A COMPARISON OF THE CHANGES IN BIOCHEMISTRY AND PLASTID ULTRASTRUCTURE DURING THE DEVELOPMENT OF GREEN AND ALBINO GENETICAL STRAINS OF NICOTIANA TABACUM

A Thesis

Presented to

the Faculty of the Department of Biology

The University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Barbara Wood

Aug. 1969

ACKNOWLEDGEMENTS

This thesis is dedicated to Dr. D. Wiseman and Dr. M. J. Merrett for converting me from a chemist to a botanist. I would also like to express my appreciation to Dr. S. Venketeswaran for enabling me to carry out this research in the United States.

I am greatly indebted to N. Raghu, D. Ponce, W. Law and D. Hanselka for their help in the preparation of this manuscript.

This research was supported in part by the Brown Hazen Fund, Research Corporation, California and NASA Contract # 9-8264.

A COMPARISON OF THE CHANGES IN BIOCHEMISTRY AND PLASTID ULTRASTRUCTURE DURING THE DEVELOPMENT OF GREEN AND ALBINO GENETICAL STRAINS OF *NICOTIANA TABACUM*

An Abstract of a Thesis

Presented to

the Faculty of the Department of Biology

The University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Barbara Wood

Aug. 1969

ABSTRACT

The development of highly isogenic green and albino strains of Nicotiana tabacum was compared. A method of growing albino plants in bulk under asceptic conditions in culture was devised. This allowed greater growth than methods used by other investigators. Both strains were found to show maximum growth in liquid compared to solid medium. Optimum growth of both strains was obtained in modified liquid Hoagland's medium compared to modified liquid MUK medium. The albino plant showed less growth than the green under all conditions and developed only a reduced capacity for photosynthesis.

The green plant was found to follow the same general pattern of changes in dry weight, protein, RNA and DNA content as that of other higher plants. The albino initially contained more protein and dry weight than the green, but followed the same overall pattern of metabolism as the green plant. The nucleic acids of the albino showed a different pattern during development to that of the green. The albino had a lower initial DNA content which later showed a large increase after the 16th day. This large increase in DNA content was followed by an increase in RNA content at the 23rd day. The increase in DNA occurred at the same time as the appearance of the third leaf with green patches.

The plastids of the green plant followed a normal course of development. The albino formed mainly vesicle-containing plastids and a few grana-containing plastids in the cotyledons at germination. The grana apparently degenerated as only vesicle-containing plastids were

observed in later stages of the cotyledons. The green patches of the later leaves of the albino contained both grana- and vesicle-containing plastids. A correlation between chlorophyll synthesis and grana formation was established in the albino. A possible correlation between nucleic acid content and normal plastid formation was observed.

TABLE OF CONTENTS

	Page
A. Abstract	1
B. Introduction	3
C. Statement of the Problem	8
D. Materials and Methods	
I. Culture techniques	10
II. $^{14}CO_2$ fixation	13
III. Dry weight determinations	14
IV. Protein analyses	14
V. Nucleic acid analyses	15
VI. Electron microscopy	15
E. Results	
I. Visual observations	16
II. Optimum growth conditions	16
III. $^{14}CO_2$ fixation	20
IV. Dry weight	24
V. Protein analyses	26
VI. DNA analyses	26
VII. RNA analyses	28
VIII. Plastid ultrastructure	
1) Green plant	
a) 2 day old	31
b) 9 day old	33

TABLE OF CONTENTS (Cont'd)

	Page
c) 16 day old	38
d) 23 day old	38
2) Albino plant	
a) 2 day old	48
b) 9 day old	54
c) 16 day old	60
d) 23 day old	60
e) 30 day - green patches	68
F. Discussion	79
G. References	85

LIST OF FIGURES

Figure		Page
1	Apparatus for growth of albino plants in liquid culture under asceptic conditions	12
2	Mature albino plant	17
3	Mature green plant	17
4	Comparison of growth of green and albino plants on solid MUK medium	18
5	Comparison of increase in height of green and albino plants with time in liquid Hoagland's and MUK medium	19
6	Comparison of increase in number of leaves of green and albino plants with time in liquid Hoagland's and MUK medium	21
7	Comparison of changes in percentage dry weight with time of green and albino plants	25
8	Comparison of changes in protein content with time of green and albino plants	27
9	Comparison of changes in DNA content with time of green and albino plants	29
10	Comparison of changes in RNA content with time of green and albino plants	30
11	Two-day old green plant [x 49,500]. Plastids containing vesicles or grana	34
12	Two-day old green plant [x 39,000]. Plastid showing vesicle transformation into lamellae	35

LIST OF FIGURES (Cont'd)

Figure		Page
13	Two-day old green plant [x 33,000]. Plastids containing small grana	35
14	Nine-day old green plant [x 256,000]. Well developed granum showing discs extending into the stroma.	37
15	Nine-day old green plant [x 105,000]	39
16	Sixteen-day old green plant [x 30,000]	41
17	Sixteen-day old green plant [x 50,000]	42
18	Sixteen-day old green plant [x 15,750]	43
19	Sixteen-day old green plant [x 30,000]	44
20	Sixteen-day old green plant [x 31,500]	45
21	Twenty-three day old green plant [x 45,000]	47
22	Two-day old albino plant [x 40,000]. Dividing proplastid	50
23	Two-day old albino plant [x 28,000]. Cell containing plastids with small and large vesicles.	52
24	Two-day old albino plant [x 37,500]. Plastid containing grana.	53
25	Nine-day old albino plant [x 30,000]. Plastid containing a few vesicles.	55
26	Nine-day old albino plant [x 25,000]. Plastid containing vesicles.	57

LIST OF FIGURES (Cont'd)

Figure		Page
27	Nine-day old albino plant [x 30,000]	58
28	Nine-day old albino plant [x 28,000]	59
29	Sixteen-day old albino plant [x 12,500]	62
30	Sixteen-day old albino plant [x 13,500]	63
31	Twenty-three day old albino plant [x 9,750]. Cell containing a large central vacuole and an aberrant plastid.	65
32	Twenty-three day old albino plant [x 7,000]. Cell containing two aberrant plastids with vesicles.	65
33	Twenty-three day old albino plant [x 24,000]. Plastid with vesicles.	66
34	Twenty-three day old albino plant [x 16,000]. Plastids and circular mitochondria.	66
35	Twenty-three day old albino plant [x 15,000]	67
36	Green patches of the thirty-day old albino plant [x 9,750]. Cell containing only plastids with vesicles.	71
37	Green patches of the thirty-day old albino plant [x 37,500]. Fusion of prolamellar-like bodies to form vesicles.	72
38	Green patches of the thirty-day old albino plant [x 27,500]. Plastid with flattened vesicle forming lamellae.	73

LIST OF FIGURES (Cont'd)

Figure		Page
39	Green patches of the thirty-day old albino plant [x 11,000]. Cell containing plastids with grana and vesicles.	73
40	Green patches of the thirty-day old albino plant [x 11,000]. Grana containing plastid.	75
41	Green patches of the thirty-day old albino plant [x 19,500]. Grana containing plastid.	75
42	Green patches of the thirty-day old albino plant [x 21,000]. Plastids containing normal grana.	76
43	Green patches of the thirty-day old albino plant [x 60,000].	77

LIST OF TABLES

Table		Page
1	Comparison of the photosynthetic capacity of green and albino plant	23
2	Measurements of the plastids of the two-day old green plant	32
3	Measurements of the plastids of the nine-day old green plant	36
4	Measurements of the plastids of the 16 day old green plant	40
5	Measurements of the plastids of the 23 day old green plant	46
6	Summary of changes in plastid structure of the green plant throughout the culture period	49
7	Measurements of the plastids of the two-day old albino plant	51
8	Measurements of the plastids of the nine-day old albino plant	56
9	Measurements of the plastids of the 16 day old albino plant	61
10	Measurements of the plastids of the 23 day old albino plant	64
11	Measurements of the plastids of the green patches of the 30 day old albino plant	69

LIST OF TABLES (Cont'd)

Table		Page
12	Summary of the measurements of the plastids of the 30 day old albino plant .	70
13	Summary of the measurements of the plastids of the albino plant throughout the culture period	78

INTRODUCTION

Higher plants have the ability to photosynthesize, i.e., evolve oxygen in the light and fix carbon dioxide in the dark. These processes have been shown to occur within the chloroplast in higher plants (2). Chloroplasts are cytoplasmic organelles reaching a diameter of 4-5 microns when mature. Electron microscopy has shown that they consist of a lipoprotein membranous network set in a granular stroma and surrounded by a double membrane. The membrane or lamellae form discs which aggregate together to form the grana characteristic of higher plants. A plastid contains 40-60 grana (16) and 2-100 discs or thylakoids per granum. Starch grains and osmiophilic granules are frequently found within chloroplasts, depending on the metabolic state of the plastid. Recently, the presence of characteristically small ribosomes (70S) and 25 A° diameter fibrils of DNA (25) have been demonstrated in chloroplasts. Electron microscopy has shown that the above ultrastructure is not fixed, but the components vary within the lifetime of the plant. Thus, the lamellae and grana may disappear and starch grains predominate, resulting in the formation of amyloplasts. In all cases of chloroplast transformation, grana production is accompanied by concomitant chlorophyll synthesis.

Chloroplasts are both structurally and functionally differentiated. The different biochemical reactions of photosynthesis occur at specific sites within the plastid. Light absorption by chlorophyll and accessory pigments is restricted to the grana (7). Photolysis of water, the Hill reaction and cyclic phosphorylation enzymes occur in the lamellae.

Ferredoxin is loosely bound to the lamellar system (27) whereas the last steps of noncyclic photophosphorylation occur in the stroma. A block in ultrastructural development will therefore be reflected in a specific block in photosynthetic function.

The development of chloroplasts has been studied by combining electron microscopy with controlled changes in environmental conditions, i.e., effects of light and temperature. Such studies (41) have revealed a correlation between light, chlorophyll synthesis, and ultrastructural changes summarized below:

1) Formation of proplastids which are small (1 micron) organelles with no internal structure, bounded by a double membrane.

2) The inner membrane invaginates to form small "blebs", which become tube-like.

3) Tubes are transformed into vesicles by the action of light absorbed by protochlorophyll, which is transformed to chlorophyll a.

4) The vesicles disperse and form the primary lamellae under the influence of light.

5) The lamellae form discs which aggregate into grana accompanied by chlorophyll synthesis. This process is enzyme controlled and accompanied by protein synthesis (12).

The study of green plants has thus shown distinct steps involved in ultrastructural development accompanied by various biochemical changes.

Albino plants, i.e., pigment mutants, have been investigated to determine whether lack of chlorophyll is reflected in the state of ultrastructural development of the albino. Ultrastructural studies

of the development can be correlated with the biochemical state of the mutant to give an understanding of the control of plastid development. Mutations at the photochemical or photoreduction level, which do not effect chlorophyll synthesis rarely effect the plastid structure. Mutant algae (15) and higher plants incapable of oxygen production, aerobic carbon dioxide fixation or pyridine nucleotide reduction still possessed green plastids with normal ultrastructure.

Mutants deficient in chlorophyll synthesis frequently show changes in plastid development. Mutant barley, xantha-10 blocked at the protoporphyrin to protochlorophyll step have proplastids incapable of forming prolamellar bodies (41) (44). This Mendelian-inherited defect manifests itself in production of concentric lamellae only and has been recently shown (1) to repress the uptake of C^{14} acetate to phospho, sulpho and galactolipids. The acetate is diverted from forming the polar membrane components to a steriod and fatty acid fraction. Xantha mutants of Picea also show a correlation between lack of chlorophyll and nonformation of prolamellar bodies in the dark (36). Picea mutants of the virescens type which greens later in development are heterozygotes, having a reduced amount of chlorophyll a and can form aberrant lamellae. These mutants comfirm the correlation of chlorophyll a synthesis and grana production and demonstrate a simple recessive homozygous control.

An albino lacking chlorophyll b production in Arabidopsis is shown to be controlled by several alleles and result in a variety of plastids, ranging from normal plastids to plastids with reduced grana (29). Albino barley lacking chlorophyll b also possessed fewer, smaller grana (8).

The lack of chlorophyll is often due to a secondary photodestruction rather than a lack of synthesis of chlorophyll. The albina variety of Helianthus annuus can synthesize protochlorophyll but not carotenoids (35). These plastids can form prolamellae in the dark and a few grana and plastoglobuli. However, in the light the grana disintegrate and vesicles form. Xantha varieties of Helianthus can form xanthophyll but not carotenoids (33). This variety synthesizes small amounts of chlorophyll (9) which enable the formation of larger grana than the albina. In strong light the xanthophyll cannot protect the chlorophyll from photodestruction and the grana disintegrate to form vesicles. Carotene deficient maize mutants can form prolamellae in the dark, but in the light no grana are formed (26).

No direct measurement of nucleic acids of albinos have been reported in the literature and there are no examples of albinos caused by lack or excess of nucleic acids. Plastids of the white stripes of iojap maize have been shown to have no ribosomes (28), and some mutants of Arabidopsis have increased ribonuclease activity (24). Both these plants are variegated rather than pure albino plants.

Many albinos are caused by mutations affecting the general metabolism of the cell, rather than specific plastid mutations described above. Such plants will grow on complete media as green auxotrophs but not on sugar alone, as do the plastid mutants. A spontaneous, Mendelian-inherited viridoalbina of barley (albina-7) was found to be deficient in the synthesis of aspartic acid (34). This mutant, possessing 3%

chlorophyll and having no grana, turned green and simultaneously developed grana on aspartic acid containing medium. Another barley mutant, eg xantha-23, increased its chlorophyll content from 25 to 75% when grown on leucine. If grown on leucine medium from germination, the abnormal giant grana characteristic of this mutant became normalized. The protein affected was probably in the plastid, as 75% of the leaf protein is found in the plastids (10). When two such temperature-sensitive leucine auxotrophs were analyzed, they were found to have widely different amounts of polar lipids (1) reflecting the different alleles involved.

Albino mutants requiring thiamine exist in tomato (5) and Arabidopsis (14). These non-allelic thiamine-requiring mutants show chlorosis and grana degeneration which can be reversed by addition of thiamine. Thiamine is a carboxylase coenzyme in respiration and its absence affecting plastid structure demonstrates the interdependence between photosynthesis and respiration. Other general metabolism mutants are an iron-requiring corn (3) and an arginine requiring Chlamydomonas (15). Some albinos appear to spontaneously correct their defect. A pale yellow barley mutant of the virescens type (18) had an initial, abnormally high, serine content and formed plastids with vesicles instead of grana. With age, the mutant spontaneously normalized its amino acid content and formed both chlorophyll and grana; reflecting an early development block possible in the carrier protein.

The few studies on albino plants reported in the literature have been rarely carried out under asceptic conditions and none involved the

use of culture conditions throughout the period of investigation. Culture conditions ensure a defined uniform environment for all the plants. To determine exactly how the lack of chlorophyll affects plastid structure, it is necessary to compare the development of plastids of the albino with those of the genotypically green plant under the same conditions. Of the studies on albino plants cited above, only Walles (34) carried out such a systematic study. The present study attempts to compare the development of albino and green genetic strains of tobacco over a time period when both are grown under defined culture conditions. The aspects of development determined here consisted of the changes of concentration of various biochemical components and changes in plastid ultrastructure of both the strains.

STATEMENT OF THE PROBLEM

Clausen and Cameron (6) obtained a colorless mutant by crossing two varieties of Nicotiana tabacum (Purpurea x Chinchao). They established that chlorophyll inheritance in this strain was controlled by duplicate factors and that the inheritance of these factors was by a typical 3:1 Mendelian ratio of green and white. Venketeswaran and Mahlberg (32) were able to induce proliferation of both albino and green strains in tissue culture. Pigment content of both green and albino plants grown asceptically in a modified Murashige and Skoog (21) medium (referred to later as MUK) was analyzed by Mahlberg and Venketeswaran (19). They observed that the 2-day old albino cotyledons contained only 9% of the chlorophyll content of the green. The chlorophyll a:b ratio and the

chlorophyll a+b/carotenoid ratio were lower in the albino cotyledons than in the green. The chlorophyll content of the mosaic leaves of the albino of different ages in culture had a concentration of 18% to that of the green plants in culture. Although chlorophyll content of these mosaic leaves of the albino was found to be higher, the carotenoid content was found to be the same as the albino cotyledons.

Whereas the albinos were grown under asceptic conditions only, the green plants were grown both under asceptic conditions and in the green house. Chlorophyll content of the green plants grown under asceptic conditions were found to have less chlorophyll content (only 64% that of the plants grown in the greenhouse). This suggested that suboptimum conditions for chlorophyll synthesis existed in culture conditions under fluorescent light. Thus chlorophyll content of both strains was already established before this present study.

The aim of this investigation was to study the biochemical changes and differences in plastid structure of green and albino genetic strains of N. tabacum during development under optimum asceptic conditions. The study was divided into the following sections:

I. Establishment of the optimum conditions for growth of both strains under asceptic conditions. Previous work had used a solid medium designed for tissue culture (modified MUK medium containing 1% agar). This study has attempted to determine whether optimum growth could be achieved by using a liquid medium designed for normal plants (Hoagland's medium) (11) under asceptic conditions without using agar as a support. The presence

of agar would result in a non-defined medium as it contains amino acids and would reduce the availability of the nutrients to the plant.

II. Comparison of biochemical changes throughout development of the green and albino plants. Analyses of these changes were determined to observe whether the small amount of chlorophyll in the mutant would result in abnormal biochemical development.

III. Comparison of the plastid ultrastructure of green and albino plants. Electron microscope observations were carried out to establish how the reduced amount of chlorophyll affected the differentiation of plastids during development. Thus far, the plastid structure of this albino strain has not been studied; efforts were directed to determine whether this albino strain lacked the ability to form normal plastids or whether normal plastids developed and later degenerated. The plastid ultrastructure of the cotyledons of both green and albino plants were investigated at the same time intervals as the biochemical studies to observe whether any correlation existed between specific biochemical events and stages of plastid development. The plastids of the green patches which occur in later leaves of the albino were examined to see whether the presence of chlorophyll influenced the development of normal plastids.

MATERIALS AND METHODS

Seeds of Nicotiana tabacum were obtained from Dr. Paul Mahlberg, Indiana University, Bloomington, Indiana, and from Carolina Biological Supply Company. Seeds were surface sterilized with 5% calcium hypochlorite for 10 minutes or with 0.1% mercuric chloride for two minutes before

the surface-sterilization procedure. Seeds were germinated on solid modified MUK medium containing 3% sucrose and 1% agar. The composition of the medium was as follows: (mg/l): NH_4NO_3, 800; $Ca(NO_3)_2.4H_2O$, 100; KNO_3, 80; KCl, 65; KH_2PO_4, 300; $MgSO_4.7H_2O$, 35; $ZnSO_4.7H_2O$, 0.1; H_3BO_3, 0.1; $MnSO_4.H_2O$, 0.01; $CuSO_4.5H_2O$, 0.003; $AlCl_3$, 0.003; $NiCl_2.6H_2O$, 0.003; KI, 0.001; $FeCl_3.6H_2O$, 0.01. The pH was adjusted to 5.5 and the medium was supplemented by: (mg/l) glycine, 2; nicotinic acid, 0.5; pyridoxine HCl, 0.1; and thiamine HCl, 0.1. Seeds were originally grown on solid modified MUK media in petri plates to compare rates of development of green and albino plants. To find the optimum medium for growth of green and albino plants, two-day old seedlings were transferred under asceptic conditions into the following culture system. Three-four layers of cheesecloth were stretched tightly over the inverted petri plate which was inside a crystallization dish. This provided a mechanical support for the plants and also allowed the roots to reach the liquid medium without interfering with the chemical constitution of the medium. The whole apparatus was autoclaved. Seedlings (two days old) were transferred into it and the top of the crystallization dish was covered with a sterile polypropylene cover. This allowed gaseous exchange to occur, but prevented contamination. Up to twenty seedlings could be grown together in one such apparatus as shown in Figure (1). Albino and green plants were grown in separate dishes on either liquid modified MUK or in liquid Hoagland's medium (11). The composition of the medium was as follows: (ml/l) 1M $Ca(NO_3)_2$, 1.25; 1M KNO_3, 1.25; 1M $MgSO_4$, 0.5;

FIGURE 1. APPARATUS FOR GROWTH OF ALBINO PLANTS IN LIQUID CULTURE UNDER ASCEPTIC CONDITIONS.

KH_2PO_4, 0.25; $FeCl_3^*$, 1 ml; micronutrients, 1.

