

In Vitro Protein Synthesis by Plastids of *Phaseolus vulgaris*

IV. AMINO ACID INCORPORATION BY ETIOPLASTS AND EFFECT OF ILLUMINATION OF LEAVES ON INCORPORATION BY PLASTIDS¹

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ABSTRACT

Protein synthesis *in vitro* by etioplasts and chloroplasts from *Phaseolus vulgaris* was examined to study the factors regulating the development of etioplasts into chloroplasts. The properties of incorporation of ¹⁴C-leucine into protein by etioplasts from plants grown 6.5 days in darkness are similar to those of chloroplasts from plants of the same age that were illuminated for 12 hours. However, the rate of incorporation per plastid by chloroplasts is 4 times higher than the rate of amino acid incorporation by etioplasts. When 6-day-old plants are placed in light, this 4-fold increase occurs within 6 hours and is maintained up to 36 hours. The difference in rate of amino acid incorporation into protein between etioplasts and chloroplasts represents a real difference in the ability of etioplasts and chloroplasts to synthesize protein. A difference in pool size of leucine between etioplasts and chloroplasts does not account for the difference in amino acid incorporation between etioplasts and chloroplasts. Also the difference in photosynthetic capabilities of etioplasts and chloroplasts does not account for the difference in the ability to incorporate amino acid into protein. Furthermore, there are no factors in homogenates of etiolated leaves which inactivate amino acid incorporation into protein by chloroplasts. The difference in rates of amino acid incorporation between etioplasts and chloroplasts is correlated with the state of development of the plastids. The plastids have increased ability to incorporate amino acid into protein when the plastids are undergoing growth and differentiation.

Etioplasts are converted to chloroplasts when etiolated leaves of higher plants are placed in the light. Chlorophyll is formed during this conversion, the ability to photosynthesize develops (2, 8, 19, 26, 31), and the plastids increase in size and form their characteristic lamellar structures (9, 24). Development of the ability to photosynthesize depends both on the formation of chlorophyll and on the formation of protein (11, 20, 21). Transformation of etioplasts into chloroplasts is an example of subcellular differentiation triggered by an external stimulus: light.

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In the system studied in this paper, differentiation of plastids of primary leaves of *Phaseolus vulgaris*, this differentiation occurs without plastid division (10).

There is a considerable amount of information that plastids are semiautonomous structures with differentiation regulated partly by nuclear DNA and partly by their own DNA (14). Chloroplasts have been shown to be capable of synthesizing at least some of their proteins (6, 23). Since etioplasts, like chloroplasts, have the components necessary for protein synthesis (*e.g.*, ribosomes) (14), one would expect that etioplasts are also capable of protein synthesis. In this paper protein synthesis *in vitro* by crude etioplast and chloroplast preparations is studied and compared with the aim of finding a system *in vitro* for the study of the factors which limit protein synthesis by plastids when leaves of higher plants are grown in darkness.

MATERIAL AND METHODS

Plant Material. Plants of *Phaseolus vulgaris* var. Black Valentine were used throughout. They were grown in darkness for 6 to 8 days, or they were grown in darkness for 6 days and then illuminated for up to 48 hr. The temperature in darkness was 25 C and in light 28 C. Plants were illuminated with 1000 ft-c of white fluorescent light. Otherwise conditions of growth were the same as described previously (25).

Preparation of Plastids. Chloroplasts and etioplasts were prepared from plants that were 6.5 days old, except where mentioned otherwise. Preparation of etioplasts was carried out in dim green light (5). Otherwise, both etioplasts and chloroplasts were prepared in the same way. Leaves were picked and stored and plastids were isolated as described previously (25) except for the following modifications. Ten layers of facial tissue were used for filtering the homogenate. This filtration removed almost all the whole leaf cells, which were predominantly gland and hair cells. The homogenate was centrifuged at 3500g for 3 min. The pellet was resuspended in 2.5 ml of Honda medium containing tris, 0.05 M, pH 7.8, at 0 C (25) for each 30 g of leaves (fresh weight). One milliliter of etioplast suspension contained 1.4 ± 0.2 μg of protochlorophyll and 1.5 ± 0.2 mg of protein (average of four experiments). One milliliter of chloroplast suspension contained 191 ± 38 μg of chlorophyll and 1.6 ± 0.2 mg of protein (four experiments). The yield of plastids from leaves of plants that were 6.5 days old was between 1 and 2% for both chloroplasts and etioplasts. The yield from plants increased with the age of the leaves. The yield from green leaves of plants that were 8 days old (illuminated for 2 days) was 5 to 6%, and the yield from 8-day-old etiolated leaves was 4 to 5%.

Electron Microscopy. Etioplast suspensions were fixed with glutaraldehyde immediately after isolation or at the end of an incubation period of 1 hr at 25 C. Leaf pieces were also fixed with glutaraldehyde. Suspensions and leaf pieces were postfixed

with OsO_4 , and sections were stained with uranyl acetate and lead hydroxide (22).

