	64±15	108±18
8	108±23	...	...
10	...	62±10	120±16
20	84±31	43±24	88±7
40	70±10	...	...
50	...	25±6	48±6
100	...	22±8	24±5
400	18±14	...	...
2,000	6	...	...
4,000	9±5	...	...

* See text for details.

μg/ml. In some experiments, significant inhibition was not observed at 10 but at 20 μg/ml. A 60 % inhibition was observed at a concentration of 4 mg/ml. Occasionally inhibition as great as 80 % was observed at this concentration. A variation of light intensity from 125 to 940 ft-c produced only small changes in the quantity of chlorophyll synthesized both in the absence and presence of chloramphenicol (table V). Light intensity was varied by covering petri dishes containing leaves with cheesecloth, or cheesecloth and typing paper.

The relative abilities of chlorophyll, formed in the presence of different concentrations of chloramphenicol, to catalyze photosynthesis are presented in table VI. The data presented under the heading, Experiment Series 1, represent the pooled results of four individual experiments in which the ability to fix carbon dioxide was tested in manometer flasks. The value for chlorophyll content and for carbon dioxide fixed/g fresh weight of leaf for the control (no chloramphenicol) in each experiment was set equal to 100, and other values adjusted accordingly. In each experiment the ratio of photosynthesis to chlorophyll for each concentration of chloramphenicol was calculated. Then the average value of this ratio for each concentration of chloramphenicol was calculated for the four experiments and is presented in the table.

Each column under the heading, Experiment Series 2, represents an individual experiment in which carbon dioxide fixation was conducted in irradiation chambers. This procedure permitted uniform exposure to light and atmosphere containing $C^{14}O_2$ of three replicates for each of six groups of leaves, each treated with a different concentration of chloramphenicol. An equal number of samples were exposed to the same atmosphere, at the same time, in the dark. Ratios of photosynthesis to chlorophyll content for each leaf sample were calculated and, then the average ratio for each concentration of chloramphenicol. The ratio for zero concentration of antibiotic was set equal to 100 and the values for other concentrations adjusted accordingly.

Significant inhibition can be obtained at a concentration as low as 5 μg/ml. Inhibition is always observed at a concentration of 40 μg/ml or lower. Inhibition of 90 % is consistently observed at 4 mg/ml.

The possible causes of variability within experiments has not been investigated. One possible source is the procedure by which chlorophyll content is estimated. For this estimation, leaf samples other than those actually exposed to carbon dioxide-C^{14} were used.

Ferricyanide Hill reaction activity of green particles from leaves treated with chloramphenicol and irradiated for 24 hours is only a tenth that of particles from control leaves not treated with antibiotic (table VII). Activities of particles from control leaves were about 200 μmoles ferricyanide reduced/mg chlorophyll/hour. The same value is obtained for chloroplasts from mature primary leaves (11). The nearly inactive particles from chloramphenicol-treated leaves did not have an inhibitory effect on active particles. With quinone as oxidant, particles from control leaves evolved oxygen in the light at the same rate as particles from plants grown in a greenhouse. Thus, it would seem probable that reduction of ferricyanide is also accompanied by oxygen evolution.

Table VII

Effect of Illumination of Leaves With or Without Chloramphenicol on Ferricyanide Hill Reaction Activity of Green Particles From Them

Source of particles		Changes in optical density at 400 mμ (0–5 min illumination)	μmoles ferricyanide reduced/mg chlorophyll/hr
Leaves treated with water	Leaves treated with 4 mg/ml chloramphenicol		
μg chlorophyll/3.0 ml			
27	...	−0.151±0.010	213±17
...	23	−0.014±0.010	23±14
27	23	−0.160±0.011	123±14

Discussion

Chloramphenicol inhibits only some of the light dependent responses of bean seedlings. Responses affected by antibiotic include chlorophyll synthesis, and the development of photosynthetic activity. The former is only partially, and the latter completely inhibited. The inhibition of photosynthetic activity can also be demonstrated on a subcellular level by a failure of chloroplasts to develop the ability to carry out the Hill reaction. These light dependent responses inhibited by chloramphenicol are associated with the maturation of the chloroplast. In contrast, other light dependent responses (opening of the hypocotyl hook, leaf expansion, and anthocyanin formation in the hypocotyl), at most, are slightly affected.