*Stock 5mg/ml.

The micronutrient stock solution contained: (g/l) H_3BO_3, 2.86; $MnCl_2 4H_2O$, 1.81; $ZnCl_2$, 0.11; $CuCl_2 2H_2O$, 0.05; $Na_2MbO_4 2H_2O$, 0.025.

Both these media contained 3% sucrose, and were replenished every 3 weeks. Plants were measured every week by observing the height and number of leaves on each plant.

Comparison of the ability to photosynthesize

Albino and green seedlings were separated when two days old, transferred to the previously described apparatus and allowed to grow for four weeks on liquid Hoagland's medium. The polypropylene cover was removed and the medium poured off to prevent it from absorbing $^{14}CO_2$. The whole apparatus was placed in a vacuum dessicator and all joints were sealed with stop cock grease. Light was supplied by four fluorescent and two incandescent lamps and the temperature of the system was maintained at 70° F. After preillumination for 10 minutes to stimulate photosynthesis, 10 ml of concentrated sulphuric acid was injected with a hypodermic needle into a vial containing 1mC of $Ba^{14}CO_3$ (specific activity 35mC/mM). The high radioactivity was used as the albino was expected to have an extremely reduced ability to photosynthesize. After 15 minutes of exposure to $^{14}CO_2$, 50 ml of 1N sodium hydroxide were poured into the dessicator to remove the residual $^{14}CO_2$. The plants were removed after the apparatus had been transferred into a fume cupboard. The plants were then weighed and soluble components were extracted three times in 10 ml of boiling

80% ethanol. The residue was removed by centrifugation and the combined supernatants were evaporated to 10 ml. Two hundred lambda of each sample were counted on a Beckman scintillation counter at 92% efficiency. The experiment was first run using nonradioactive material to insure that the system would function. The experiment was repeated in the dark and photosynthesis was expressed as a light to dark ratio in disintegrations per minute (dpm) per mg fresh weight.

Comparison of changes in dry weight during development

Seeds were grown on solid modified MUK medium in petri plates after surface sterilization. Each week, seedlings were separated into albino and green. All seedcoats were discarded and any agar on the roots was removed to prevent interference with the biochemical analyses. Seedlings were weighed, freeze-dried for 2 days in a lyophilizer and reweighed to obtain percentage dry weight. The lyophilized material was ground and used for chemical analyses. The data from all samples were analyzed and the standard error of the mean was calculated. Data used for comparison were tested for statistical significance by using the t test at the 5% probability level.

Protein analyses:

3 mg lyophilized tissue was extracted with 1 ml of water and 2 drops of 0.1 N sodium hydroxide for 10 minutes at 0° C. The residue was removed by centrifugation at 3000xg and the protein content of the supernatant was analyzed by the Lowry method (17).

Nucleic acid analyses:

Lyophilized material was delipidized by successive (10 mg to 1.5 mls) extractions with chilled 10% perchloric acid, 95% ethanol (3x), 50-50 ether and ethanol, and ether (3x). The supernatants were discarded after each centrifugation at 5000xg, and the pellet was air-dried. DNA was extracted from the pellet with hot 5% perchloric at 70° C for 10 minutes (3x) and the pooled supernatant was analyzed by the diphenylamine method (37). RNA was analyzed after hydrolysis with 0.1 N potassium hydroxide (10mg/1ml) for 20 hours at 37° C. After neutralization with 1 ml concentrated hydrochloric acid, the DNA was precipitated with 5% trichloracetic acid and removed by centrifugation at 5000xg. The supernatant was used in the orcinol test for RNA analyses.

Electron microscopy

Each week, the cotyledons of green and white plants were removed, cut into 0.1 cm pieces and fixed overnight in 3% glutaraldehyde in cacodylate buffer. Specimens were rinsed in buffer, post-fixed in 2% potassium permanganate ($KMnO_4$) for 30 minutes and rinsed again. Permanganate was used because (i) this was initially used to reveal plant cell membranes and recently chloroplast subunits (38), and (ii) permanganate retains 90% of the chlorophyll in the lamellae (23), whereas OsO_4 retains only 30%. Dehydration in alcohol was followed by embedding in a 1:1 epon-araldite mixture (20). Sections were cut on a MT2 ultramicrotome, collected on formvar coated grids and observed under an EM 6B electron microscope.

RESULTS

I. Visual observations of development:

The albino germinated with completely white or yellow cotyledons which remained white throughout the entire development of the plant. However, subsequent leaves developed small green patches which sometimes disappeared or alternatively remained to give a green and white mosaic pattern. The green patches varied in size, shape and location on the leaf. Leaves with half of the area green were common and occasionally completely green leaves have been observed (Figure 2). After 4 weeks of growth, the albino resembled a variegated plant but still had white cotyledons. The green plants germinated with completely green cotyledons and all later leaves were also a pure stable green (Figure 3).

II. Establishment of optimum conditions of growth

1) Comparison of development in solid MUK medium

Figure (4) showed that both green and white seedlings increased in height and number of leaves with time. At the time of germination, both green and white plants had two cotyledonary leaves and were of the same height. After 4 weeks the white plant had grown to only 80% of the height and produced only 88% of the number of leaves of the green plant. The albino therefore showed a slower developmental rate in solid MUK medium than the green.

2) Comparison of development in liquid Hoagland and liquid MUK medium

In these two media there was an initial lag followed by a steady increase in height (Figure 5). At the end of eight weeks, the height of

FIGURE 2. MATURE ALBINO PLANT

FIGURE 3. MATURE GREEN PLANT

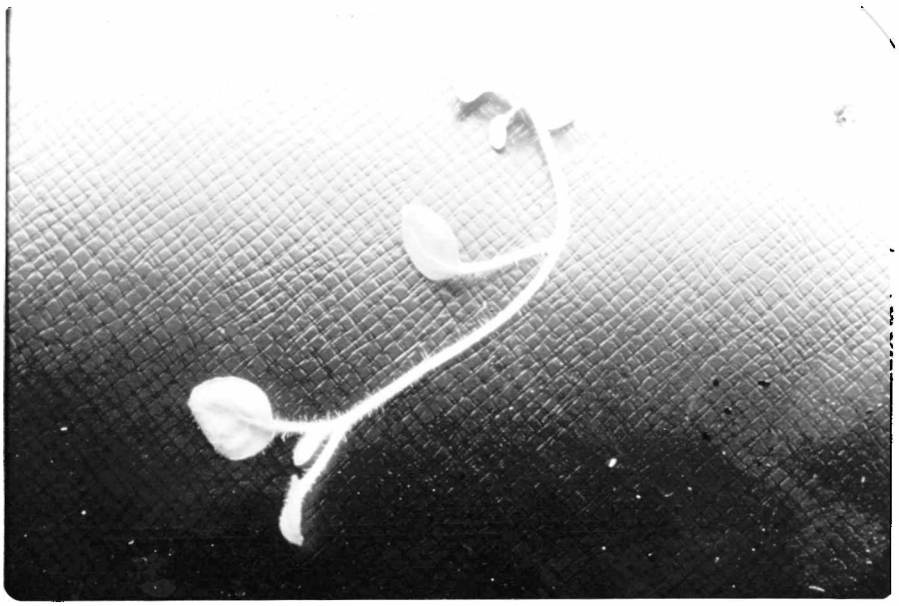


FIGURE 4. COMPARISON OF GROWTH OF GREEN AND ALBINO PLANTS ON SOLID MUK MEDIUM.

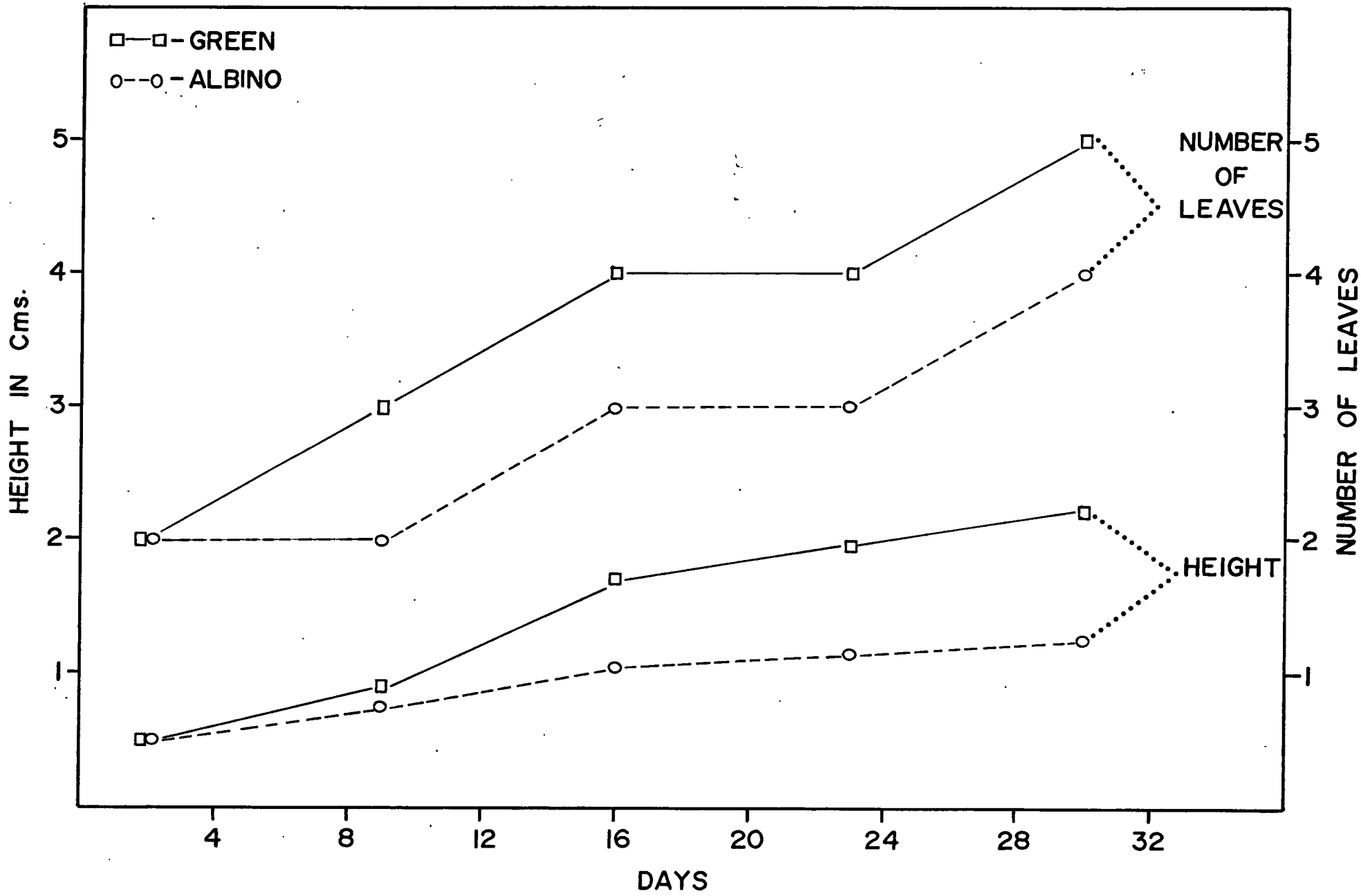
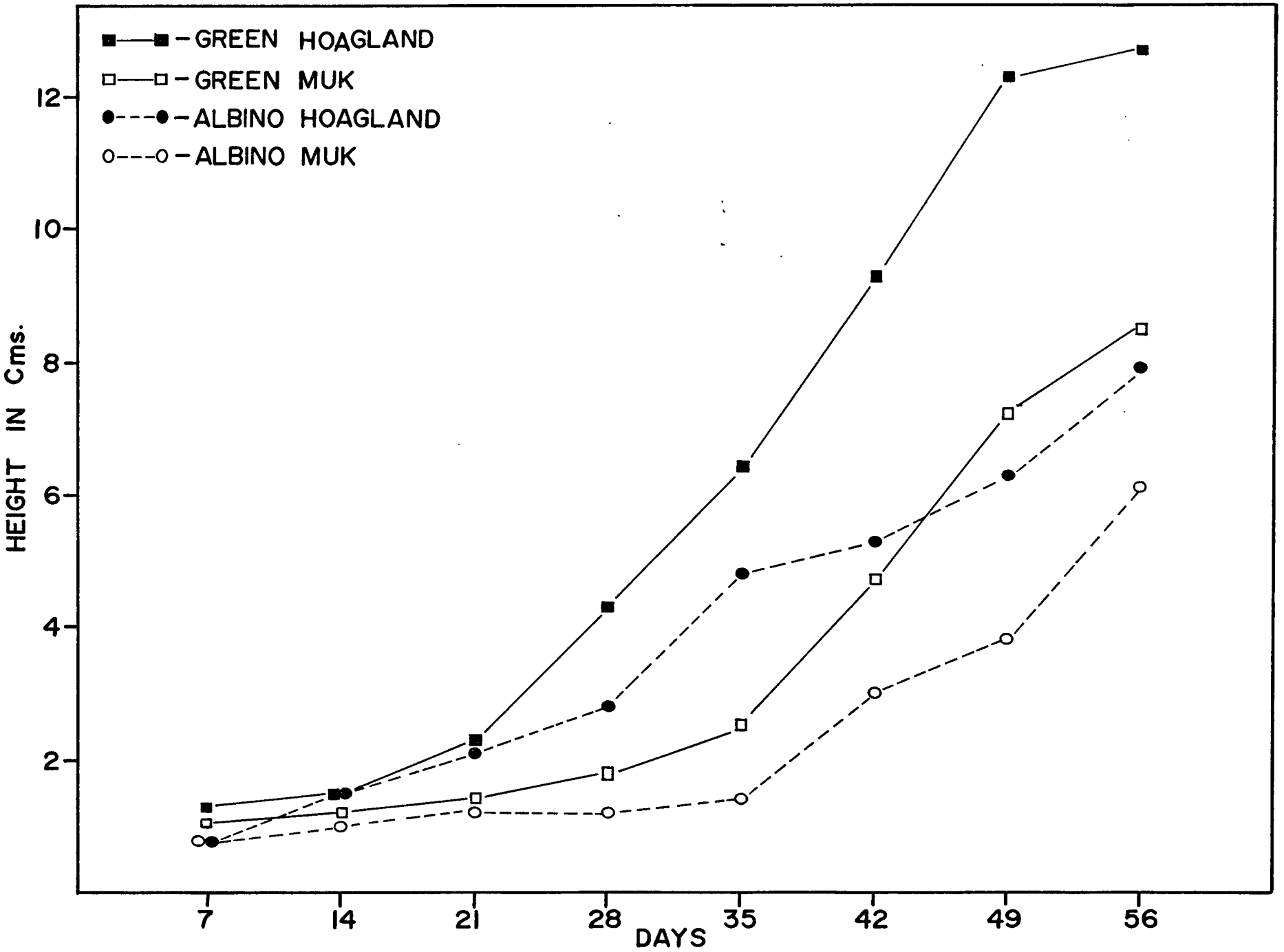

□—□ - GREEN
o--o - ALBINO
HEIGHT IN Cms.
NUMBER OF LEAVES
NUMBER OF LEAVES
HEIGHT
DAYS
1
2
3
4
5
4
8
12
16
20
24
28
32

FIGURE 5. COMPARISON OF INCREASE IN HEIGHT OF GREEN AND ALBINO PLANTS WITH TIME IN LIQUID HOAGLAND'S AND MUK MEDIUM.


GREEN HOAGLAND
GREEN MUK
ALBINO HOAGLAND
ALBINO MUK
HEIGHT IN Cms.
12
10
8
6
4
2
7
14
21
28
35
42
49
56
DAYS

both green and albino was greater in Hoagland's medium than in MUK medium. The increase for both green and albino was 25%. The albino increased its height but never reached the height of the green. This suggested that the albino achieved its optimum growth in Hoagland's medium; but the low amount of chlorophyll (18% that of the green) resulted in reduced growth compared to the green, with respect to height.

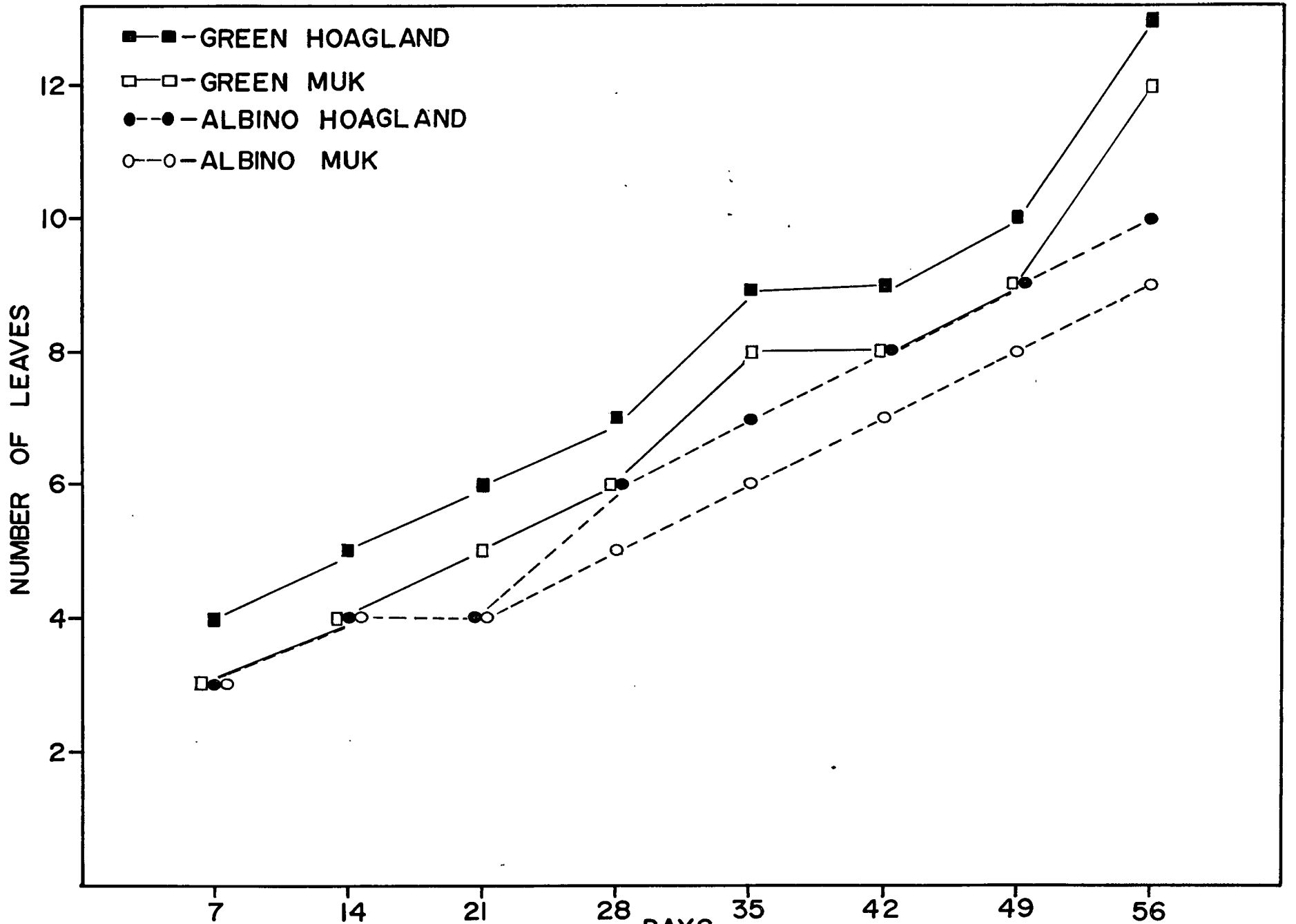
Both green and white seedlings were observed to show a progressive increase in the number of leaves with time (Figure 6). No lag phase in leaf production during early growth was observed in either media. Both the strains showed an increased number of leaves when grown in liquid Hoagland's medium than in liquid MUK medium. The increase was 30% for the green and 25% for the albino. However, the albino did not produce the same number of leaves as the green even in Hoagland's medium. This would indicate that the albino has a slower rate of development than the green.

Growth of both green and albino was greater with a 20% increase in the number of leaves in MUK liquid medium than in solid MUK medium. These results would suggest that growth is generally better in liquid media than in solid media. Liquid Hoagland's medium was used whenever large numbers of plants were required since it allowed optimum growth of both green and albino plants. When changes in the cotyledons were studied, solid MUK was used.