Standard Assay for Determination of Amino Acid Incorporation into Protein. Incorporation of ^{14}C -leucine into protein was assayed by the procedure of Spencer and Wildman (29) as described previously (23, 25). Each milliliter of reaction mixture contained 0.8 ml of plastid suspension (either chloroplasts or etioplasts) in modified Honda medium (25), and 0.2 ml of a solution containing the following reagents: ATP; CTP; GTP; UTP; phosphoenolpyruvate; ^{14}C -amino acids other than leucine; magnesium ion; potassium ion; mercaptoethanol; tris, pH 8.0 (25). Twenty micrograms of pyruvate kinase were added and the incorporation reaction was started by adding $0.5 \mu\text{g}$ ^{14}C -leucine (specific radioactivity about 250 mc/mole) and warming to 25 C. In all experiments incorporation of ^{14}C -leucine into protein was determined by extraction of protein samples on filter paper discs followed by scintillation counting, both according to the procedure of Mans and Novelli (18). Specifically, protein on discs was precipitated with 0.1 M leucine in 10% trichloroacetic acid. Discs were then extracted with hot 5% trichloroacetic acid; cold 5% trichloroacetic acid; ethanol-ether (50:50); ether. Each point for radioactivity in protein is the average of duplicate samples which were corrected for counts in samples that were taken immediately after addition of radioactive amino acid. One picomole of ^{14}C -leucine is equivalent to 392 cpm.

Estimation of Rate of Incorporation per Leaf. Protein per plastid increases during light-dependent chloroplast development (20, 24). Thus, increases in enzymatic activity measured per unit of chloroplast protein might be masked. Therefore, we were interested in comparing the incorporation rate of etioplasts and differentiating chloroplasts on a per plastid basis. This is possible without actually counting plastids, because the number of plastids in etiolated primary leaves of bean does not change during 45 hr of illumination (10). The rate of incorporation was calculated as follows:

$$\text{rate (in pmole/hr)} = \frac{A \times C}{B}$$

where A = pigment (protochlorophyllide or chlorophyll) per leaf (in μg); B = pigment per ml of plastid suspension (in μg); C = incorporation per ml of plastid suspension (in pmole of ^{14}C -leucine/hr). Thus, we can make direct comparisons between the incorporation of etioplasts and chloroplasts because these rate units for both etioplasts and chloroplasts are the rates per plastid $\times k$. This constant equals the number of plastids per leaf, which, as stated above, is the same in etiolated and greening leaves.

Preparation of Soluble Protein from Plastids Labeled with ^{14}C -Leucine. Plastids were labeled for 1 hr with ^{14}C -leucine, and soluble protein was prepared as already described (23), with these modifications. The 30,000g supernatant was recentrifuged for 1 hr at 100,000g, yielding a pellet, which in the case of chloroplasts was green. This pellet was discarded, and the supernatant solution was dialyzed against 0.05 M potassium phosphate buffer-0.001 M glutathione (pH 7) to remove unincorporated ^{14}C -leucine.

Chemicals and Chemical Determinations. Pronase (cat. No. 53702, powder, research grade, from *Streptomyces griseus*) was obtained from CalBiochem, Los Angeles, California. All other chemicals used were the same as those listed in previous papers of this series (22, 23, 25). The amount of protein in the plastid suspensions was determined by Kjeldahl digestion, followed by Nesslerization of the digest (25). At least three replicate samples were digested for each determination.

Chlorophyll content was determined spectrophotometrically after extraction of chloroplast suspensions (25) or whole leaves (21) with 80% acetone. Protochlorophyllide was determined

spectrophotometrically in two different ways. (a) The etioplast suspensions and the leaf homogenates, respectively, were extracted with 80% acetone, and the protochlorophyllide was transferred into absolute ether. Absorbance was measured in a Cary model 14 spectrophotometer, and calculations were carried out with extinction coefficients of pigments in ether (15). (b) Alternatively, we determined the absorbance of pigments in 80% acetone extracts. Calculations were made as above, with extinction coefficients in ether. We used these relative values since we were no longer interested in the absolute values of protochlorophyllide, but in the relation between pigment contents in plastid suspensions and leaves.

RESULTS

Properties of Amino Acid Incorporation into Protein by Crude Etioplast Preparations. Incorporation of ^{14}C -leucine by etioplast suspensions may continue for 2 hr (Fig. 1), but it may cease after 1 hr. The rate of incorporation decreases rapidly with time, especially after 30 min. The time dependence of incorporation by etioplasts is similar to that for chloroplasts (22, 25). Incorporation of amino acid into protein is a linear function of plastid concentration, for both etioplasts and chloroplasts (Fig. 2). Rates of incorporation by etioplasts (estimated from the first 5 min) were 53, 70, and 68 pmoles of ^{14}C -leucine per mg of protein per hr in three replicate experiments.

Etioplast preparations lose their ability to incorporate amino acids into protein when they are stored in Honda medium at 0 C. Seventy per cent of the initial activity is lost after 4 hr (Fig. 3). Chloroplasts behave in the same way. However, there is no loss in activity when plastids are stored in Honda medium to which the reagents (except ^{14}C -leucine and pyruvate kinase) normally used in the amino acid incorporation mixture have been added. This protective effect may be due to the higher magnesium concentration in the amino acid incorporation reaction mixture (3).