The mechanism of action of chloramphenicol has been most thoroughly investigated in bacteria where it has been shown to be a specific inhibitor of protein synthesis (3). It seems reasonable to conclude that chloramphenicol is inhibiting light dependent maturation of the chloroplasts of bean because it is inhibiting protein synthesis. However, conclusive evidence to support this view has not been presented.

Increase in levels of the photosynthetic enzyme, triphosphopyridine nucleotide-linked glyceraldehyde-3-phosphate dehydrogenase, have been observed when etiolated plants of bean (10) or pea (6) are irradiated. This would indicate that light dependent maturation of chloroplasts of higher plants is accompanied by protein synthesis. Therefore, chloroplast development should be sensitive to chloramphenicol if protein synthesis in these organisms can be inhibited by the antibiotic.

Almost complete inhibition of protein synthesis of bacteria can be obtained at a concentration of chloramphenicol of 10 μg/ml (3). In contrast, the incorporation of amino acids into protein by a cell-free system from maize endosperm is inhibited only 60 % by 0.4 mg/ml (14). Thus the requirement for concentrations of 0.4 to 4.0 mg/ml to obtain nearly complete inhibition of light dependent development of photosynthetic activity of bean leaves can be consistent with action through a specific inhibition of protein synthesis.

The concentration of chloramphenicol needed within the plant to prevent development of photosynthetic activity cannot be determined from the data presented. It is probable that this concentration is less than that of the solution applied, since it has been observed that the concentration of chloramphenicol within higher plants can vary from 100th to 1/3 that of the solution applied (4, 5, 12). These variations depend, in part, on the plant species used and on the mode of application of the antibiotic.

The lack of Hill reaction activity of plastid material from chloramphenicol-treated leaves can fully account for the lack of photosynthetic activity of those leaves. It can be concluded that etiolated leaves lack a substance, other than the photosynthetic pigments, which is needed for photosynthetic activity. When present in photosynthetically functional leaves, this substance, possibly a protein, is intimately bound to the chloroplast. The lack of Hill reaction activity in plastids from antibiotic-treated leaves does not mean that this reaction is the only point at which a component necessary for photosynthesis is rate-limiting.

Like the inhibitory effect of chloramphenicol on the development of photosynthetic activity, the inhibitory effect of the antibiotic on chlorophyll accumulation can also be interpreted in terms of an inhibition of protein synthesis. Two possibilities are considered: A, That the inhibition of chlorophyll formation is a result of increased photooxidation of leaf pigments because of the inability of antibiotic-treated leaves to photosynthesize. The pigments of leaves treated with inhibitors of photosynthesis are more susceptible to the photooxidation than are the photosynthetically active pigments (15). B, That chloramphenicol inhibits light induced formation of enzymes necessary for chlorophyll synthesis. Such adaptive enzyme formation has not been demonstrated, but might occur since the maximum rate of chlorophyll accumulation is not achieved for 2 to 4 hours after the start of irradiation of etiolated bean leaves (21). This second explanation is more probable. The first possibility is improbable since an eightfold variation in light intensity has almost no effect on chlorophyll accumulation whether or not antibiotic is present.

Summary

Etiolated plants that have been treated with chloramphenicol do not develop photosynthetic activity when irradiated. Synthesis of chlorophyll is markedly, but only partially, inhibited. Light dependent leaf expansion, opening of the hypocotyl hook, and anthocyanin formation are not inhibited by the antibiotic. The inhibitory action of chloramphenical on chlorophyll synthesis is not affected by large changes in the intensity of irradiation. The inhibition of the development of photosynthetic activity is not due to an inhibitory action of chloramphenicol on photosynthesis, nor to a lack of chlorophylls. Green particles from photosynthetically inactive leaves, obtained by irradiation in the presence of chloramphenicol, do not carry out the Hill reaction. This lack of Hill reaction activity can completely account for the lack of photosynthetic activity of the leaves. The inhibitory action of chloramphenicol on the development of photosynthesis and on chlorophyll formation has been interpreted in terms of the known inhibitory effect of the antibiotic on protein synthesis in bacteria.

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