III. Comparison of the development of photosynthetic ability

The results of $^{14}CO_2$ fixation in the light and dark by four week old

FIGURE 6. COMPARISON OF INCREASE IN NUMBER OF LEAVES OF GREEN AND ALBINO PLANTS WITH TIME IN LIQUID HOAGLAND'S AND MUK MEDIUM.


GREEN HOAGLAND
GREEN MUK
ALBINO HOAGLAND
ALBINO MUK
NUMBER OF LEAVES
12
10
8
6
4
2
7
14
21
28
35
42
49
56

green and albino seedlings have been shown in Table (1). The CPM/mg were higher in the light-treated plants than in the dark-treated plants in both strains. The difference was far greater in the green plants than in the albino. However, the light-dark difference in CPM/mg for the albino was statistically significant at the 5% level.

The uptake of $^{14}CO_2$ by the green plants studied here in culture conditions was less than that reported by Laetsch and Stetler (13) on mature tobacco plants grown in the greenhouse (i.e. 13.87 CPM/mg compared to 24 CPM/mg). However, Laetsch and Stetler (13) were investigating mature tobacco plants and used CPM/mg dry weight whereas the plants analyzed in this study were young and a CPM/mg fresh weight was used. Based on this difference in analyses it was reasonable to presume that the total photosynthetic capacity of the young green plants in culture conditions was comparable to that of adult greenhouse grown plants.

The albino had a greatly reduced photosynthetic capacity compared to that of the green plant (i.e. 442 dpm/mg to 20,041 dpm/mg). Thus after four weeks of optimum growth the albino had the capacity to photosynthesize but only at a rate of 2% of the green. The growth of the albino was due to the exogenous supply of sucrose and not to its ability to photosynthesize. It is possible that the small green patches and sectors were responsible for the small photosynthetic capacity and that the photosynthetic mechanisms could function normally if they were allowed to develop.

TABLE 1. COMPARISON OF THE PHOTOSYNTHETIC CAPACITY OF GREEN AND ALBINO PLANTS.

Table (1)

Measurements	Light Conditions	Albino	Green	
Fresh weight in grams	Dark	0.391	0.150	
	Light	0.417	0.1377	
Average CPM/200	Dark	938.	4,300	
	Light	4,375	54,750	
CPM/10ml	Dark	46,900	215,000	average CPM at 2% level corrected for background
	Light	219,750	2,737,500	
CPM/mg fresh weight	Dark	120.0*	1,433	
	Light	527.0*	19,870	
$^{14}CO_2$CPM due to photosynthesis	Dark-light	407	18,437	
	L/D ratio	4.394/1	13.87/1	
$^{14}CO_2$ DPM/mg due to photosynthesis		442/1	20,040/1	(92% efficiency)

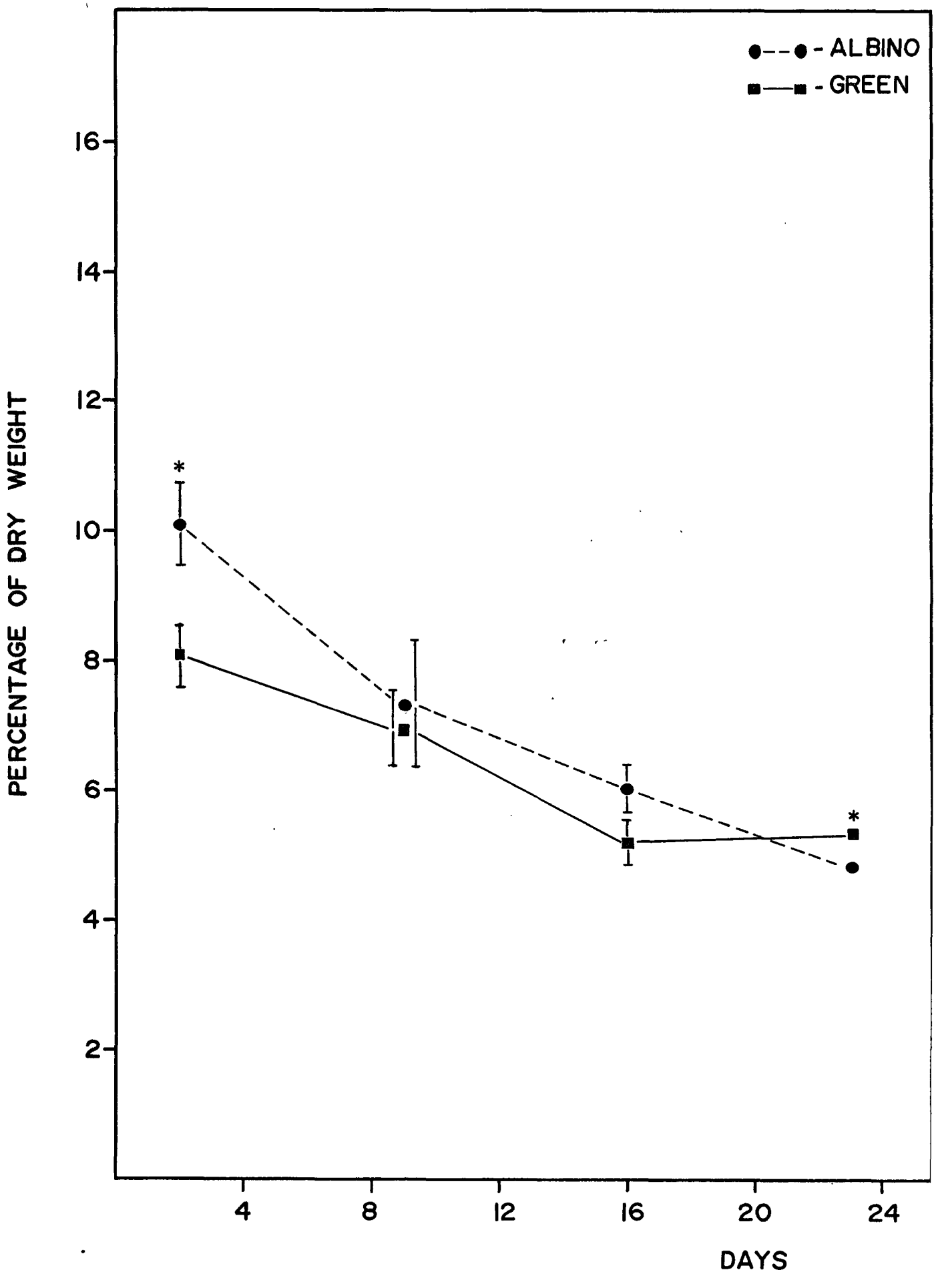
* These figures are statistically significant using the sample t test at the 5% level.

IV. Comparison of percentage of dry weight during development

Olsson and Boulter (22) showed that higher plants underwent a sharp decrease in dry weight during the initial periods of development, followed by a later increase. They attributed the decrease to a depletion of cotyledonary supplies and an increase in absorption of water. They explained the subsequent increase as being due to an accumulation of food materials formed during photosynthesis. Both green and albino plants showed a large initial decrease immediately after germination and continued this decrease until 16 to 23 days (Figure 7). Therefore both strains showed a normal development with respect to dry weight for the first two weeks. The green plant showed no further decrease in dry weight after 16 days and a slight increase was observed at 23 days. Thus the green strain showed normal development throughout the entire culture period with respect to dry weight.

Although the albino showed a normal decrease with time it differed from the green in two respects. Immediately after germination, the two-day old albino had a higher dry weight than the green. The difference was a constant 2% which occurred in every determination and was statistically significant at the 5% level. The albino also differed from the green plant in that after 16 days growth it did not show an increase in dry weight but was still decreasing. At 23 days the albino had a smaller dry weight than the green, and this difference was again statistically significant at the 5% level. No standard error of the mean was shown in the graph at 23 days, as all measurements were identical. The excess material in the albino at the time of germination was catabolized in the same

FIGURE 7. COMPARISON OF CHANGES IN PERCENTAGE DRY WEIGHT WITH TIME OF GREEN AND ALBINO PLANTS.


●--● - ALBINO
■—■ - GREEN
PERCENTAGE OF DRY WEIGHT
16
14
12
10
8
6
4
2
*
*
4
8
12
16
20
24
DAYS

manner as the green plant. However, the depletion was still occurring at the end of the culture period, resulting in a retarded growth curve with respect to dry weight.

V. Comparison of protein changes during development

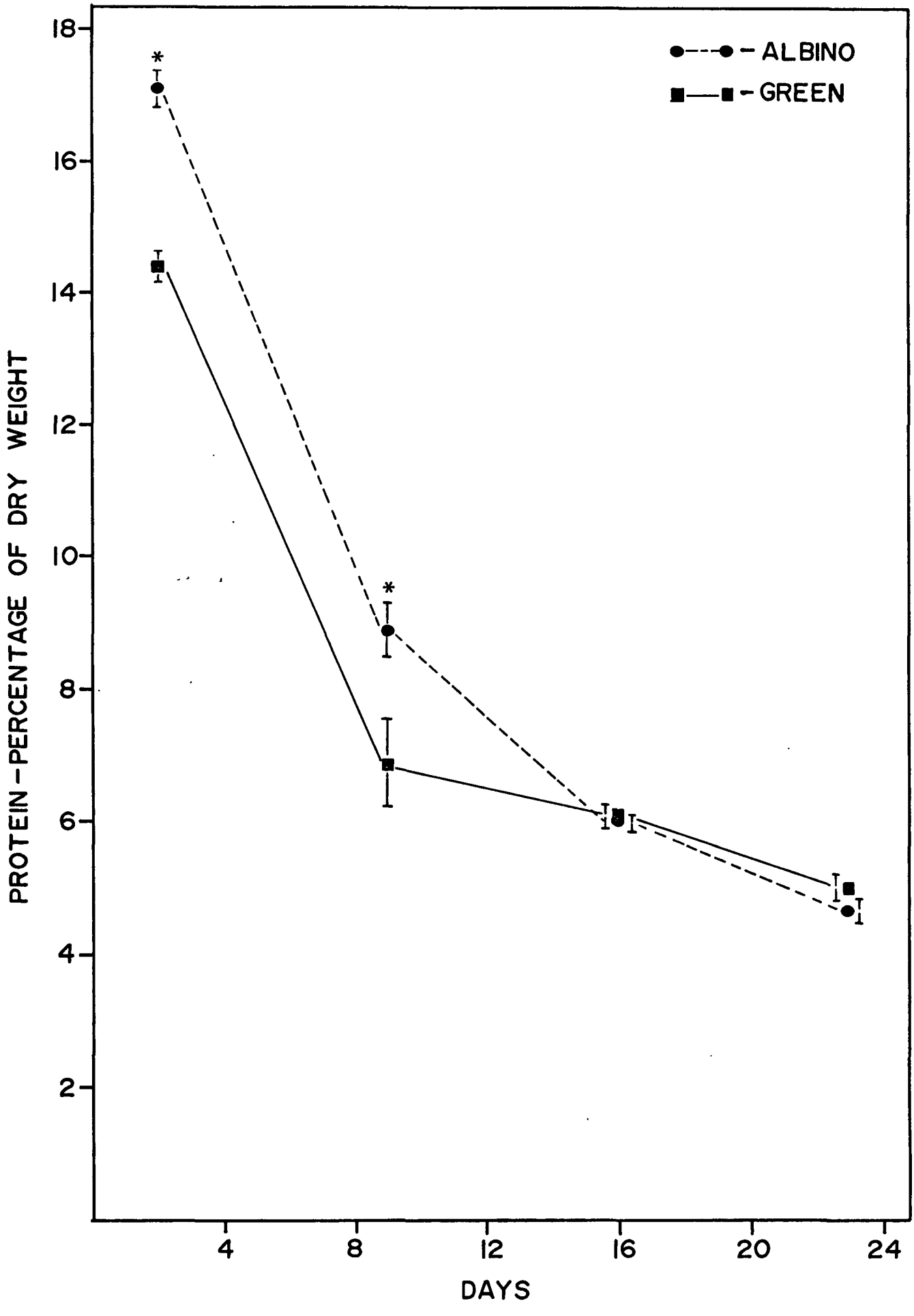
Both green and albino plants showed a decrease in protein content during development until the 23rd day (Figure 8). The protein curve for the green plant resembled the dry weight curve, except that depletion of initial materials had not ceased and an increase in protein content had not begun by the 23rd day. However, the rate of decrease was far smaller from the 9th to the 23rd day.

The albino differed from the green in that it had a higher protein content immediately after germination and also after 9 days of growth. Both these differences were statistically significant at the 5% level. The protein content of the albino continued to decrease from the 9th to the 23rd day, resulting in a final protein content statistically the same as the green. The initial excess protein was therefore catabolized in the same manner as the green plants to the same final level as the green. This suggested that the initial abnormal amount of protein of the albino plant became normal after four weeks of growth. The standard error of the mean of both green and albino plants was consistently small, suggesting that both strains consisted of uniform populations.

VI. Comparison of DNA content during development

The green plant showed an initial large decrease in DNA content from the 2nd to the 9th day and continued to decrease until the 16th day

FIGURE 8. COMPARISON OF CHANGES IN PROTEIN CONTENT WITH TIME OF GREEN AND ALBINO PLANTS.


ALBINO
GREEN
PROTEIN-PERCENTAGE OF DRY WEIGHT
DAYS
18
16
14
12
10
8
6
4
2
4
8
12
16
20
24
*
*

(Figure 9). From the 16th to the 23rd day an increase in DNA content occurred. This pattern of development with respect to DNA content was identical to that reported by Olsson and Boulter (22) for other higher plants. The standard of the mean was extremely small and constant, indicating a uniform population throughout the entire culture period.

The albino showed a completely different pattern of DNA metabolism during development from that of the green plant and the investigations of Olsson and Boulter (22). After germination the two-day old albino plants had only 66% of the DNA content of the green. This difference was statistically significant at the 5% level. Very little change occurred from the 2nd to the 9th day, resulting in a DNA content approximately the same as the green. From the 9th to the 16th day there was a large increase in DNA content from 1.9% to 2.8% which declined slightly to 2.6% at 23 days. Both figures were statistically significant at the 5% level. The albino plant showed a completely abnormal developmental pattern as it changed from an initially smaller DNA content to a final larger DNA content when compared to that of the green. The standard error at the mean was greatest at the 16 day time interval but was still very small, suggesting that greater variation occurred in the population at that time.

VII. Comparison of RNA content during development

The green plant showed a decrease in RNA content throughout the culture period (Figure 10). The decrease was greater from the 2nd to the 9th day than from the 9th to 23rd day. Olsson and Boulter (22) reported

FIGURE 9. COMPARISON OF CHANGES IN DNA CONTENT WITH TIME OF GREEN AND ALBINO PLANTS.

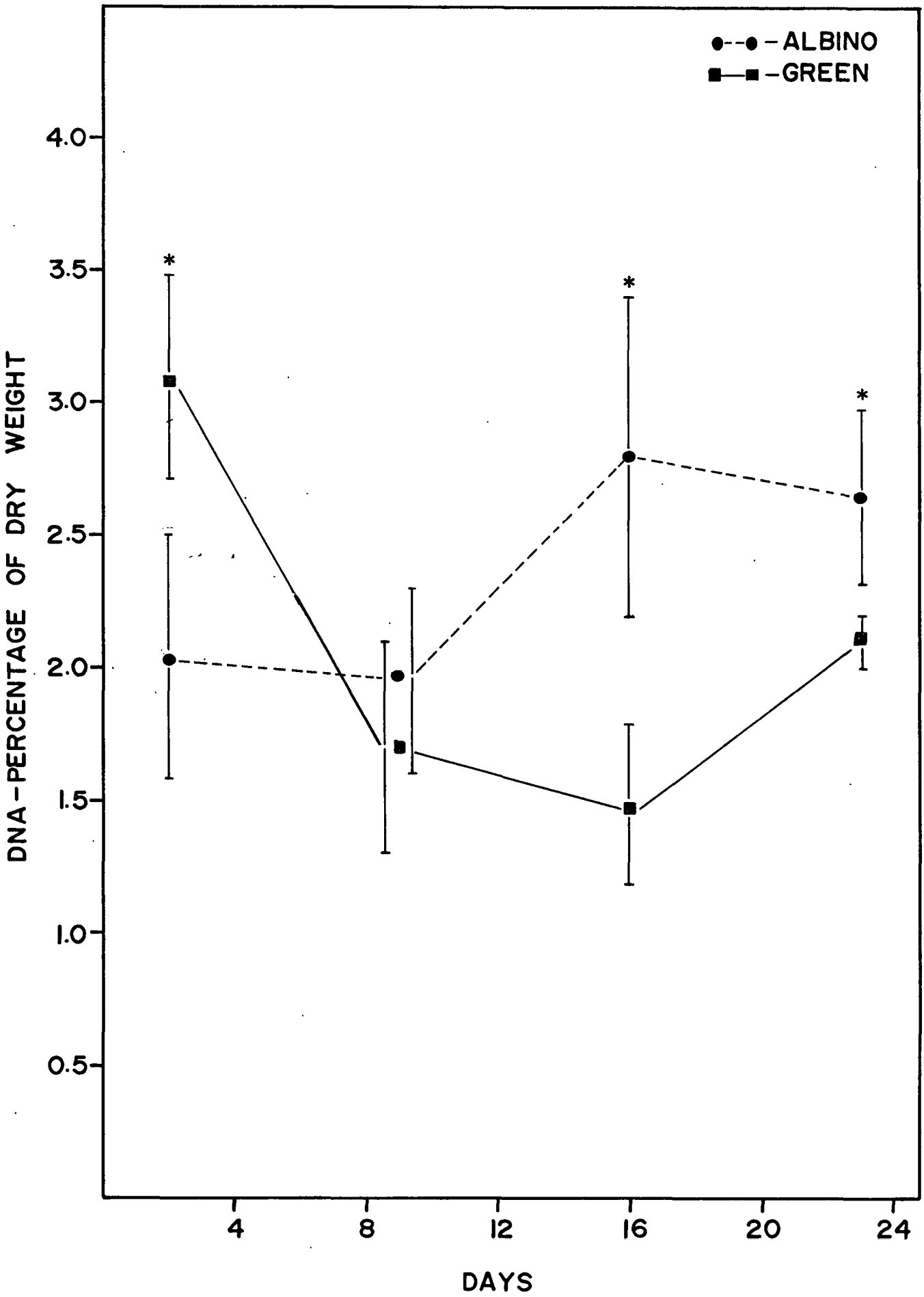
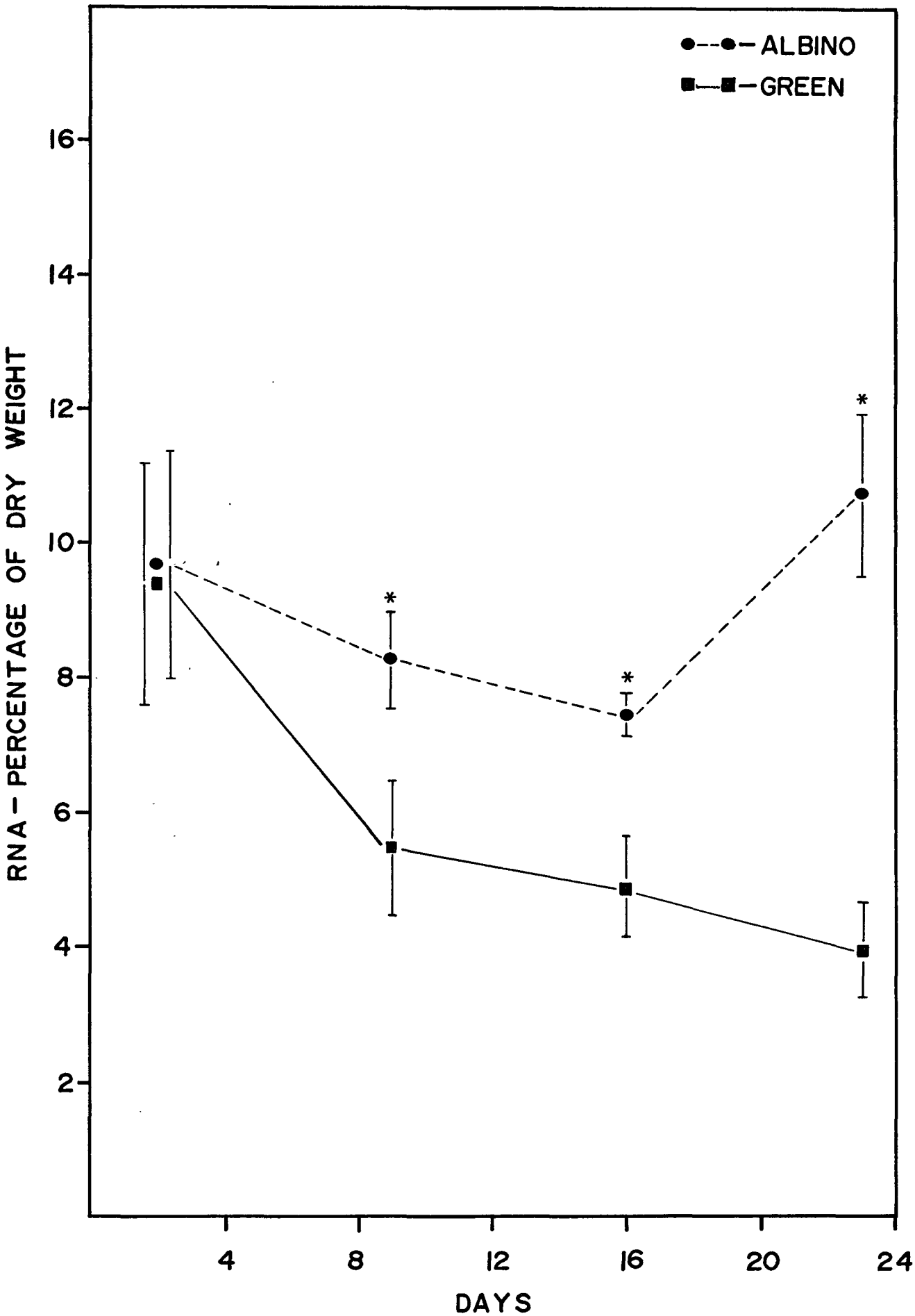

●--● - ALBINO
■—■ - GREEN
DNA - PERCENTAGE OF DRY WEIGHT
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
*
*
*
4
8
12
16
20
24
DAYS

FIGURE 10. COMPARISON OF CHANGES IN RNA CONTENT WITH TIME OF GREEN AND ALBINO PLANTS.