Etioplasts incorporate some radioactive amino acid into soluble protein (protein that is not sedimented at 100,000g in 1 hr), and some amino acid into insoluble protein (protein that is sedimented at 100,000g in 1 hr). About 20% of the radioactivity incorporated into protein by etioplasts or chloroplasts is found in soluble protein (Table I). The radioactive soluble protein of

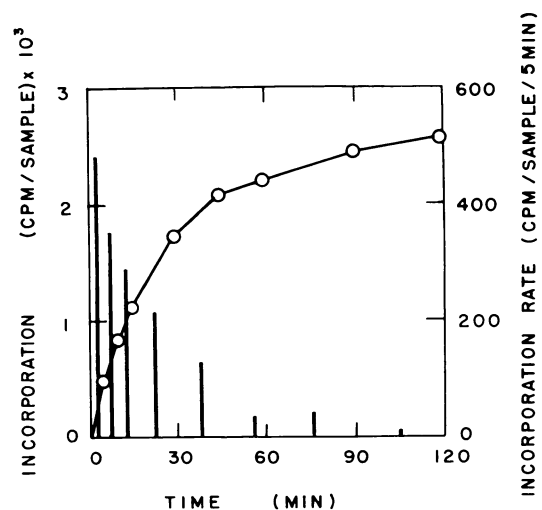


FIG. 1. Time dependence of incorporation of ^{14}C -leucine into protein by an etioplast suspension. One milliliter of suspension contained 1.21 mg of protein and 1.71 μg of protochlorophyll. Sample size was 0.1 ml. \circ : Incorporation at specified time intervals. Black bars represent incorporation rates in the intervals between two points.

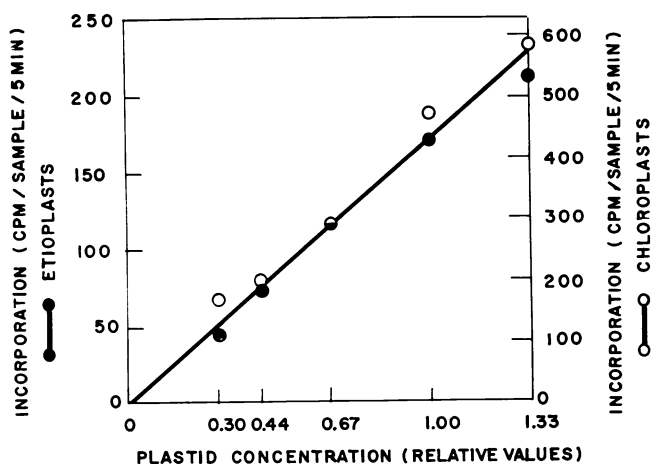


FIG. 2. Incorporation as a function of plastid concentration. ○: Chloroplasts; ●: etioplasts. The relative value "1.00" on the abscissa represents the concentration of plastids with which we normally work (see "Material and Methods"). Incorporation was measured at the end of 5 min at 25 C.

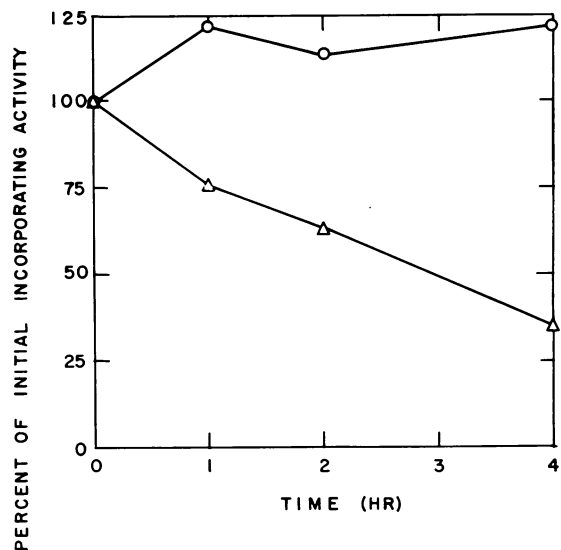


FIG. 3. Effect of storage at 0 C on incorporation activity. △: Stored in Honda medium; ○: stored in Honda medium containing incorporation reagents (see "Material and Methods"); values calculated from incorporation during the first 5 min.

etioplasts and chloroplasts is digested by pronase (see Reference 27 for methods). Only 10 to 20% of the radioactivity in soluble protein is still precipitated with trichloroacetic acid after digestion with pronase for 6 hr at 37 C.

Since we worked with crude plastid preparations, the contribution of whole leaf cells, nuclei, and bacteria to the observed incorporation of amino acid into protein was evaluated. For this purpose we used the nonionic detergent Triton X-100, which breaks plastids but leaves whole leaf cells, nuclei, and bacteria unaffected (1, 25, 29). The latter are still sedimentable at low speeds (Triton-insoluble fraction) while the plastids no longer are (Triton-soluble fraction). Two per cent of the radioactivity incorporated into protein is found in the Triton-insoluble fraction (bacteria, whole leaf cells, nuclei) after 5 min of incorporation, and 10% after 1 hr (Fig. 4). Rates of amino acid incorporation into protein were calculated from the incorporation that occurred during the first 5 min, unless specified otherwise, so that the contribution of bacteria, etc. would be negligible.

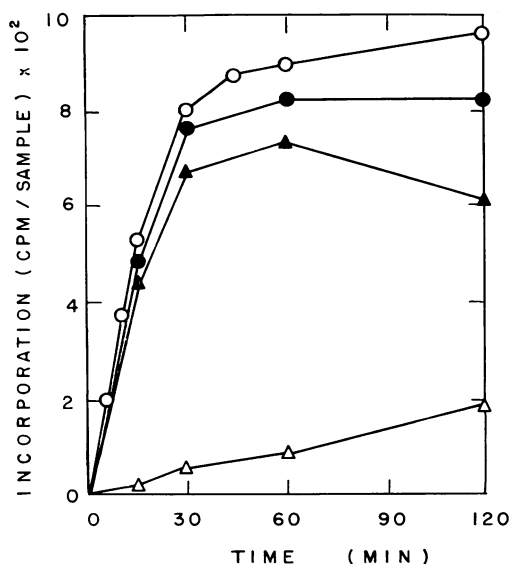


FIG. 4. Evaluation of the contribution of bacteria, leaf cells, and nuclei to amino acid incorporation of an etioplast preparation. Samples were taken and amino acid incorporation was determined in the usual manner (○), or samples were taken and made to 0.1% with respect to Triton X-100 and were fractionated into Triton-soluble (▲) (plastids) and Triton-insoluble (△) (bacteria, leaf cells, and nuclei) fractions as already described (25). The sum of radioactivity in protein of insoluble and soluble fractions was calculated (●).

Table I. Incorporation of ¹⁴C-Leucine into Soluble Protein of Etioplasts and Chloroplasts

The incorporation reaction was carried out for 1 hr.

Fraction	Radioactivity in Reaction Mixture	
	Etioplasts	Chloroplasts
	<i>cpm/ml</i>	
Total incorporation	26,800	57,300
Insoluble protein	18,300	36,300
Soluble protein	5,200	11,600
Recovery	23,500	47,900
Soluble protein, % of total in incorporation	19.5	20.0

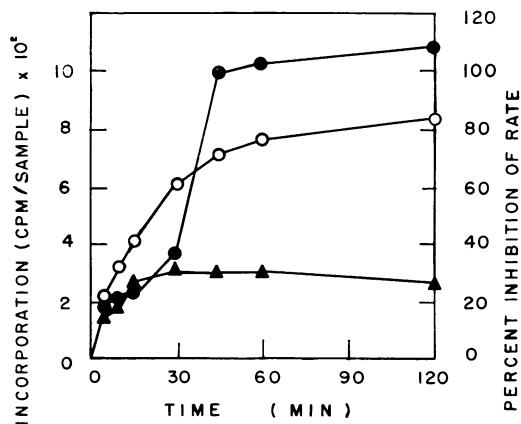


FIG. 5. Effects of ribonuclease (ribonuclease A; EC 2.7.1.40; 400 μg/ml) on incorporation by etioplasts. ▲: With ribonuclease; ○: without ribonuclease; ●: percentage inhibition of rate of incorporation during the preceding time intervals. Ribonuclease was added a minute or two before incorporation was started by adding labeled leucine and warming the reaction mixture to 25 C.