ALBINO
GREEN
RNA - PERCENTAGE OF DRY WEIGHT
16
14
12
10
8
6
4
2
4
8
12
16
20
24
DAYS
*
*
*

an increase in RNA content after the initial decrease, but no such increase was observed in these green plants under culture conditions. The RNA metabolism was similar in that respect to the protein metabolism. The standard error of the mean in the RNA determinations was always very large, suggesting that the population had a wide range of RNA content.

The RNA content of the albino showed a similar decrease until the 16th day to that of the green plant, but the rate of decrease was less. This reduced rate resulted in higher RNA contents at both the 9th and 16th days. From the 16th to the 23rd day there was a large increase in RNA content resulting in a final RNA content of almost 3 times that of the green. The last 3 points were statistically significant at the 5% level. This increase in RNA synthesis followed the increase in DNA synthesis at 9 to 16 days. The standard of the mean was large but constant, indicating a uniform population.

VIII. Comparison of changes in plastid ultrastructure during development

1) Changes in plastid ultrastructure of the green plants

a) The two day old plant

The measurements of the plastids of the two day old plants were given in Table (2). Each cell was found to contain five to six plastids which had a wide range of size from 2 to 4 microns and an average of three microns. Within one cell all stages of plastid differentiation were observed; therefore the development of the plastids was not synchronous. This phenomenon has been observed by other investigators (40).

Very few proplastids were observed at this stage. Several plastids

TABLE 2. MEASUREMENTS OF THE PLASTIDS OF THE TWO DAY OLD GREEN PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (2)
Two-day old Green Plant

Size of Plastid in microns	Number and size of large vesicles	Number and size of small vesicles	Number of grana per plastid	Number of of discs per granum	Width of granum in Angstroms	Length of granum in Angstroms
3 4			12 13 12			
2 3 2		10 4000	18	2-6		
3		10 2000	1			
3			13 9 11	4 4 4 4 6 8	1500	3000
4			20 17 46 22 35	5 6 3 5 6 7 4 8	3000	6000 3000 4000 7500

of approximately 2 microns in size displayed invaginations of the inner membrane. These invaginations developed into flat sacs or discs (Figure 11). The vesicles were small, ranging from 2-4000 A°. The next stage of development was the formation of lamellae and grana by fusion of the vesicles (Figure 12). The grana increased in size due to the addition of more discs (Figure 13). Later stages of development involved elongation of the grana.

Most of the plastids were in the later stages of differentiation. The average number of grana per plastid was 19 and each granum contained from 2 to 8 discs (the average was 5). The range of grana width was large (1500 to 3000 A°) as was the grana length (3000 to 7500 A°). The shape of the grana was generally rectangular (2300 A° x 4000 A°).

b) The nine day old plant

After 9 days development, it was observed that the plastids in the green strain had increased in size to an average of 4 microns (Table 3). Fewer plastids were observed in each cell, but all plastids were at the last stages of differentiation (i.e. grana formation and elaboration). No vesicles were observed at this stage. The plastids showed a large increase in number of grana per plastid to an average of 28. The grana did not increase in length but showed a large increase in width (from 2300 A° to 3700 A°). The increase in width was due to the addition of discs to the grana. The average number of discs per granum had increased from 5 to 12 by 9 days. A typical grana at this stage (Figure 14) consists of 11 discs which have been fused to give a granum which was wider than its length.

FIGURE 13. TWO DAY OLD GREEN PLANT [x 49,500]. PLASTIDS CONTAINING VESICLES OR GRANA. v=vesicle. g=granum. Scale = 1000A°

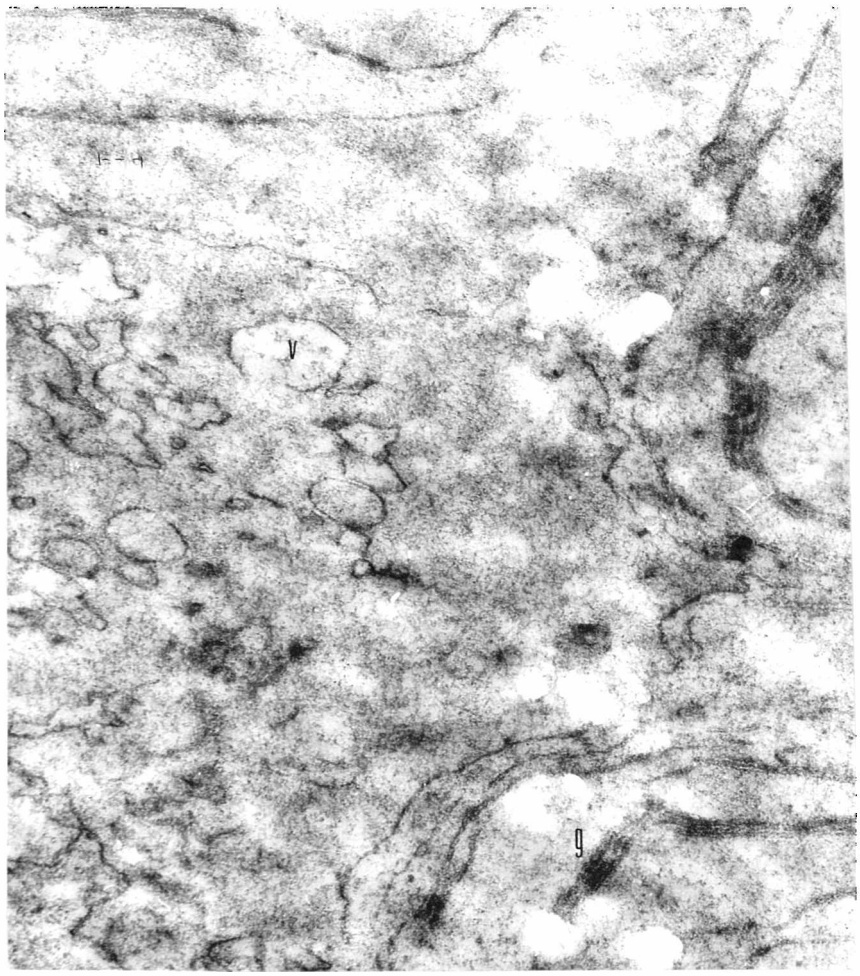
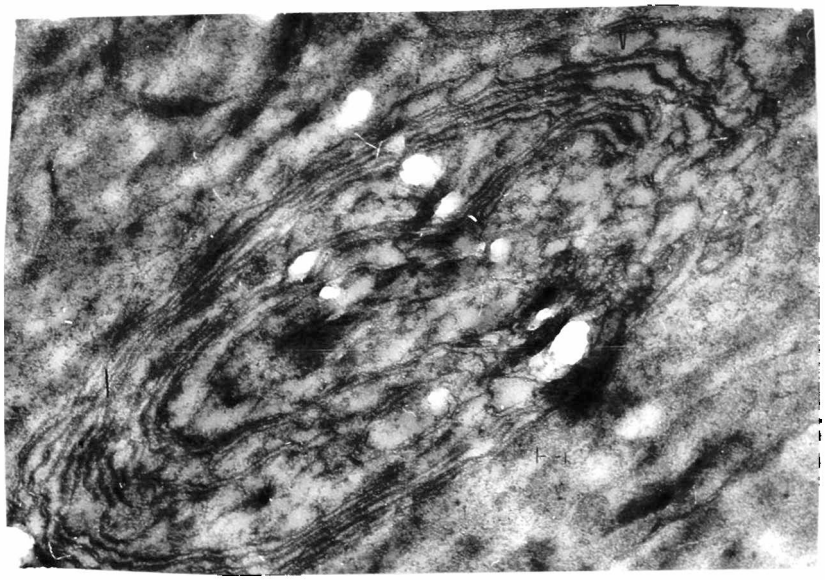

V
g

FIGURE 12. TWO DAY OLD GREEN PLANT [x 39,000]. PLASTID SHOWING VESICLE TRANSFORMATION INTO LAMELLAE. v=vesicle. l=lamellae Scale = 1000 A°

FIGURE 13. TWO DAY OLD GREEN PLANT [x 33,000]. PLASTIDS CONTAINING SMALL GRANA. g=granum. l=lamella. Scale = 1000 A°



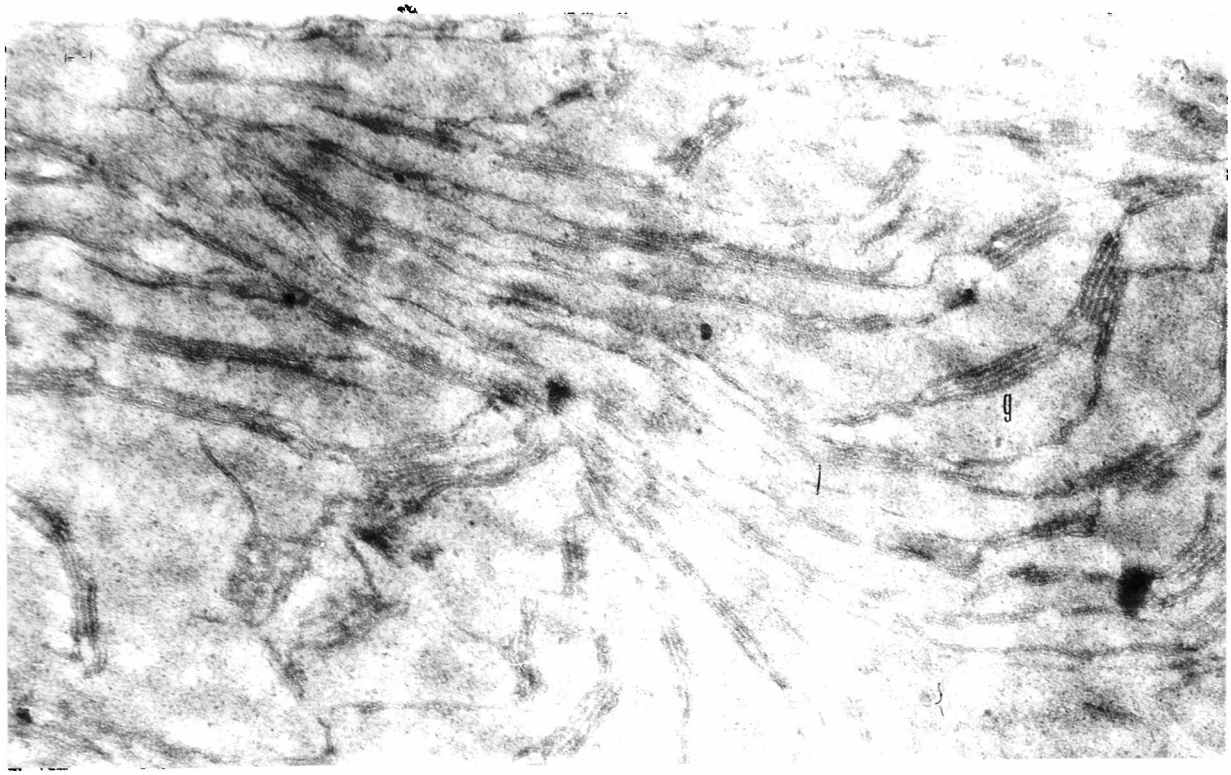


TABLE 3. MEASUREMENTS OF THE PLASTIDS OF THE NINE DAY OLD GREEN PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (3)
Nine-day Green Plant

Size of Plastids in microns	Number of Vesicles	No. of grana per plastid	No. of discs per grana	Width of grana in Angstroms	Length of grana in Angstroms
2		15	11	2800	2000
6		33	15	2400	
4		24	8 15 16	4500	5000
		40	6 8 10 12 23	2400 4000 6000	3000 3000

FIGURE 14. NINE DAY OLD GREEN PLANT [x 256,000]. WELL DEVELOPED GRANUM SHOWING DISCS EXTENDING INTO THE STROMA. s=stroma, d=disc. l=lamella. Scale = 1000 A°


S
I
d

Some of the discs extend into the stroma and into other grana (Figure 15). These connecting lamellae may be very short or alternately may extend across the plastid. Mitochondria were often found adjacent to the plastids and showed normal development (Figure 15).

c) The 16 day old plant

After 16 days growth the plastids had again increased in number per cell (Table 4), and had undergone further internal differentiation. The plastids were arranged around the periphery of the cell, lying close to the cell wall. Their size had increased to 4.7 microns and the number of grana per plastid had increased to 32 (Figure 16). The number of grana per disc and the grana width had increased only slightly. However, grana elongation had greatly increased from 3500 A° to 6000 A° (Figures 16 & 17). Grana elongation was therefore the predominant stage of plastid differentiation at the 16 day time interval. At the leaf tip where growth of the cotyledon is initiated, some cells were observed to have younger stages of grana development (Figures 18 & 19). Other cell organelles found near the plastids were normal, eg. mitochondria (Figures 17, 18, & 19), and nucleus (Figure 20).

d) The 23 day old plant

After 23 days growth, plastids had undergone an increase in size to 5.0 microns (Table 5). The number of grana had greatly increased to an average of 55 per plastid. Elongation of the grana was not pronounced and addition of discs and grana width was not increased (Figure 21). The major stage of differentiation at this time interval was an increase in

FIGURE 15. NINE DAY OLD GREEN PLANT [x 105,000]. cm=chloroplast membrane. m=mitochondrion. l=lamella. Scale = 1000 A°


CM
I
M

TABLE 4. MEASUREMENTS OF THE PLASTIDS OF THE SIXTEEN DAY OLD GREEN PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (4)
Sixteen-day Green Plant

Size of Plastids in Microns	Number of grana per plastid	Number of discs per granum	Grana Width in Angstroms	Grana Length in Angstroms
	42	9	4500	4000
		12	5000	4000
6		16	2000	10,000
7		21	2000	4000
5		15	4000	10,000
7			3000	6000
			2000	6000
3.5		21	3000	9000
3.5		7	11,000	5000
2		5	6000	4000
2		10	6000	10,000
8	28	23	5000	5000
5.3	30	5	4000	5000
	20	12	7500	5000
3	32	22	4000	5000
3			4000	5000
7	40		2500	5000
5			3000	3000
4			3000	5000
2			3000	

FIGURE 16. SIXTEEN DAY OLD GREEN PLANT [x 30,000]. s=stroma.
Scale = 1 micron


S

FIGURE 17. SIXTEEN DAY OLD GREEN PLANT [x 50,000]. Scale = 1000 A°

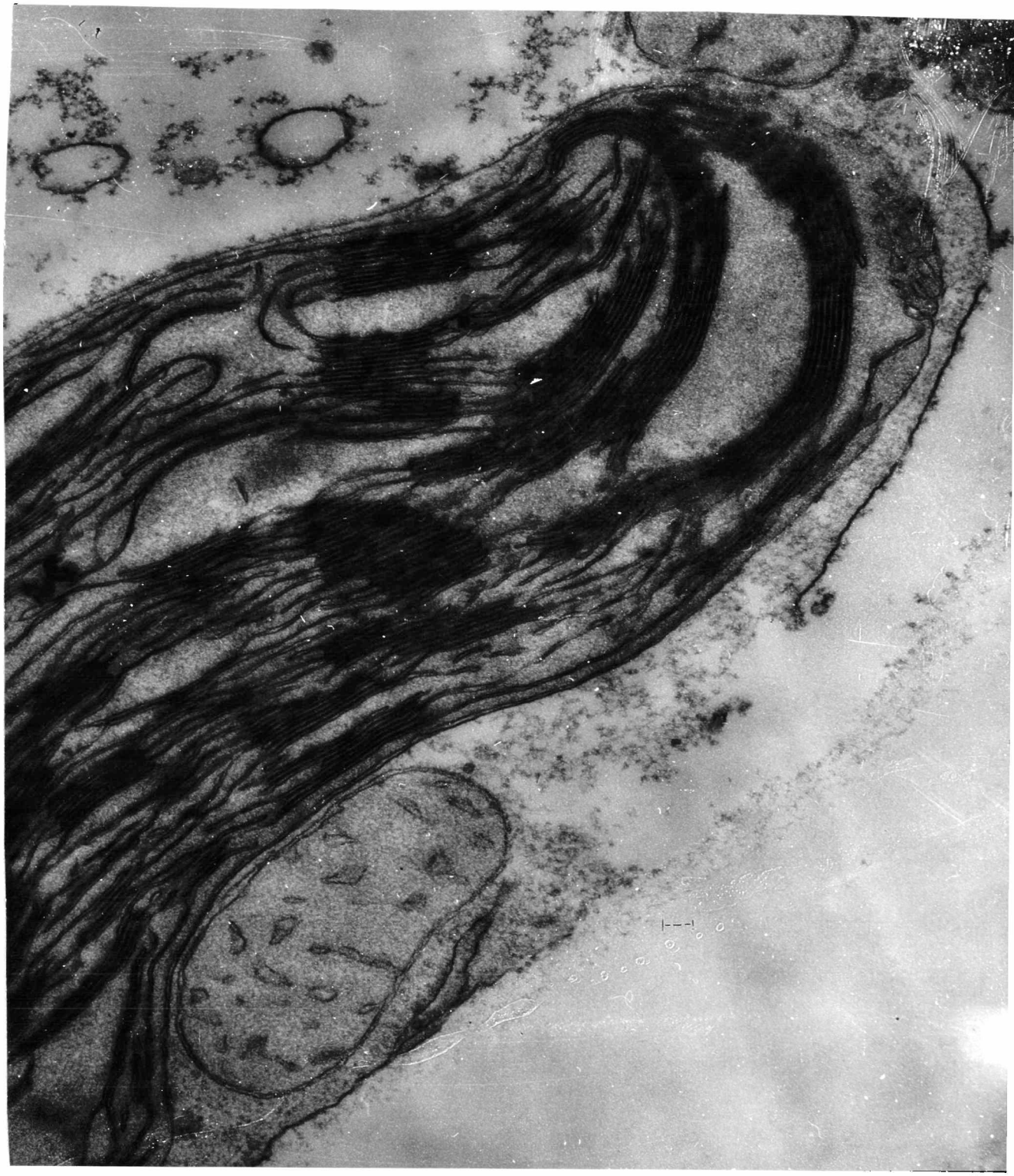


FIGURE 18. SIXTEEN DAY OLD GREEN PLANT [x 15,750]. cw=cell wall. Scale = 1 micron


CW

FIGURE 19. SIXTEEN DAY OLD GREEN PLANT [x 30,000]. g=golgi body.
Scale = 1 micron