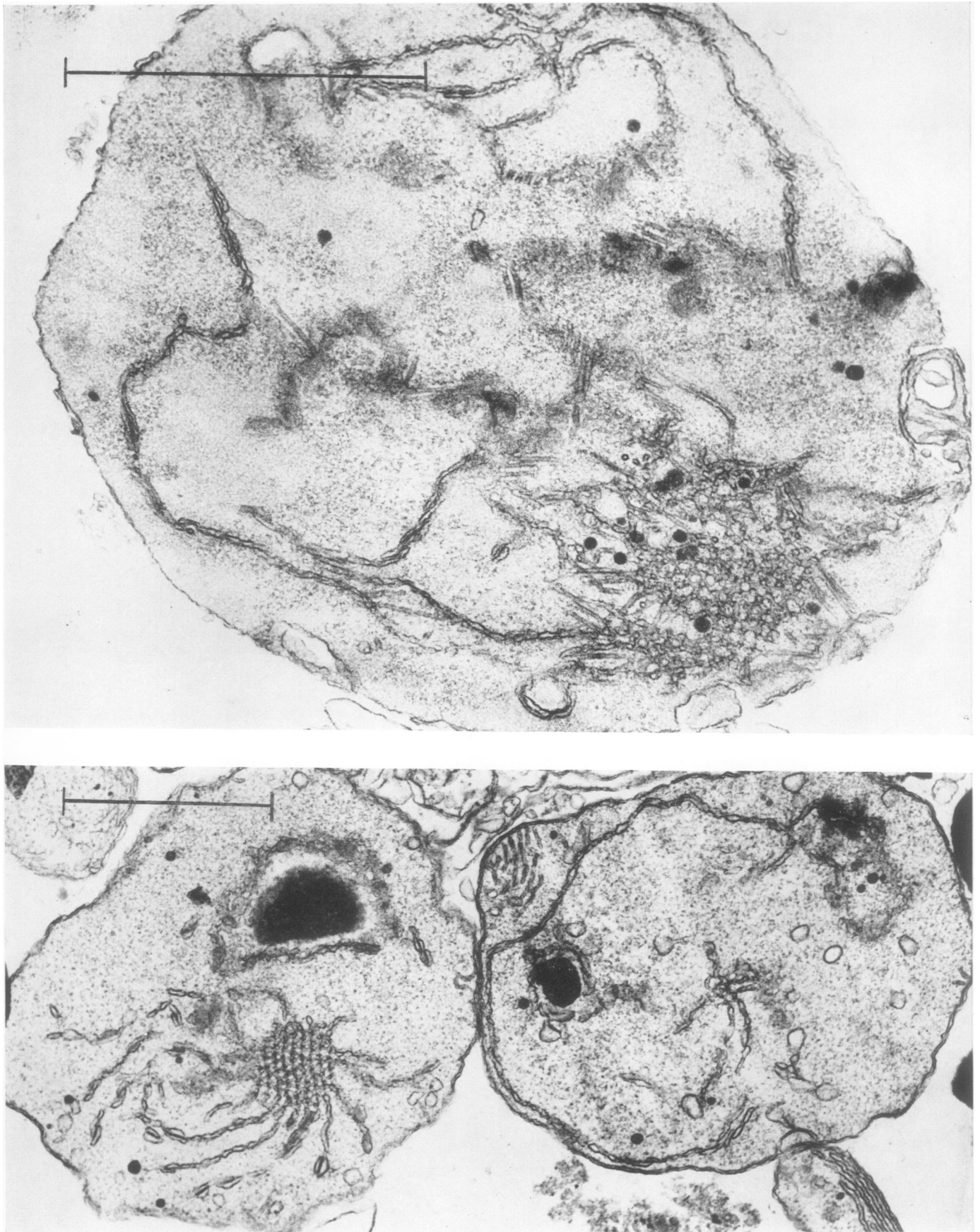


FIG. 6. Electron micrograph of crude etioplast preparation, fixed immediately after preparation (upper) and after incubation for 1 hr at 25 C (lower). Bars represent 1 μ .

There is only slight inhibition of amino acid incorporation by ribonuclease during the first 5 min of the incorporation reaction (Fig. 5). This result suggests that the contribution of cytoplasmic ribosomes to incorporation is small, since an extremely high concentration of ribonuclease (400 $\mu\text{g}/\text{ml}$) was used. However, the percentage inhibition of rates of incorporation by ribonuclease reaches 100% within 30 to 60 min (Fig. 5). Possibly this result is obtained because the etioplast membrane is damaged or at least altered during the incubation at 25 C, as has been hypothesized for chloroplasts (22). But in contrast to chloroplasts, no clear damage to etioplast membranes could be demonstrated by electron microscopy. Etioplasts incubated for 60 min at 25 C appeared to have their membranes intact (Fig. 6, lower), as did etioplasts before incubation (Fig. 6, upper). In contrast, chloroplasts are severely damaged when they are incubated for 60 min at 25 C (22).

Effect of Illumination of Etiolated Leaves on Rate of Incorporation by Plastids. Illumination of etiolated leaves increases the ability of plastids isolated from them to incorporate amino acid into protein. In these experiments, rates of incorporation *in vitro* are compared on the basis of plastids present in one leaf since leaves at the various stages of development under observation contain equal numbers of plastids (see "Material and Methods"). When 6-day-old etiolated plants are left in darkness for another 48 hr, the plastids, prepared at any time during this 48-hr period, incorporate amino acid into protein at an equal rate (Fig. 7). However, when 6-day-old etiolated plants are illuminated for 48 hr, the ability of plastids isolated from them to incorporate amino acids into protein varies with the time plants are illuminated. A 4-fold increase in amino acid incorporation per plastid is observed after 6 hr. The rate of incorporation of amino acid into protein then remains fairly constant at this increased level when plastids are isolated anytime between 6 and 36 hr of the onset of illumination. The rate of incorporation by plastids isolated 48 hr after the onset of illumination is less than the rate of incorporation of plastids isolated 6 to 36 hr after the onset of

illumination. A rate difference between etioplasts and chloroplasts is also observed when one expresses rates of amino acid incorporation on the basis of protein. The rate for etioplasts for 6.5-day-old plants was 63.5, while the rate for chloroplasts from plants grown in darkness for 6 days and exposed to light for an additional half day was 176 pmoles of ^{14}C -leucine per mg of protein per hr. Each value is the average of three experiments. The following factors were tested to see if they might account for the difference in rate of amino acid incorporation between etioplasts and chloroplasts: differences in photosynthetic capacity; possible presence of inhibitors in etiolated leaves; and differences in leucine pool size.

Chloroplasts can produce ATP by photosynthetic phosphorylation. Etioplasts, on the other hand, cannot. Both etioplast and chloroplast preparations were standardly supplied with ATP and an ATP-generating system since ATP is required to obtain amino acid incorporation into protein. However, incorporation of amino acid into protein was normally carried out in room light, which might cause significant photosynthetic phosphorylation. Therefore, incubation of both systems, chloroplasts and etioplasts, was carried out in complete darkness and under room light (cool white fluorescent lamps, average intensity at working surface of 75 ft-c). No differences in incorporation of amino acid into protein were observed between illuminated and nonilluminated samples (Fig. 8). Thus, synthesis of ATP by chloroplasts via photophosphorylation *in vitro* does not account for the difference in amino acid-incorporating activity of etioplasts and chloroplasts.

Another possibility that could explain the rate difference is the presence of an inhibitor in homogenates of etiolated leaves. Mixing of etioplast homogenates with chloroplast homogenates does not suppress the higher incorporating activity of chloroplasts isolated from this mixed plastid suspension (Table II).

Even if there were no difference between etioplasts and chloroplasts in their respective rates of protein synthesis, a difference in rate of incorporation of ^{14}C -leucine into protein would be observed if etioplasts and chloroplasts differed in the amounts of unlabeled leucine they contained. Etioplasts would show a rate of incorporation lower than that of chloroplasts, if etio-

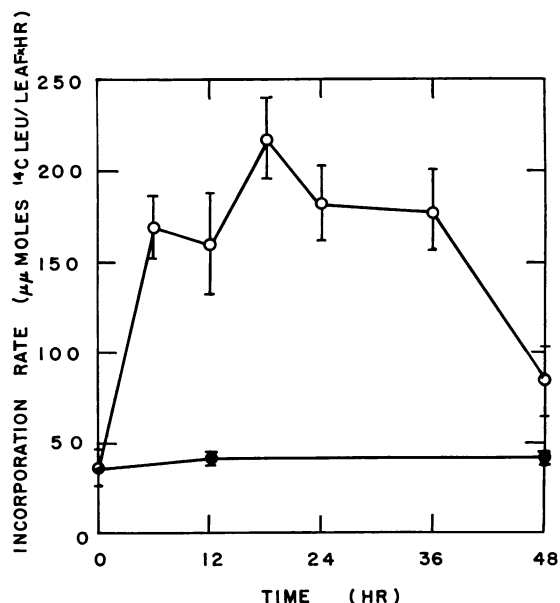


FIG. 7. Effect of illumination of etiolated plants on amino acid incorporation by plastid suspensions. ○: Suspensions from illuminated plants; ●: suspensions from plants left in darkness. Incorporation rates were determined from incorporation occurring during the first 5 min. Rates are calculated as incorporation per leaf, which is related to incorporation per plastid by a constant factor in both etiolated and illuminated leaves (see "Material and Methods"). Each point is the average of at least four independent experiments. Dispersion is standard deviation.

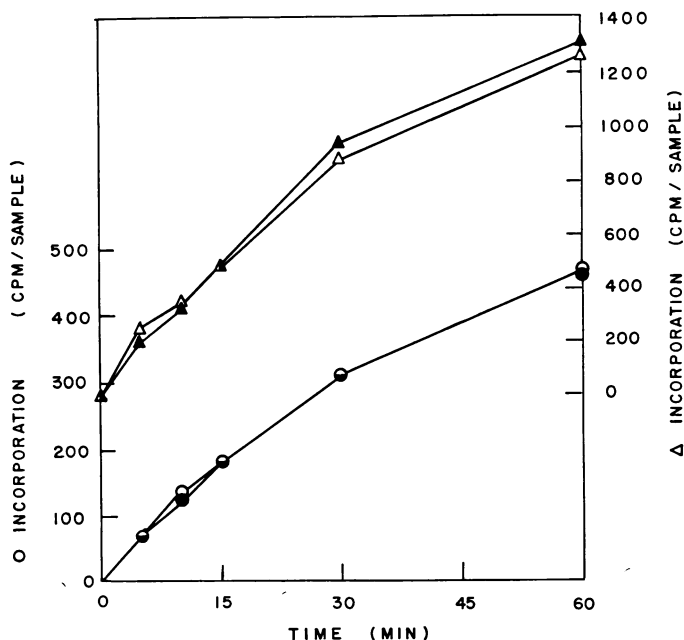


FIG. 8. Effect of room light (75 ft-c) on incorporation into protein by plastids. Etioplasts: ●: incubation in darkness; ○: incubation in room light. Chloroplasts: ▲: incubation in darkness; △: incubation in room light. Incorporation was measured at the end of 5 min at 25 C.