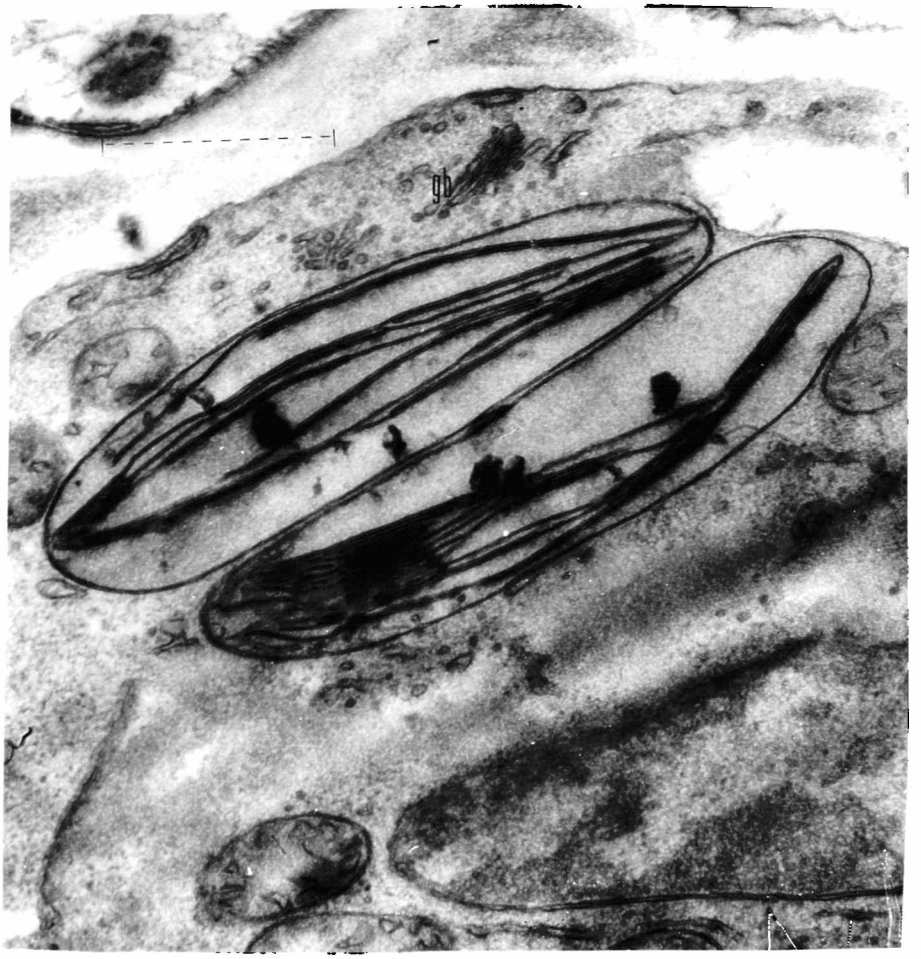

gb

FIGURE 20. SIXTEEN DAY OLD GREEN PLANT [x 31,500]. NUCLEUS SHOWING CHROMATIN (chr) AND NUCLEAR PORES (n.p.). Scale = 1000 A°

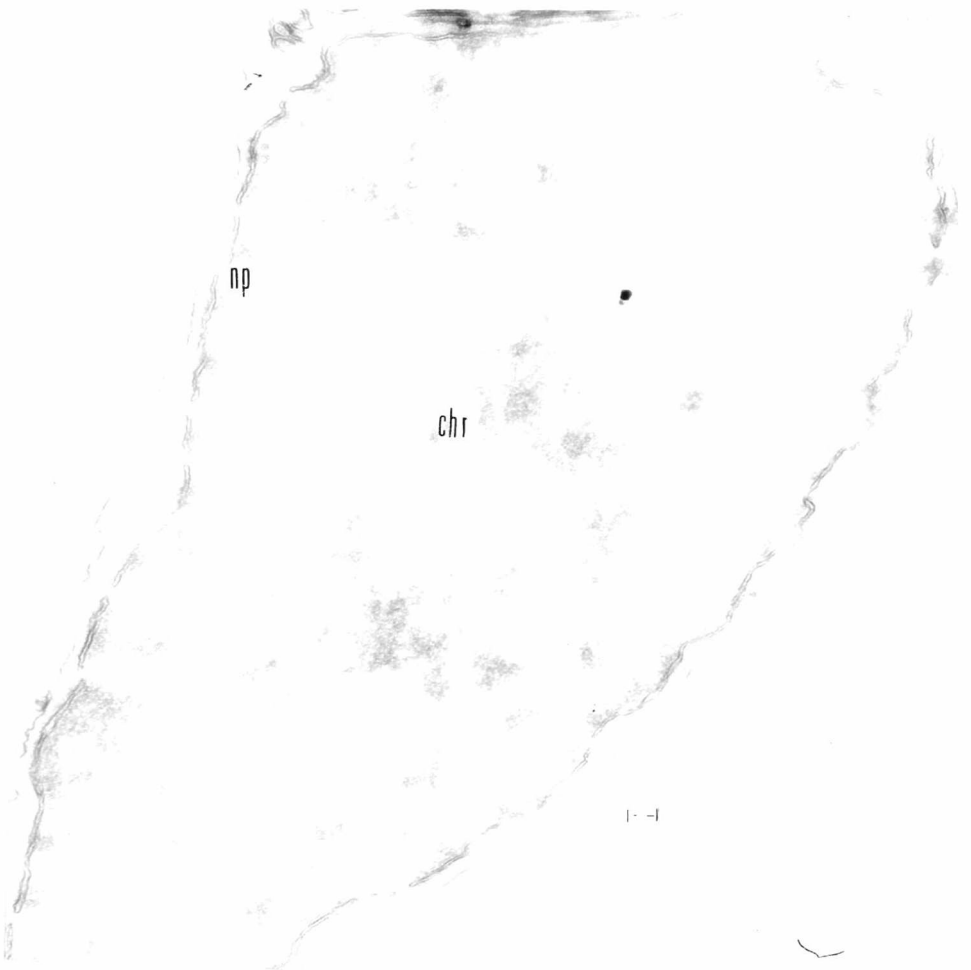
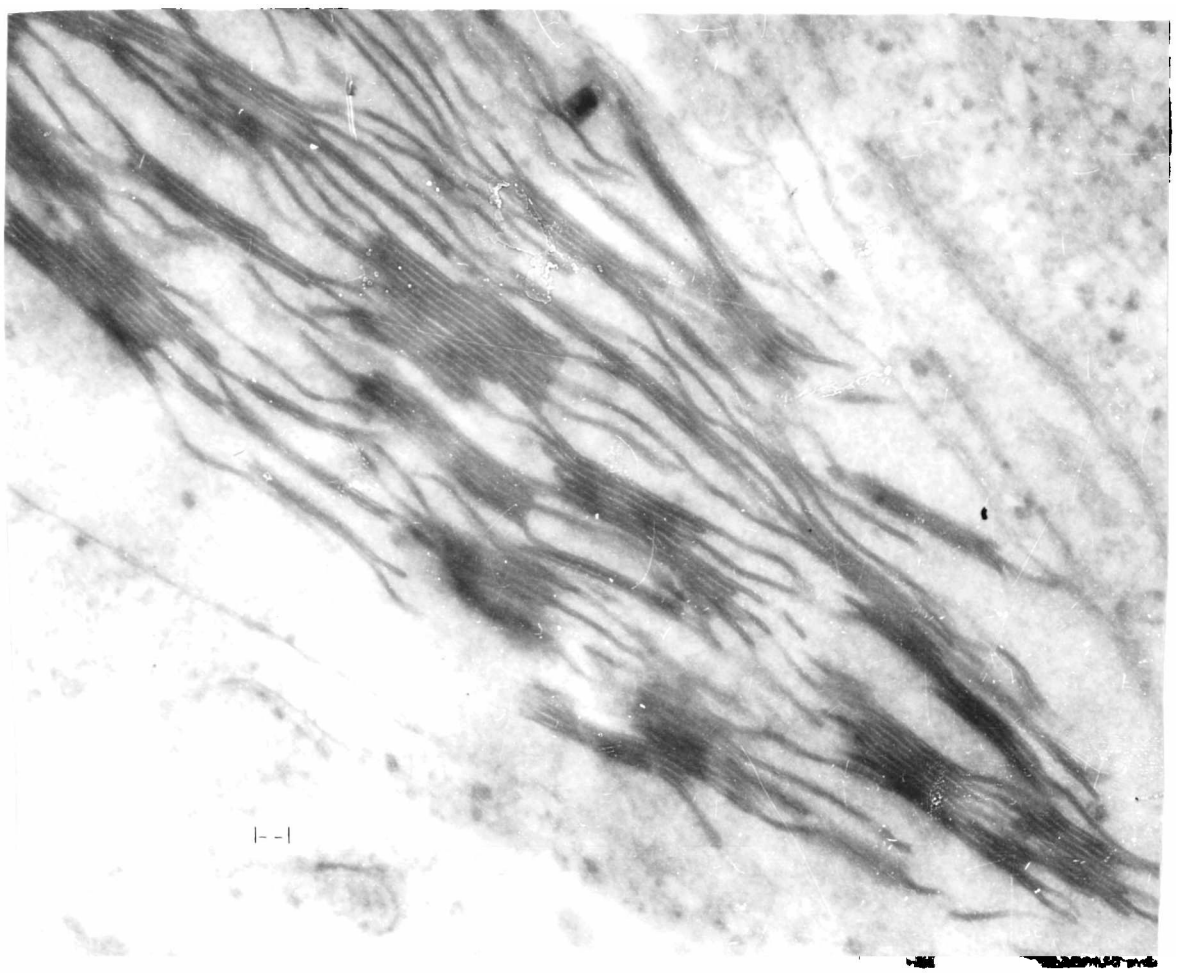

np
chr

TABLE 5. MEASUREMENTS OF THE PLASTIDS OF THE TWENTY-THIRD DAY OLD GREEN PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (5)
Twenty-three day old Green Plant

Size of Plastids in Microns	Number of grana per Plastids	Number of discs per granum	Grana width in Angstroms	Grana Length in Angstroms
4	50	19	4000	5000
5	55	20	3500	5800
6	55	21	4500	6000
	60	18		

FIGURE 21. TWENTY-THREE DAY OLD GREEN PLANT [x 45,000]. Scale = 1000 A°



plastid size and an increase in the number of grana per plastid. This resulted in the slightly decreased average grana length as the younger grana were wider than they were long.

The stages of plastid differentiation in the green strain during the entire culture period were summarized in Table (6).

2) Changes in plastid ultrastructure of the albino plants

a) The two day old plant

Many proplastids were observed at the two day stage, some of which were dividing (Figure 22). They had little internal structure and were 1 to 3 microns long.

Two kinds of plastid were observed at the two day time interval. The first kind was numerous, ranging from 6 to 11 per cell (Table 7). There were therefore more plastids per cell than the comparable green strain at the two day stage. The size of these abnormal plastids was less than that of the plastids of the green strain (2 microns compared to 3 microns). This first kind had very little internal structure compared to the green strain plastids. Usually numerous small vesicles (300 A°) were present (Figure 23) resembling the prolamellar bodies found in plastids of etiolated plants. Occasionally larger vesicles were observed inside the plastids ranging from 3000 to 7000 A° (Figure 23). The shape of these plastids varied from circular to heart shape. No grana or lamellae were observed in these plastids.

The second type of plastid contained grana (Figure 24) and had a size comparable to that of the two day green strain plastids (4 microns).

TABLE 6. SUMMARY OF CHANGES IN PLASTID STRUCTURE OF THE GREEN PLANT THROUGHOUT THE CULTURE PERIOD.

Table (6)
"Plastid Changes during Development of The Green Plant"

Age in days:	2	9	16	23
Number of Plastids Per cell:	5-6	4	3	*
Plastid size in Microns:	3 (2-4)	4-0 (2-6)	4.7 (2-8)	5.0 (3-5)
Number of Vesicles Per Plastid:	1	--	--	--
Size of Vesicles in Angstroms:	2-4000	--	--	--
Number of Grana per Plastid:	19 (1-46)	28 (15-40)	32 (20-42)	55 (50-60)
Lamellae:	Prolamellae present	lamellae	lamellae	lamellae
Grana length in Angstroms:	4000 (3-7,500)	3000 (2-5000)	6000 (3-10,000)	5800
Grana width in Angstroms:	1500 (0-3000)	3700 (2-4500)	4200 (2500-11,000)	4500
Number of discs per granum:	5 (2-8)	12 (8-23)	14 (5-23)	18

*No observations on the number of plastids per cell were made at this time interval.

FIGURE 22. TWO DAY OLD ALBINO PLANT [x 40,000]. DIVIDING PROPLASTID. cw=cell wall. Scale = 1 micron

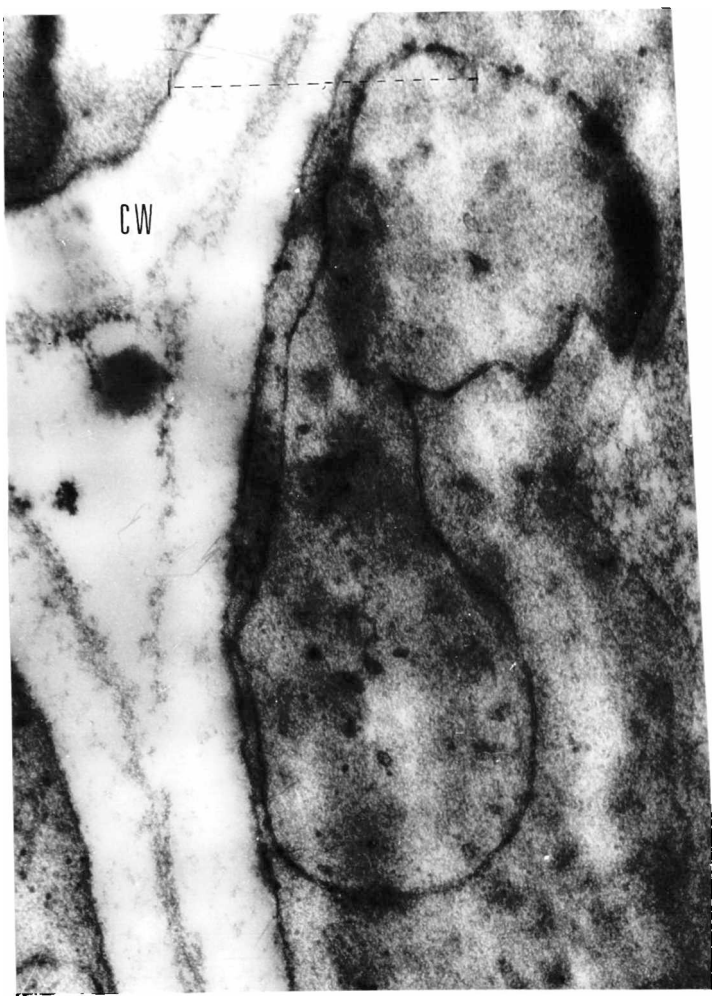

CW

TABLE 7. MEASUREMENTS OF THE PLASTIDS OF THE TWO DAY OLD ALBINO PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (7)
Two-day old Albino Plants

Vesicle-containing plastids			Grana-containing plastids					
Size of Plastid in Microns	Number and size of vesicles in Angstroms	Number of dividing proplastids	Size of plastid in microns	Number and size of vesicles in Angstroms	Number of grana per plastid	Width of grana in Angstroms	Length of grana in Angstroms	Number of discs per granum
2.5	1 (3000)	1	2	--	16	3000	2500	6
1.0	1 (7000)	1	5	--	15	2000	2900	8
0.5	2 (1000)	1	4	--		2500	2100	5
2.0						1300		9
1.3								12
1.7								2
3.0								
1.8								

FIGURE 23. TWO DAY OLD ALBINO PLANT [x 28,000]. CELL CONTAINING PLASTIDS WITH SMALL AND LARGE VESICLES. v=vesicle. Scale = 1 micron

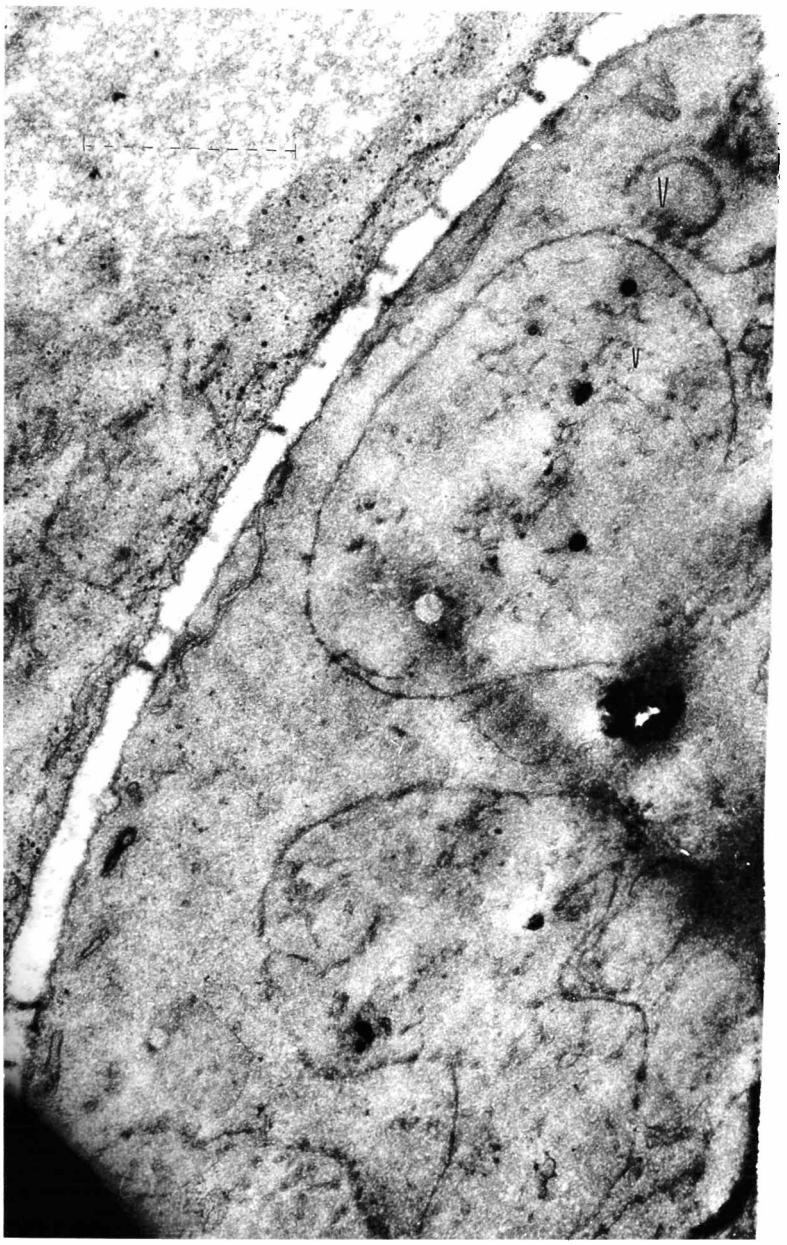
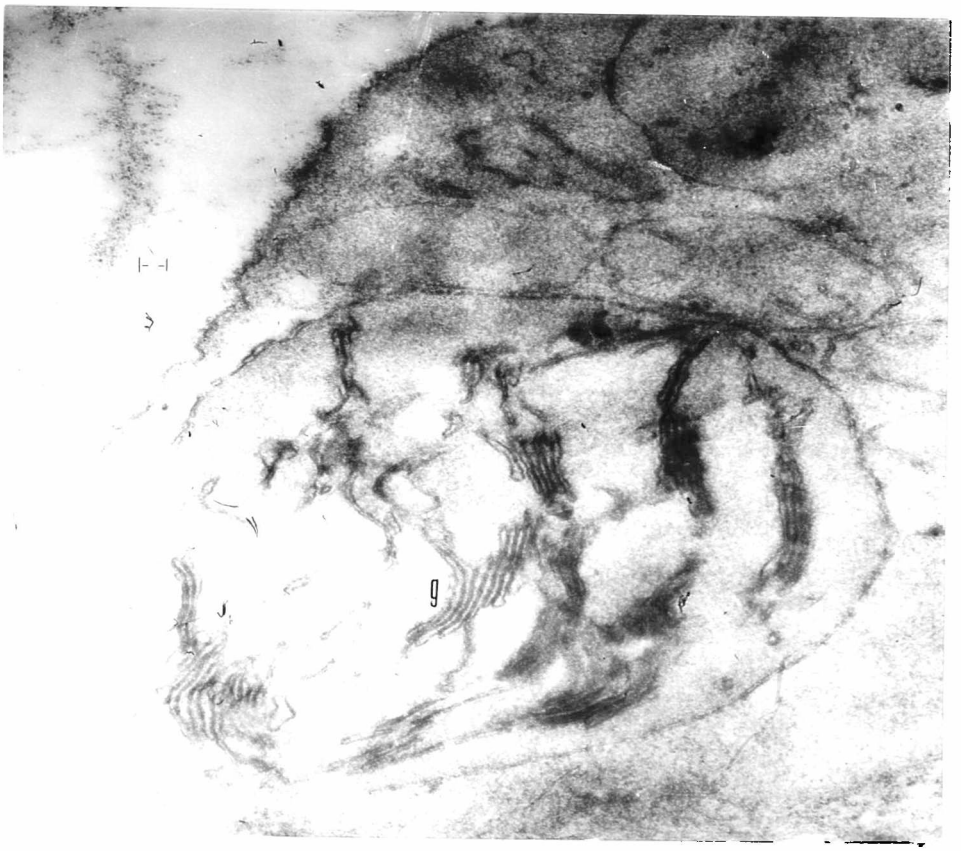