plants contained more unlabeled leucine than chloroplasts. Therefore, incorporation of ^{14}C -leucine into protein by etioplasts and chloroplasts was measured as a function of labeled leucine (of constant specific radioactivity) added to the reaction mixture (Fig. 9). The curve obtained with etioplasts is different in absolute magnitude from the curve obtained with chloroplasts. However, both curves have the same shape, since when each point on the etioplast curve is multiplied by a constant factor, the curves for etioplasts and chloroplasts are the same. This factor, 2.74, is the ratio, incorporation obtained with chloroplasts/incorporation obtained with etioplasts, at the standardly used concentration of ^{14}C -leucine (100 nc per sample). A difference in the shape of the two normalized curves of sufficient magnitude to have been detected by our measurements would have been observed if the amount of ^{12}C -leucine in etioplasts were twice the amount of ^{12}C -leucine in chloroplasts. This con-

clusion rests on the assumption that the chloroplast membrane is freely permeable to both ^{12}C -leucine and ^{14}C -leucine, and also on an estimation that the amount of ^{14}C -leucine added (at the standardly used concentration of ^{14}C -leucine) was twice the amount of ^{12}C -leucine present in the chloroplasts.³ Under these conditions the rate of incorporation by etioplasts would be 75% of the rate of incorporation by chloroplasts. The difference in rates of incorporation of ^{14}C -leucine between chloroplasts and etioplasts is greater than this. Therefore, the difference in rates of incorporation of amino acid into protein between etioplasts and chloroplasts is not accounted for by a difference in content of ^{12}C -leucine between the two.

DISCUSSION

Evidence is presented in this report that etioplasts, like chloroplasts, have the ability to incorporate amino acid into protein. No reports concerning this metabolic activity of isolated etioplasts have appeared previously. However, there had been every reason to believe that etioplasts would be capable of protein synthesis; *e.g.*, etioplasts are reported to have DNA (10, 12) and different kinds of RNA (13, 14).

It was necessary to demonstrate that etioplasts were the sites of amino acid incorporation since we used crude preparations of plastids. It had been demonstrated previously that chloroplasts in crude preparations (22, 23, 25) from bean leaves are the principal sites of amino acid incorporation into protein. Therefore, we believed that it was necessary to establish only a limited number of similarities between the amino acid incorporation of the etioplast preparations and the chloroplast preparations to demonstrate that etioplasts were the principal sites of amino acid incorporation into protein. Bacteria, whole leaf cells, nuclei, and cytoplasmic ribosomes were ruled out as important sites of incorporation (Figs. 4 and 5) in the same way as had previously been done for chloroplasts (22, 25). Other similarities to chloroplast incorporation are the time dependence of incorporation, the products formed (soluble and insoluble protein), and the rapid loss of ability of etioplasts to incorporate amino acid into protein when they are stored at 0 C.

The maintenance of structural integrity of etioplasts during 1 hr at 25 C was the only dissimilarity between the behavior of etioplasts and chloroplasts. Etioplasts remain intact during this time, while chloroplasts lose stroma contents and the chloroplast envelope. These observations do not affect the conclusion that etioplasts in crude etioplast preparations of bean leaves are the principal sites of amino acid incorporation into protein. However, it does require a re-evaluation of the relationship of the chloroplast envelope to ribonuclease sensitivity of incorporation. Ribonuclease inhibits incorporation by chloroplasts (22) and etioplasts (Fig. 5) very little during the first 5 min of incorporation. Inhibition of incorporation increases with each succeeding time interval. With chloroplasts, this increased inhibition of

Table II. *Lack of Inhibitory Effect of Homogenate of Etiolated Leaves on Amino Acid Incorporation by Chloroplasts*

Equal volumes of leaf homogenate filtrates were prepared from etiolated and green leaves. One-third of each filtrate was removed and the remaining two-thirds were combined. Plastids were pelleted from the mixed filtrate, etiolated leaf filtrate, and green leaf filtrate. Each pellet was suspended in an equal volume. Incorporation was corrected for zero time.

Incorporation time Min. at 25°	Incorporation				Inhibition ¹ %
	Chloroplasts	Etioplasts	Chloroplasts + etioplasts		
			Observed	Calculated ²	
	<i>cpm/sample</i>				
5	851	408	559	630	17
10	1,430	723	910	1,080	23
15	3,106	1,360	2,070	2,230	9

¹ Percentage inhibition =

$$\frac{\text{cpm chlor}/2 - (\text{cpm mixture} - \text{cpm etio}/2)}{\text{cpm chlor}/2} \times 100.$$

² Calculated = $\text{cpm chlor}/2 + \text{cpm etio}/2$.

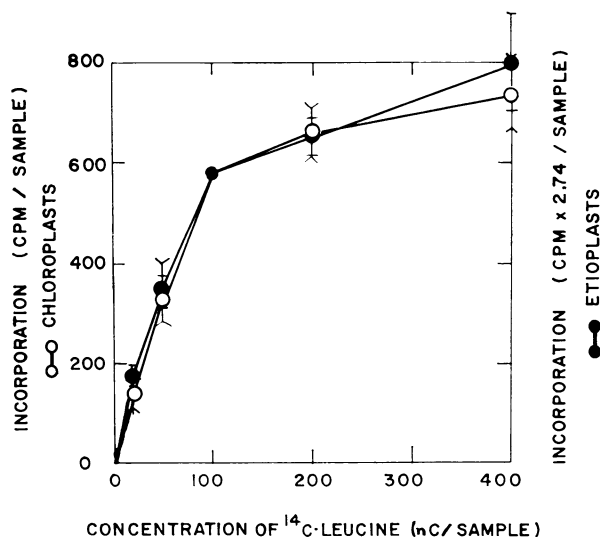


FIG. 9. Dependence of incorporation by etioplasts and chloroplasts on concentration of ^{14}C -leucine. Incorporation was measured at the end of 5 min.

³ A curve for incorporation of amino acid into protein as a function of ^{14}C -leucine added was derived by calculation. First any value for the amount of ^{12}C -leucine in chloroplasts was assumed. Then the amount of ^{14}C -leucine of constant specific radioactivity was varied, and incorporation was calculated for each level of ^{14}C -leucine. The curve obtained was matched by normalization to the curve obtained from actual measurements of incorporation. It was found that the point on the curve (that was experimentally determined) which corresponded to the standardly used ^{14}C -leucine concentration corresponded to a ratio of ^{14}C -leucine/ ^{12}C -leucine equal to 2 (on the curve that had been calculated). There are 40 nmoles of leucine per mg of chlorophyll according to the method of calculation used here. Bamji and Jagendorf (1) measured the amount of amino acids in wheat chloroplasts. Their figures yield a value of 100 nmoles of leucine per mg of chlorophyll if it is assumed that each of 20 amino acids is present in equal amounts.

incorporation is related to destruction of the chloroplast envelope (22). At the start of the incorporation, the chloroplast envelope is intact, but at the end of 1 hr at 25 C, the chloroplast envelope is visibly damaged. It was hypothesized that the chloroplast envelope is almost impermeable to ribonuclease. However, the envelopes of the etioplasts are still intact after 1 hr at 25 C. One possibility is that there has been a physical change in the etioplast envelope which affects its permeability to ribonuclease, but not its appearance in electron micrographs.

The rate of amino acid incorporation by developing chloroplasts is about 200 pmoles of leucine per mg of protein per hr. The rate is 2000 pmoles per mg of RNA per hr if it is assumed that the protein content and RNA content of chloroplasts are 50 and 5% of plastid dry weight, respectively (14). Higher rates have been obtained with chloroplasts from *Acetabularia* (6, 7) and tobacco (30). The results with tobacco are questionable since incorporation was not dependent on ATP. Our results clearly show that amino acid incorporation activity depends on the developmental stage of the plastids. This result suggests that variations which have been observed in amino acid-incorporating activity of plastids with age of plants (1, 28, 29) may be due to differences in the stage of plastid development.

Maximum or near maximum increase of the ability of plastids to incorporate amino acid into protein occurs within 6 hr of the onset of continuous illumination. It has been reported that the ability of ribosomes from corn, bean, and soybean leaves to incorporate amino acids into protein increases when etiolated leaves are illuminated (17, 32). Maximum increase in activity occurred 3 hr after a 30-min illumination. Possibly the maximum increase in bean leaf plastid amino acid incorporation also occurs in such a short time. The increase in the ability of leaf ribosomes to incorporate amino acid into protein as a result of illumination of leaves might be due to increased ability of plastid ribosomes to incorporate amino acid into protein. But since leaf ribosomes are a mixture of chloroplast and cytoplasmic ribosomes, ribosomes from the cytoplasm may also contribute to the increase in activity of leaf ribosomes upon illumination of leaves.

It is interesting that the dependence of amino acid incorporation activity by plastids *in vitro* on time of illumination has a maximum. The ability of plastids to incorporate amino acid *in vitro* decreases during the interval 36 to 48 hr after the start of

illumination of leaves (Fig. 7). This decrease is correlated with a marked diminution in the rate of chlorophyll accumulation by leaves (Fig. 10). The formation of some proteins associated with photosynthesis parallels the formation of chlorophyll during greening of etiolated cells (4, 16). Thus, the relationship between chlorophyll formation and amino acid-incorporating activities suggests that the ability of plastids to synthesize protein increases during the period of most active plastid growth and differentiation and again decreases when growth and differentiation are completed.

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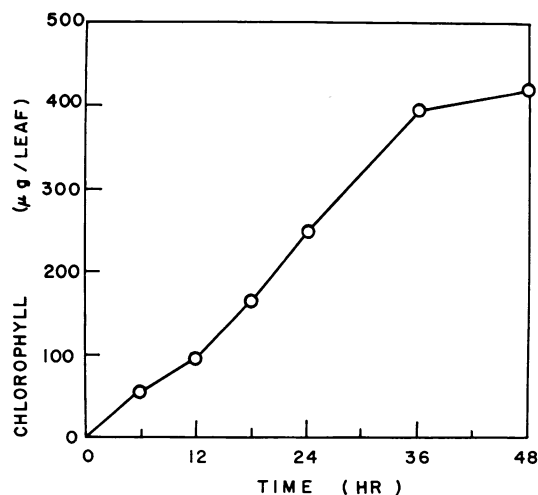


FIG. 10. Time dependence of accumulation of chlorophyll on illumination of etiolated plants. Each point is the average of three independent experiments and are taken from some of the experiments presented in Figure 7.

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