FIGURE 24. TWO DAY OLD ALBINO PLANT [x 37,500]. PLASTID CONTAINING GRANA. g=grana. Scale = 1000 A°



However, very few of these normal plastids were observed. Usually only 8% of the cells contained these normal plastids and only 1 or 2 such plastids occurred in a cell. Approximately the same number of grana per plastid were observed in these plastids (15-16 in the albino as compared to 19 in the green strain). The number of discs per grana was approximately the same as the green strain plastids, both having a range of 2 to 12 per grana. Similarly, grana width was comparable, both having a range of 1500 to 3000 A°. However, the grana were usually not as long as the green strain plastids (2500 to 4000 A°). Thus, although grana containing plastids were observed in the albino plants at the two day time period, these plastids differed in number per cell and in having a shorter grana length.

b) The nine day old plant

After nine days growth, only the first type of plastid was observed (Figure 25). These plastids had increased to the same size as the plastids of the green strain (4 microns) but had developed an internal structure entirely different from the normal green strain. They contained large vesicles of an average diameter of 7000 A° (Table 8) which varied in shape from round to irregular. Each plastid contained a few of these large vesicles ranging from 1 to 10 in number (Figures 25, 26, & 28). Often the vesicles originated from the inner lining of the double membrane (Figure 27) and arranged themselves in loop-like formations. These vesicles were often found in the center of the plastid accompanied by the small vesicles (300 A°) observed in the two day old

FIGURE 25. NINE DAY OLD ALBINO PLANT [x 30,000]. PLASTID CONTAINING A FEW VESICLES. v=vesicle. Scale = 1 micron

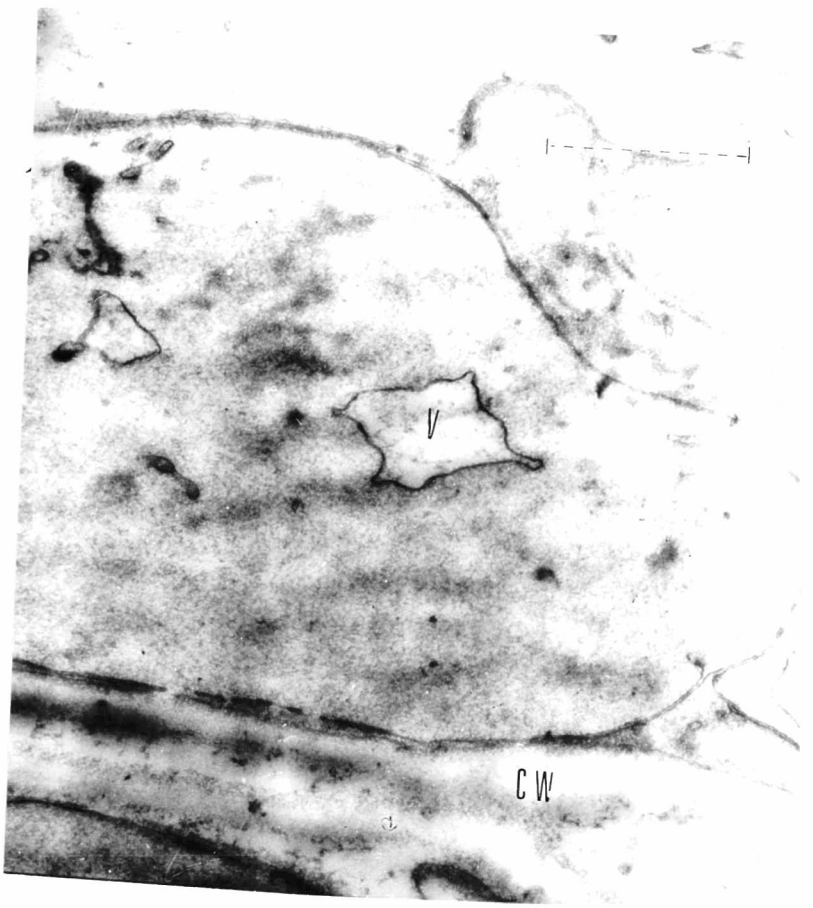

V
CW

TABLE 8. MEASUREMENTS OF THE PLASTIDS OF THE NINE DAY OLD ALBINO PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (8)
Nine-day old Albino Plant

Plastid Size in Microns	Number of vesicles per plastid	Size of vesicles in Angstroms
3	7	4000
4		9000
3.5	10	1000
2.5	6	
1.25		3000
5	4	1000
3	2	1000
	5	500
4	1	
5	1	
	3	8000
	4	

FIGURE 26. NINE DAY OLD ALBINO PLANT [x 25,000]. PLASTID CONTAINING VESICLES. v=vesicle. Scale = 1 micron

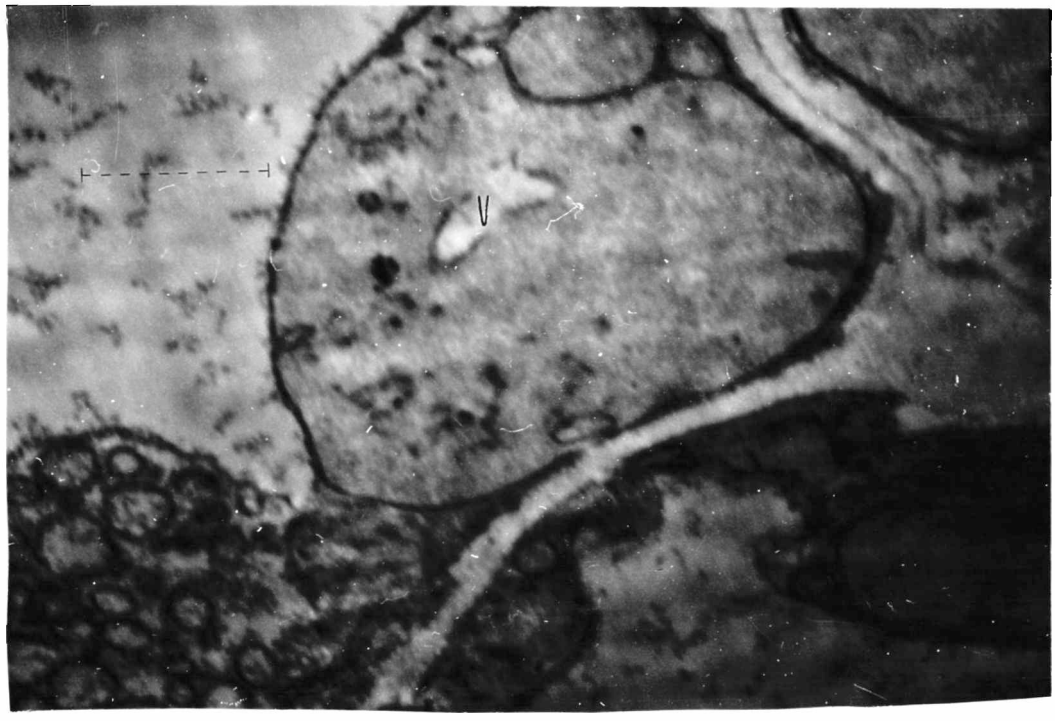

V

FIGURE 27. NINE DAY OLD ALBINO PLANT [x 30,000]. v=vesicle. Scale = 1 micron

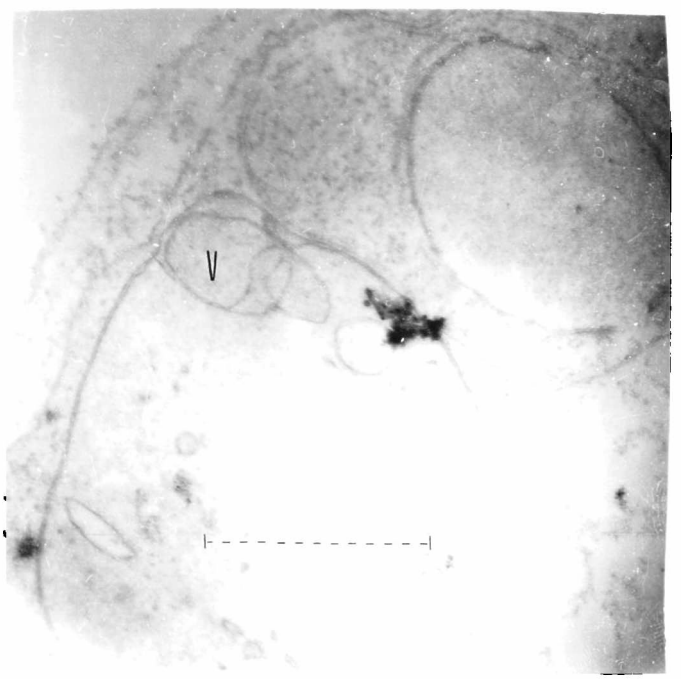
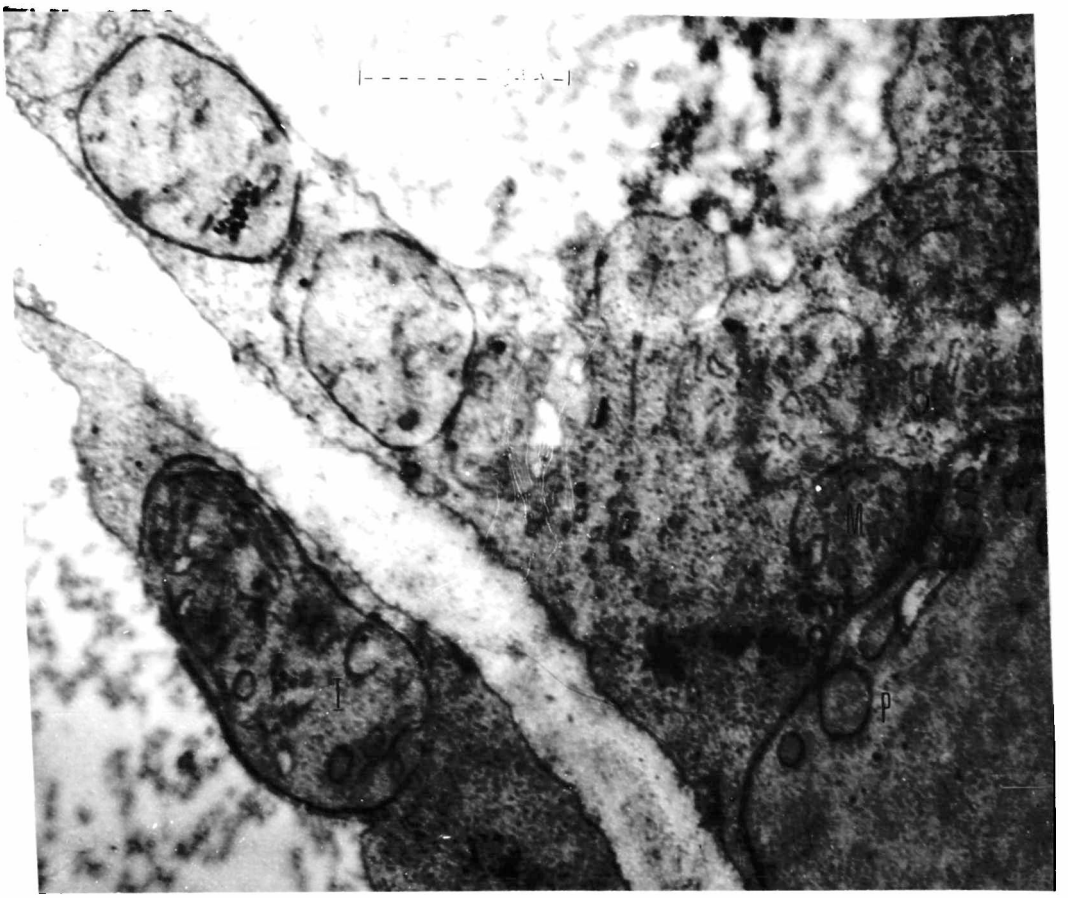

V

FIGURE 28. NINE DAY OLD ALBINO PLANT [x 28,000]. p=plastid. m=mitochondrion. i=organelles intermediate between mitochondria and plastids. Scale = 1 micron


I
III
P

albino plastids. Plastids showing a shape and size intermediate between mitochondria and the vesicular plastids were observed (Figure 28). The large vesicles were apparently aggregating in certain areas of these plastids. It is possible that the grana containing plastids observed in the two day old albino plant had degenerated, giving rise to the plastids containing the large vesicles.

c) The 16 day old albino plant

The plastids at this stage had not increased in size (Table 9) and were smaller than the plastids of the normal green strain of the same age. Again, no grana containing plastids were observed at this stage. The number of vesicles inside the plastids was approximately the same as the 9 day old albino (Figures 29 & 30). However, the diameter of the vesicles had greatly increased to 9000 A° (ranging from 6-13,000 A°). The smaller vesicles of 300 A° were more numerous than at the 9 day stage.

d) The 23 day old albino plant

The abnormal plastids had increased in size to 4 microns (Table 10), but were still smaller than the plastids of the green strain. Usually, few plastids were seen per cell, and the largest component of the cells were the large vacuoles (Figure 31). The number of vesicles per plastid had greatly increased to an average of 13 (Figures 32, 33, 34, & 35). The size of these vesicles was approximately the same as that of 16 day old albino plastids. The smaller vesicles (300 A°) were also numerous, suggesting that the predominant stage of development from 9 to 16 days was the proliferation of large vesicles. The shape of the plastids was

TABLE 9. MEASUREMENTS OF THE PLASTIDS OF THE SIXTEEN DAY OLD ALBINO PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (9)
Sixteen-day old Albino Plant

Size of Plastid in Microns	Number of large vesicles	Size of vesicles in Angstroms
2.7	7	10,000
3.0	7	6,000
2.3	5	6,000
	6	7,000
3.8	4	12,000
3.8	2	13,000
4.0	2	12,000
	3	10,000
	4	
2.5	4	9,000
2.0	5	
3.0	0	
	0	

FIGURE 29. SIXTEEN DAY OLD ALBINO PLANT [x 12,500]. PLASTIDS CONTAINING VESICLES (v). vac=vacuole. Scale = 1 micron

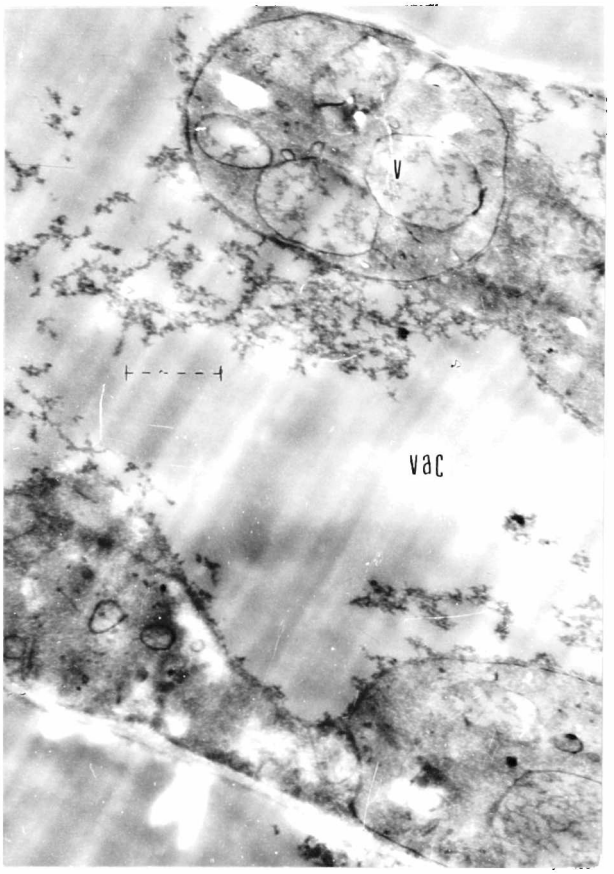

V
vac

FIGURE 30. SIXTEEN DAY OLD ALBINO PLANT [x 13,500]. Scale = 1 micron

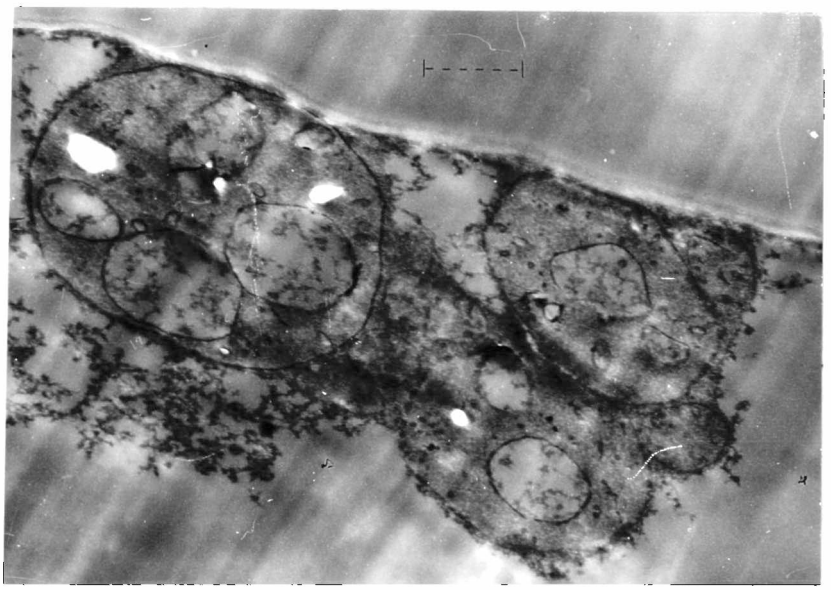


TABLE 10. MEASUREMENTS OF THE PLASTIDS OF THE TWENTY-THREE DAY OLD ALBINO PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (10)
Twenty-three day old Albino Plant

Size of Plastid in Microns	Number of Large vesicles	Size of Vesicles in Angstroms
3	2	5000
		4000
5	29	5000
5		5000
6		5000
5		5000
4.5	5	2500
3.5	11	3000
4	12	5000
5	20	8000
		9000
		7000
		9000
		13,000
		5000
		10,000
		10,000
		6000
		8000

FIGURE 31. TWENTY-THREE DAY OLD ALBINO PLANT [x 9,750]. CELL CONTAINING A LARGE CENTRAL VACUOLE AND AN ABERRANT PLASTID. vac=vacuole. p=plastid. Scale = 1 micron

FIGURE 32. TWENTY-THREE DAY OLD ALBINO PLANT [x 7000]. CELL CONTAINING TWO ABERRANT PLASTIDS WITH VESICLES. Scale = 1 micron


p
vac

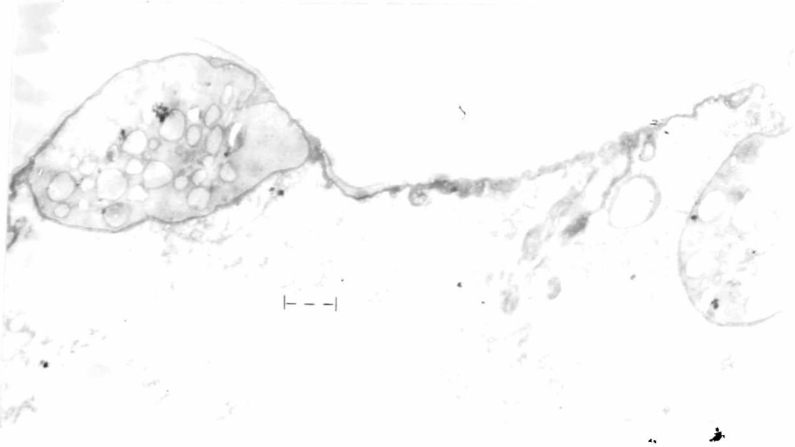
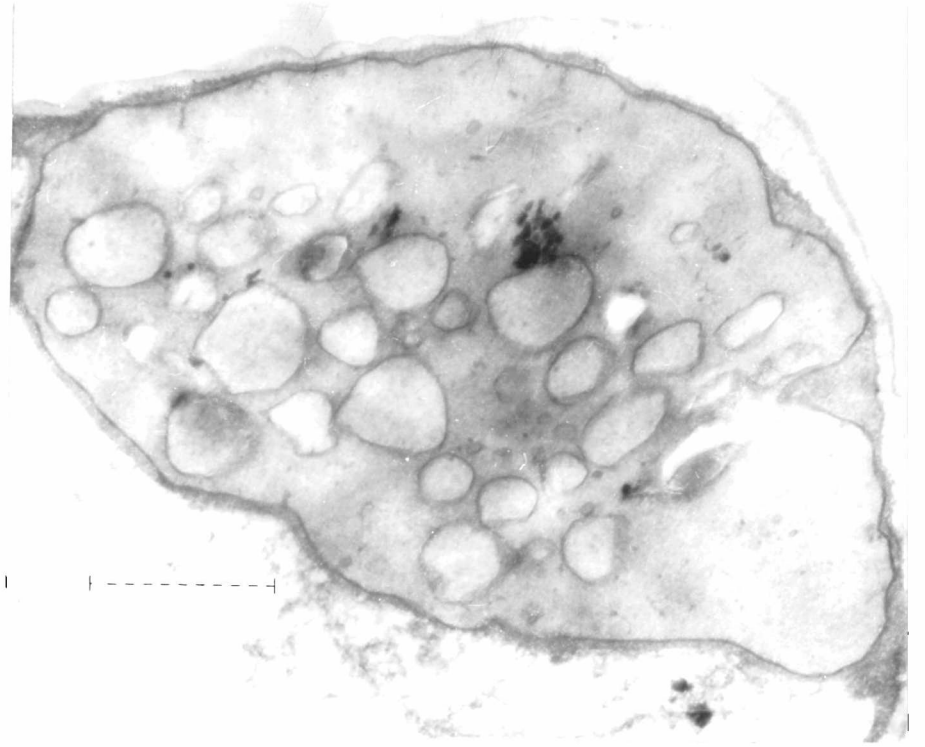


FIGURE 33. TWENTY-THREE DAY OLD ALBINO PLANT [x 24,000]. PLASTID WITH VESICLES. Scale = 1 micron

FIGURE 34. TWENTY-THREE DAY OLD ALBINO PLANT [x 16,000]. PLASTIDS AND CIRCULAR MITOCHONDRIA (m). Scale = 1 micron



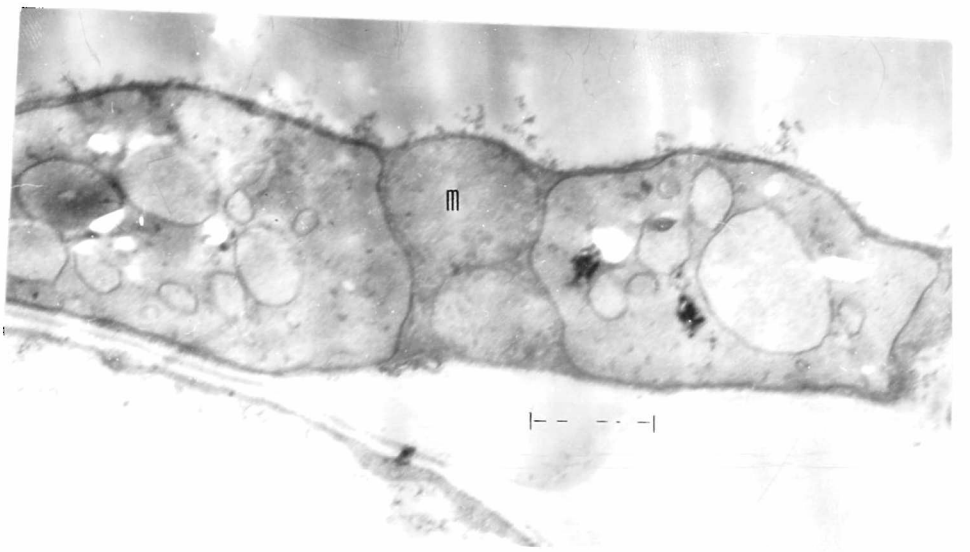
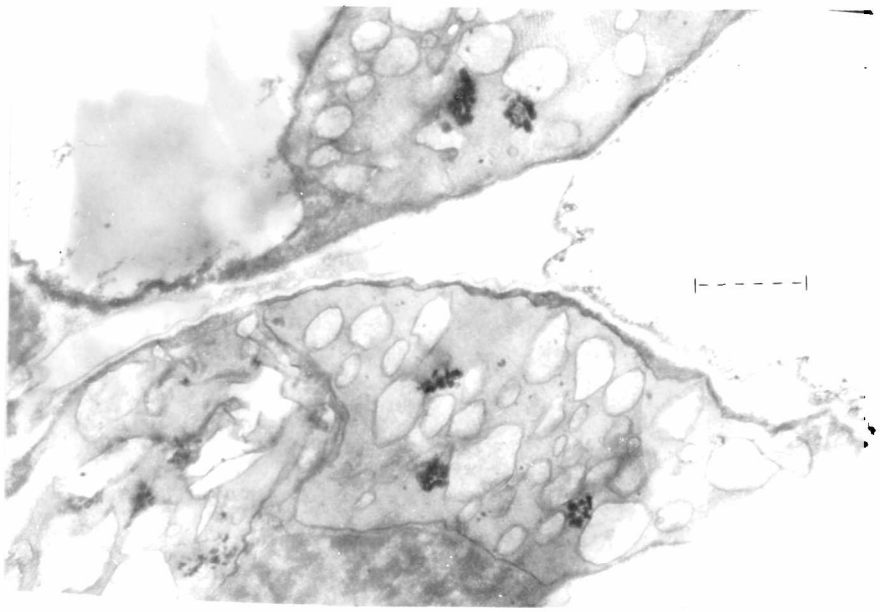

m

FIGURE 35. TWENTY-THREE DAY OLD ALBINO PLANT [× 15,000]. Scale = 1 micron



abnormal, ranging from circular to triangular. The mitochondria were observed to have an abnormal shape (circular) and a reduced number of cristae (Figure 34).

e) The 30 day old albino plants containing green patches

When the green patches on the adult leaves of the albino in culture were examined, they were found to contain two types of plastids. (The measurements of both plastids are given in Table (11) and summarized in Table (12).) The first type resembled the plastids found in the cotyledons of the albino as they contained vesicles (Figures 36, 37). They were smaller in size (3.8 microns) than the plastids found in the normal green strain (5 microns). The average number of vesicles per plastid was only 2, but the range varied from 1 to 9. They therefore had fewer vesicles than the plastids of the 23 day old albino cotyledons, which had developed 13 vesicles per plastid. The size of the vesicles was the largest observed in this study (22,000 A° compared to the largest observed in the 16 day old albino cotyledon of 9000 A°). Many small vesicles (300 A°) resembling prolamellar bodies of etioplasts were also present in these plastids. These prolamellar bodies were observed to flatten out into sacs and fuse to give a lamellae-like structure (Figure 38). These vesicle-containing plastids were observed to be the only type of plastid in some cells (Figure 36).

However, in some cells both vesicle-containing plastids and the second type of plastid (those containing grana) were observed (Figure 39). Such mixed cells have been found in other albinos, eg. maize (28),

TABLE 11. MEASUREMENTS OF THE PLASTIDS OF THE GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT. THESE MEASUREMENTS WERE TAKEN FROM DIFFERENT MICROGRAPHS AND DO NOT CORRESPOND HORIZONTALLY.

Table (11)
Green Patches of the Thirty-day old Albino Plant

Vesicle-containing plastids			Grana-containing plastids				
Size of Plastid in Microns	Number of vesicles	Size of Vesicles in Angstroms	Size of plastid in Microns	Number of grana per plastid	Number of discs per granum	Width of grana in Angstroms	Length of grana in Angstroms
4.0	2	28,000	4	20	6	2,500	7500
6.6	1	15,000	3		11	2,000	8000
3.3	1	30,000	4	21	14	1,000	3000
3.3	9	28,000	3.3		7	1,000	7500
2.0	2	25,000	3.3		7	2,000	1100
					8	2,000	6000
					7	1,000	9000
3.5	1	20,000			5	3,000	9000
3.5	1	10,000			4	3,300	7500
					6	3,000	7500
					5	1,300	7500
3.3	1	20,000			8	2,000	8000
		15,000			9	2,500	8000
		30,000			5	2,000	8500
					4	1,000	10000
					12		
					13		
					7		

TABLE 12. SUMMARY OF THE MEASUREMENTS OF THE PLASTIDS OF THE THIRTY DAY OLD ALBINO PLANT.

Table (12)
Summary of Data of the Green Patches of
The Thirty-day Old Albino Plant

Age in days:	Plastids containing vesicles	Thirty-day Plastids Containing grana
Plastid Size in Microns:	3.8	3.5
Number of Vesicles per Plastid:	2 1-9	--
Size of Vesicles in Angstroms:	22,000	--
Number of Grana per Plastid:	--	21
Lamellae	Prolamellae	Prolamellae
Grana length In Angstroms:	--	6,600
Grana width In Angstroms:	--	1,900
Number of discs Per granum:	--	7 (2-12)

FIGURE 36. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 9,750]. CELLS CONTAINING ONLY PLASTIDS WITH VESICLES (v). Scale = 1 micron

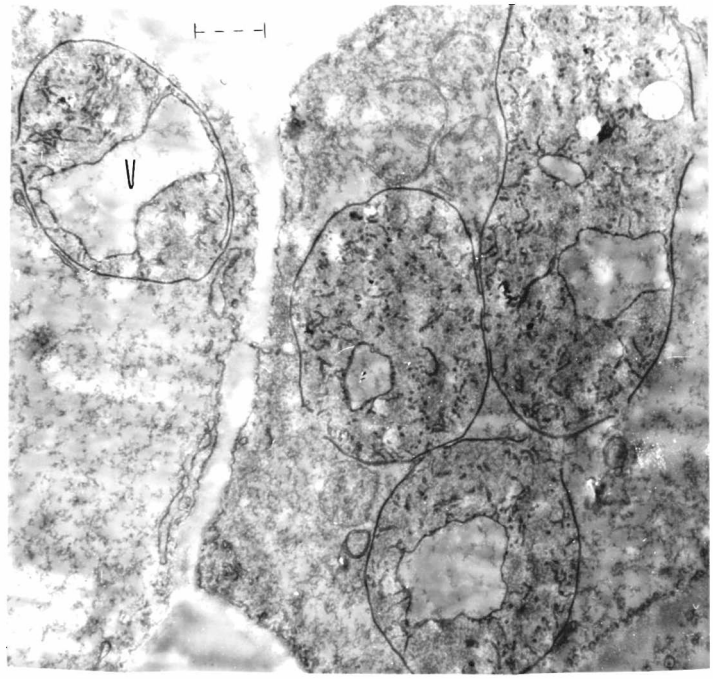

V

FIGURE 37. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 37,500]. FUSION OF PROLAMELLAR LIKE BODIES TO FORM VESICLES. f=fusion. Scale = 1 micron

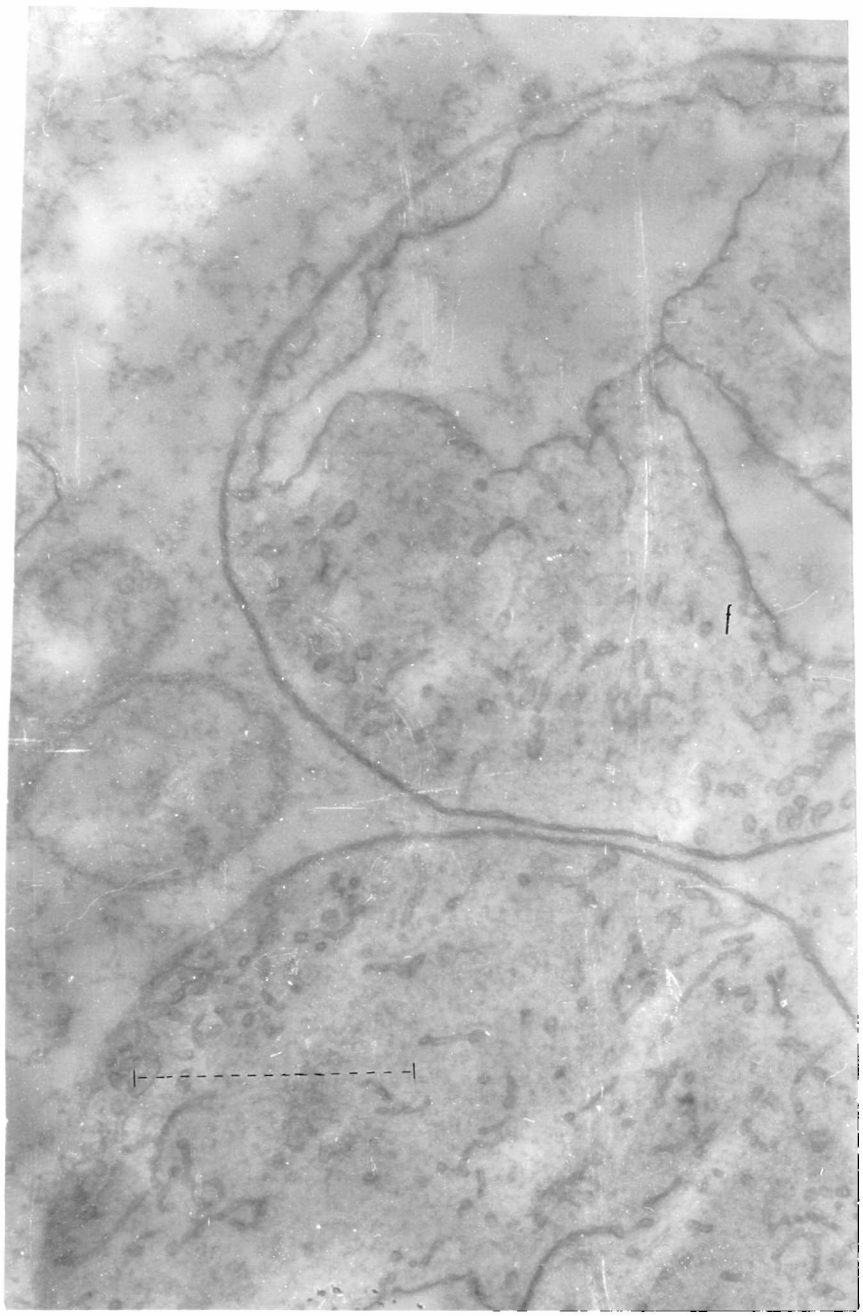
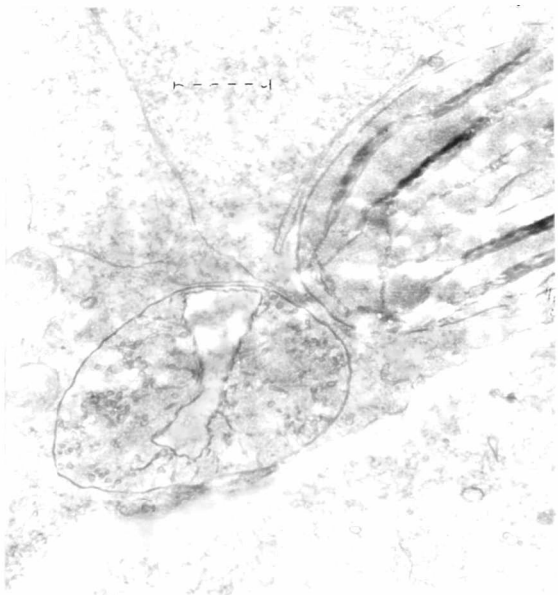


FIGURE 38. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 27,500]. PLASTID WITH FLATTENED VESICLE (v) FORMING LAMELLAE.
Scale = 1 micron

FIGURE 39. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 11,000]. CELL CONTAINING PLASTIDS WITH GRANA AND VESICLES.
Scale = 1 micron


V

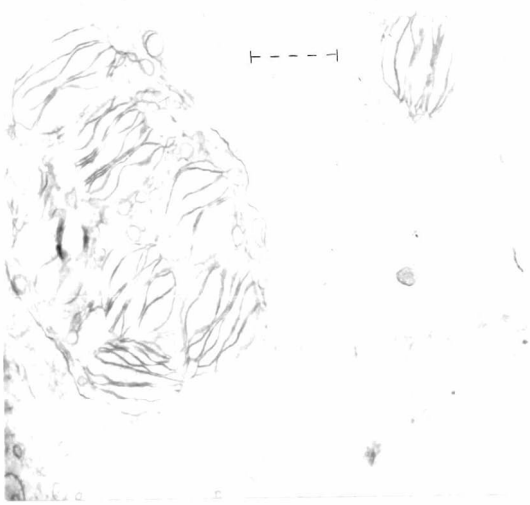


tobacco (43), and hybrid clover (4).

The grana-containing plastids were the same size as the vesicular plastids, but smaller than those of the normal green strain (Figure 39). There were fewer grana-containing plastids than vescile-containing plastids; on an average, there were two per cell. They sometimes contained prolamellar bodies which fused to give lamellae (Figure 40, 41), suggesting that many of the vesicle-containing plastids are earlier stages of development in the formation of the grana-containing plastids. Plastids containing unusual grana were observed in these green patches (Figures 40, 41). Here the discs were extremely large (6600 A° compared to the largest length, 6000 A°, ever reported for the normal green strain). In these plastids (Figures 40, 41), small vesicles were observed to fuse to form very wide discs. Apparently, certain factors which cause the normal formation of narrow lamellae and small vesicles is not functioning correctly, resulting in the formation of large vesicles, lamellae and discs. Some plastids had developed grana identical to those of the green strain (Figures 42, 43). The measurements of the grana, however, were not identical with that of any stage of the normal green strain. They had approximately the same number of discs and slightly less grana width than the two day old normal green strain. However, the grana length was larger than any recorded for the normal green strain. Apparently the grana-containing plastids of the green patches were comparable to those of the two day old normal green strain except that they were abnormally long. The measurements of the albino are summarized in Table (13).

FIGURE 40. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 11,000]. GRANA-CONTAINING PLASTID. Scale = 1 micron

FIGURE 41. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 19,500]. GRANA-CONTAINING PLASTID. Scale = 1 micron



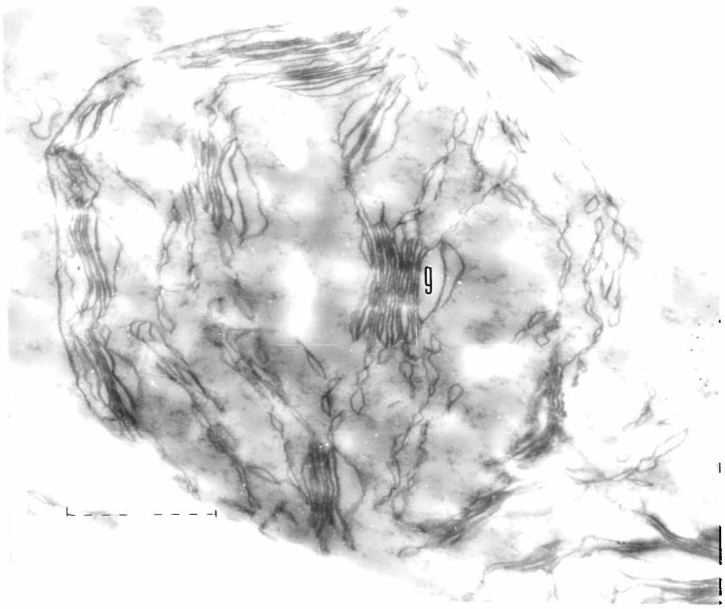

g

FIGURE 42. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [x 21,000]. PLASTIDS CONTAINING NORMAL GRANA. Scale = 1 micron

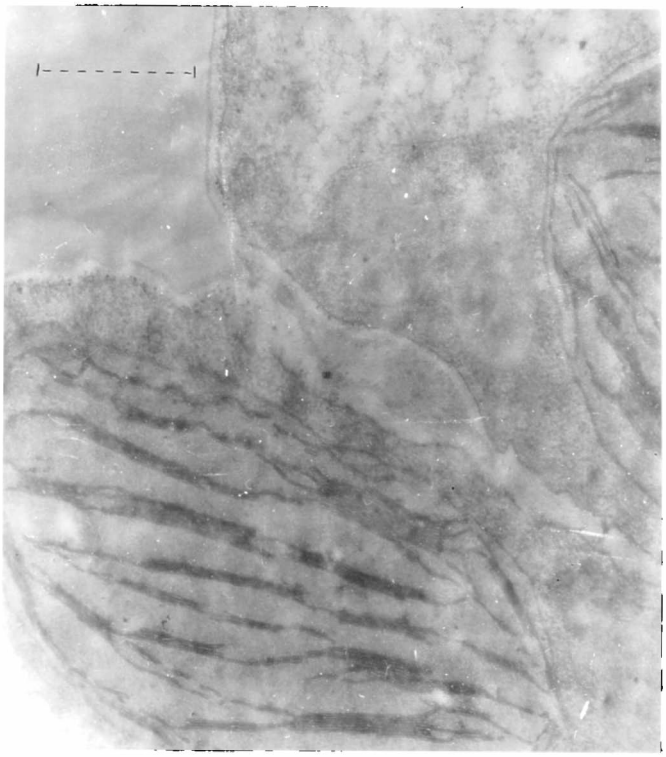


FIGURE 43. GREEN PATCHES OF THE THIRTY DAY OLD ALBINO PLANT [× 60,000]. Scale = 1 micron

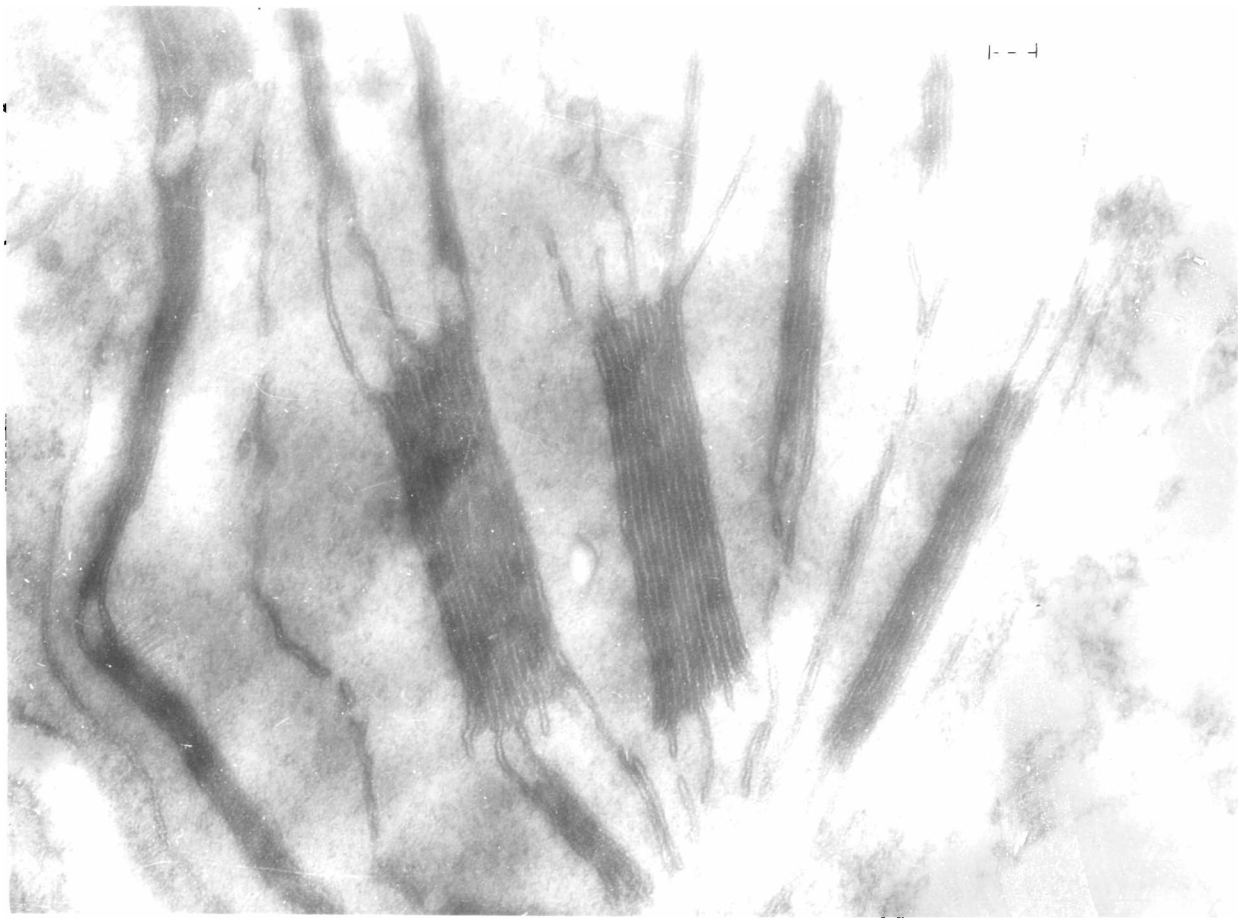


TABLE 13. SUMMARY OF THE MEASUREMENTS OF THE PLASTIDS OF THE ALBINO PLANT THROUGHOUT THE CULTURE PERIOD.

Table 13
"Plastid Changes During Development of the Albino Plant"

Age in days:	2		9	16	23
	(a)	(b)			
Number of plastids per cell:	8 (6-11)	2	--	6	3
Plastid size in microns:	2 (0.5-3.0)	4 (3-5)	4 (2.5-5.0)	3 (2-4.0)	4 (3-6)
Number of vesicles per plastid:	1	1 in 5 plastids	4 (1-10)	3.7 (0-7)	13
Size of vesicles in Angstroms:	3000-7000	4000	7000 (5-10,000)	9000 (6-13,000)	6000 4-14,000
Number of grana per plastid:	--	15-16	--	--	--
Lamellae:	Pro-lamellae	Pro-lamellae	Pro-lamellae	Pro-lamellae	Pro-lamellae
Grana length in Angstroms:	--	2,500	--	--	--
Grana width in Angstroms:	--	2,200	--	--	--
Number of discs per granum:	--	7 (2-12)	--	--	--

a) -- measurement of vesicle-containing plastids
b) -- measurement of grana-containing plastids

DISCUSSION

Highly isogenic strains of N. tabacum possessing genotypes for chlorophyll synthesis were grown in liquid and solid MUK medium. Growth was found to be greater in liquid than in solid medium. Other investigators have reported similar findings in that liquid medium allowed greater growth of plant cells in tissue culture than in solid media (30). Maximum growth of both strains was observed in modified liquid Hoagland's medium. This was to be expected as Hoagland's was designed primarily for mature plants, whereas MUK was designed to give maximum growth of plant cells in tissue culture. Under these optimum conditions, the albino developed 10 leaves within 8 weeks compared to 12 leaves in 25 weeks in solid media, as reported by Venketeswaran (31). Therefore, this study has established a reproducible method of growing albino plants under optimum conditions using asceptic techniques.

The green plant grown under these conditions was able to develop a photosynthetic capacity comparable to that of mature plants grown in the greenhouse (13). The albino developed only a very small photosynthetic capacity. Therefore it was dependent on the exogenous supply of sucrose for its development. This was reflected in the slower growth rate of the albino as compared to the green. The slower growth rate was observed in all media.

The general pattern of biochemical changes in the green plant during development was similar to that reported for other higher plants (22). The high initial contents of all chemical constituents

showed a large decrease with time. However, only the DNA content and dry weight showed an increase after the initial decrease as reported by other authors (22). The plants used for biochemical analyses were grown on a solid agar medium, under which conditions optimum growth is not achieved. This could therefore be responsible for the nonappearance of a later increase in RNA and protein content.

The albino had large amounts of dry weight and protein than the green plant immediately after germination. However, the albino followed the same general pattern as the green plant of decrease in dry weight and protein content during development. Presumably the dry weight material and protein were catabolized to the same extent as the green plants, as they resulted in comparable final amounts at the end of the culture period. Redei (24) reported that white areas of a variegated mutant of Arabidopsis had less protein than the wild green type. However, he did not specify what age the plants were. This study has revealed that the relationship of protein content between the green and albino plants was different at different times of development. Therefore, Redei's (24) figures could not be used for comparison. The nature of the excess protein in the two-day old albino was not determined here. No references to any single protein affecting albinism has been reported in the literature.

The albino showed a completely different pattern of DNA metabolism during development to that of the green. The initial amount of DNA was lower than that of the green and remained unchanged until the 9th day.

The albino then synthesized large amounts of DNA which remained almost unchanged at the end of the culture period. No measurements of DNA content of other albinos have been reported.

The albino showed a normal pattern of initial degradation of RNA but at a smaller rate during the first 16 days of development. After the burst in DNA synthesis at 16 days a large rise in RNA synthesis was observed at 23 days. It is possible that the RNA was DNA dependent messenger RNA. Future experiments using Actinomycin D in the medium and observing whether the RNA synthesis at this point was inhibited would determine this. No measurements of RNA content of other albinos have been reported in the literature, but Redei (24) reported an increase in ribonuclease activity in a mutant of Arabidopsis. His method of determining ribonuclease activity involved measuring the absorption of ribonucleotides formed after degradation of yeast RNA by the enzyme extract. The method of analyses used in this study involved the hydrolysis of RNA by sodium hydroxide to ribonucleotides and estimation of purine-bound pentoses by the orcinol test. It is possible that the orcinol was reacting with ribonucleotides which had been broken down by a ribonuclease prior to hydrolysis by the sodium hydroxide. The albino studied here could therefore have a high ribonuclease activity. However, as the RNA rise was preceded by DNA synthesis it was more probable that the orcinol was detecting a true rise in RNA content and not the result of ribonuclease activity.

The green plant showed a normal pattern of plastid differentiation

as reported by Wettstein (40) (41) for other higher plants. The growth consisted of increased size, number of grana per plastid, grana length, and grana width.

The albino was able to develop normal plastids in the cotyledons after germination. However, such normal plastids were smaller in all respects and very few in number. The albino cotyledons contained only 9% chlorophyll of the green plant as reported by Mahlberg and Venketeswaran (19). Therefore, only a few normal plastids would be expected to occur as Wettstein has established that chlorophyll synthesis is necessary for grana production (41). Chlorophyll has been demonstrated to be located in the grana and Weier (39) had suggested that chlorophyll was added to the granal subunits after the basic subunit structure was laid down. Apparently the albino could form the same subunit structure but insufficient chlorophyll was present to complete the granal structure in the majority of the plastids.

The disappearance of normal plastids in the albino after the two-day period was probably due to degeneration of the existing normal plastids, under the influence of some factor. Yoshida (45) demonstrated that plastids degenerated when left in close proximity to the nucleus during plasmolysis. If Actinomycin D were added to the cell, the plastids did not degenerate, suggesting that the nucleus was producing a messenger RNA that destroyed the plastids. He observed that plastid degeneration did not occur when the plastids were isolated from the nucleus. This factor, causing plastid degeneration, could be the high concentration of

RNA observed in this albino tobacco at all times after the two-day time interval.

The formation of vesicles in plastids of chlorophyll mutants have been recorded in iojap maize (28), barley (Gateway variety) (18), tobacco (43), hops (43), and sunflower(35). None of these investigators studied the nucleic acids or protein content of these mutants. The formation of vesicles in the absence of chlorophyll appears to be a common property of the plastid membranes. The barley (18) and sunflower (35) mutants were shown to develop normal grana and plastids later in development. This occurs after a spontaneous synthesis of chlorophyll in the barley, but in the carotene-deficient sunflower, dark conditions were required to prevent the photo-oxidation of chlorophyll. The present study showed that in this tobacco mutant, normal grana containing plastids formed in both germinating cotyledons and in the green patches of the adult albino leaves. The formation of grana was slightly disturbed as the grana length was abnormally high. This would be in agreement with other investigations that normal grana formation in albino plants can occur when clorophyll synthesis occurs (18) (28).

The factors causing chlorophyll synthesis in the green patches were not investigated here. However, this study established that normal grana and plastids can form in the albino whenever the effect of the mutation at the gene for chlorophyll synthesis is removed.

In conclusion, the green plant was found to follow similar biochemical and plastid changes during development as those reported for

other higher plants. The albino was found to show a reduced rate of growth under all conditions. Although the same overall pattern of development was observed for the albino with respect to protein and dry weight, the nucleic acid metabolism was abnormal. The DNA showed a large increase at the same time as the appearance of the third leaf of the albino which contained green patches. At this stage of green patch formation, the abnormal pattern of plastid development was not followed in these areas and normal plastids with distinct grana were observed. Therefore the disturbed nucleic acid metabolism and the abnormal plastid differentiation found in this mutant appear to be related with respect to time. Whether this nucleic acid disturbance was a cause or an effect of albinism remains to be determined.

REFERENCES

1. Appelquist, L. A., J. E. Boynton, K. W. Henningsen, P. K. Stumpf and D. v. Wettstein. 1968. Lipid biosynthesis in chloroplast mutants of barley. J. Lipid Res. 9:513-524.
2. Arnon, D. 1963. Photosynthetic phosphorylation and a uniform concept of photosynthesis. Proc. 5th Int. Congr. Biochem. Pergamon Press, Oxford. Vol. VI.
3. Bell, W. D., L. Bogorad, and W. J. Mcilrath. 1958. Response of the yellow strip maize mutant ys_1 to ferrous and ferric iron. Bot. Gaz. 120:36-39.
4. Bingham, E. T. 1968. Genetics and cytology of an albino in a diploid hybrid of Medicago sativa and M. falcata. Can. J. Genet. Cytol. 357:361.
5. Boynton, J. E. 1966. a) Chlorophyll deficient mutants in tomato requiring vitamin B_1. I. Genetics and Physiology. Hereditas, 56:171-199.
6. Clausen, R. E. and D. R. Cameron. 1950. Inheritance in Nicotiana tabacum XXIII. Duplicate factors for chlorophyll production. Genetics 35:4-10.
7. Gaffron, H. 1960. Energy shortage: Photosynthesis in Plant Physiology. F. C. Steward (Ed.). Academic Press, New York. Vol. I B.
8. Goodchild, D. J., H. R. Highkin, and N. K. Boardman. 1966. The fine structure of chloroplasts in a barley mutant lacking chlorophyll b. Expt. Cell. Res. 43:684-688.

9. Habermann, H. M. Spectra of normal and pigment deficient mutant leaves of Helianthus annuus L. Physiol. Plantar. 13:718-725.

10. Heber, U. 1960. Vergleichende Untersuchungen an Chloroplasten die durch Isolierung-Operationen in nicht-wassrigen erhalten wurden. I. Ermittlung des Verhältnisses von Chloroplastenprotein zum Gesamtprotein der Blaltzelle. Z. Naturforsch. 15:95-99.

11. Hoagland, D. R. and D. I. Arnon. 1938. The water culture method for growing plants without soil. Univ. Calif. Agric. Expt. Stat. Circ. 347.

12. Kupke, D. W. 1962. Correlation of a soluble leaf protein with chlorophyll accumulation. J. Biol. Chem. 237:32-87.

13. Laetsch, W. M. and D. A. Stetler. 1965. Chloroplast structure and function in cultured tobacco tissue. Am. J. Bot. 52:798-804.

14. Langridge, J. 1955. Biochemical mutations in the crucifer Arabidopsis thaliana (L). Heynh. Nature. 176-260.

15. Levine, R. P. and W. T. Ebersold. 1960. The genetics and cytology of Chlamydomonas. Ann. Rev. Microbiol. 197-214.

16. Leyon, H. 1953. In the Plastids. J.T.O. Kird and R.A.E. Tilney-Bassett. W. H. Freeman and Company, Ltd. 1967. 30pp.

17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Bio. Chem. 193:265-275.

18. Maclachlan, S. and S. Zalik. 1963. Plastid structure, chlorophyll concentrations and free amino acid composition of a chlorophyll mutant of barley. Can. J. Bot. VI. 1053-1062.

19. Mahlberg, P. G. and S. Venketeswaran. 1966. Pigment analyses of normal and proliferated genetical strains of Nicotiana under cultural conditions. Bot. Gaz. 127 (2-3):114-119.

20. Mollenhaur, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39:111-114.

21. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantar. 15:473-497.

22. Olsson, R. and D. Boulter. 1968. Nucleic acid metabolism of Vicia Faba during germination and growth. Physiol. Plantar. 21:428-434.

23. Ongun, A., W. E. Thomson, and J. B. Mudd. 1968. Lipid fixation of chloroplasts for electron microscopy. J. Lipid Res. 9:416-424.

24. Redei, G. P. 1967. Biochemical aspects of a genetically determined variegation in Arabidopsis. Genetics. 56:431-443.

25. Ris, H. and W. Plaut. 1962. In the Plastids. J.T.O. Kirk and R.A.E. Tilney-Bassett. W. H. Freeman and Company, Ltd. 1967. p. 305.

26. Robertson, D. S., M. D. Bachman, and I. C. Anderson. 1966. Biochemical and plastid alterations in allele mutant of the w_3 locus in maize. Genetics. 54:357-358.

27. Shin, M., K. Tagawa, and D. J. Arnon. 1963. Crystallization of ferredoxin-TPN reductase and its role in the photosynthetic apparatus of chloroplasts. Biochem. Zeitschr. 338:84-96.

28. Shumway, L. K. and T. Weier. 1967. The chloroplast structure of iojap maize. Am. J. Bot. 54:773-780.

29. Veleminsky, J. and G. Röbbelen. 1966. Beziehungen zwischen Chlorophyllgehalt und Chloroplastenstruktur in einer Chlorina-Mutante von Arabidopsis thaliana (L) Heynh. Planta. 68:15-35.

30. Venketeswaran, S. 1962. Tissue culture studies on Vicia faba L. 1) Establishment of culture. Phytomorphology. 12:3-9.

31. Venketeswaran, S. 1965. "In vitro" studies on chlorophyll instability of an albino mutant of tobacco. Proc. Symp. Mutation Process. Prague. Akademia Press. pp. 225-230.

32. Venketeswaran, S. and P. G. Mahlberg. 1962. Proliferation of albino and pigmented genetical strains of Nicotiana in tissue culture. Physiol. Plantarum. 15:639-648.

33. Wallace, R. H. and H. M. Habermann. 1959. Genetic history and general comparisons of two albino mutations in Helianthus annuus. Am. J. Bot. 46:157-162.

34. Walles, B. 1963. Macromolecular physiology of plastids. IV. On amino acid requirements of lethal chloroplast mutants in barley. Hereditas. 50:317-344.

35. Walles, B. 1965. Plastid structures of caretenoid deficient mutants of sunflower (Helianthus annuus). I. The white mutant. Hereditas. 53:247-256.

36. Walles, B. 1967. (b). The homozygous and heterozygous effects of an aurea mutation on plastid development in spruce. Stud. For Suec. 60:1-20.

37. Webb, J. M. and R. S. Levy. 1955. A sensitive method for the determination of DNA in tissues of microorganisms. J. Biol. Chem. 21:107-117.

38. Weier, T. E., A.H.P. Engelbrecht, A. Harrison, and E. B. Risley. 1965. Subunits in the membranes of chloroplasts of Phaseolus vulgaris, Pisum sativum, and Aspidistra sp. J. Ultrastruct. Res. 3:92-111.

39. Weier, T. E. and A. E. Bensen. 1967. The molecular organization of chloroplast membranes. Am. J. Bot. 4:389-402.

40. Wettstein, D. v. 1957. Genetics and the submicroscopic cytology of plastids. Hereditas. 43:303-317.

41. Wettstein, D. v. 1958. The formation of plastid structure. Brookhaven Symposium Biol. 11:138-139.

42. Wettstein, D. v. and A. Kahn. 1960. Macromolecular physiology of plastids. Proc. Eur. Reg. Conf. on Electron Microscopy. Delft Vol. II. 1051-1054.

43. Wettstein, D. v. 1961. Nuclear and cytoplasmic factors in development of chloroplast structure and function. Can. J. Bot. 39:1537-1545.

44. Wettstein, D. v. and G. Eriksson. 1964. The genetics of chloroplasts. In Genetics Today. Pergamon Press (S. J. Geerts) Ed. Vol. III. 391-612.

45. Yoshida, Y. 1961. Nuclear control of chloroplast activity in Elodea leaf cells. Protoplasma. 54:476.