The effects of certain environmental factors upon the phenotypic expression of a single-gene mutation, pale-yellow-1, of maize have been described (13). In brief, the observations showed that the mutant seedling is initially uniform pale-yellow in color when grown in either light or darkness. However, when exposed to light at 25°C the mutant seedlings become green in color approximately 8 days after planting. Plastid size and number, type of culture media used, length of previous exposure to light and the presence of the endosperm are not factors in this delayed appearance of pigments.

This paper will present quantitative information on the pigment content and pigment changes which occur in the mutant, pale-yellow-1, of maize. In particular, an attempt will be made to establish the relationship of the mutant gene to the accumulation of specific carotenoid pigments and the chlorophylls. This will be done in order to determine the basis for the differences in color between the mutant and its normal sibling (18). In addition, it is hoped that some information about the biochemical relationship of the carotenoids to the chlorophylls will be obtained from these observations.

**METHODS**

The plants used in this study were germinated from seeds which were found to produce seedlings in a ratio of 3 normals to 1 mutant. All seedlings were grown in a constant temperature chamber at 25 ± 0.5°C. Light (700 ft-l) was supplied by 13 slimline fluorescent lamps labeled 4500° white. A description of the culture methods, light source and the constant temperature chamber has been reported (13).

Samples of leaf material were analyzed for flavoxanthin c, violaxanthin b, lutein, beta carotene, neo-beta carotene, chlorophyll a, chlorophyll b, and protochlorophyll a. Neo-beta carotene and beta carotene were the only carotenes found in the leaf material and flavoxanthin c, violaxanthin b, and lutein accounted for over 90% of the total carotenol content. A detailed description of the methods used to make the pigment analyses has been presented (11). In brief, the analyses were made as follows. Fifteen leaves were removed from 15 different plants and divided into three samples of 5 leaves each. One leaf punch was taken from the distal 5 cm of each leaf with a 7 × 52-mm steel die and each sample of 5 leaf punches was analyzed for carotenoids or chlorophylls. When the samples were analyzed for carotenoids, the pigments were extracted with methanol, the chlorophylls saponified and the carotenoids transferred to a mixture of ethyl ether and petroleum ether. The carotenols were chromatographically resolved into individual components on a magnesia column, while the carotenes passed through the column and were recovered as a binary mixture in the percolate. Samples analyzed for chlorophylls were extracted with methanol and the chlorophylls transferred to petroleum ether. The chlorophylls were separated from the carotenoids and resolved into chlorophyll a and chlorophyll b by chromatographing the petroleum ether solution on a starch column. Protochlorophyll a was extracted from the leaf samples and chromatographically isolated by the method of Koski and Smith (12). The amounts of chlorophyll a, chlorophyll b, protochlorophyll a, lutein, violaxanthin b, and flavoxanthin c were calculated from the absorbance, at their respective maxima, and the specific absorption coefficients given by Zscheile and Comar (17), Strain (14) and Koski and Smith (12). The amounts of beta carotene and neo-beta carotene were calculated from the absorbance of the binary solution by the method of Beadle and Zscheile (2).

**EXPERIMENTAL AND RESULTS**

**Pigment Content of Dark-Grown Mutant and Normal Seedlings:** When grown at 25°C, 7-day-old dark-grown mutant seedlings are pale-yellow in color, while dark-grown normal seedlings are bright-yellow in color. This observation suggests that the concentration of carotenoids in the mutant seedlings is less than in the normal seedlings. However, it does not indicate how much less carotenoid is present and which carotenoid pigments are affected. Therefore, the pigment content of 7-day-old dark-grown mutant and normal seedlings was determined. The results are shown in table I. In the mutant seedlings the concentrations of flavoxanthin c, lutein, violaxanthin b, and protochlorophyll a are less than in the normal seedlings, while the concentrations of neo-beta carotene and beta carotene are the same in both types of seedlings.

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1 Received July 10, 1956.
2 Present address: Biochemistry Branch, U. S. Naval Radiological Defense Laboratory, San Francisco, California.
**TABLE I**

**Plastid Pigment Content of 7-Day-Old Light-Grown and Dark-Grown Mutant and Normal Corn Seedlings**

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Light-Grown Seedlings</th>
<th>Dark-Grown Seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Mutant</td>
</tr>
<tr>
<td></td>
<td>µg pigment/gm dry wt of leaf</td>
<td></td>
</tr>
<tr>
<td>Flavoxanthin c</td>
<td>110 ± 8.0*</td>
<td>62 ± 5.0</td>
</tr>
<tr>
<td>Lutein</td>
<td>432 ± 25.0</td>
<td>206 ± 9.0</td>
</tr>
<tr>
<td>Violaxanthin b</td>
<td>67 ± 6.0</td>
<td>42 ± 4.0</td>
</tr>
<tr>
<td>Beta Carotene</td>
<td>514 ± 20.0</td>
<td>74 ± 3.0</td>
</tr>
<tr>
<td>Neo-beta Carotene</td>
<td>126 ± 1.4</td>
<td>36 ± 1.0</td>
</tr>
<tr>
<td>Protochlorophyll a</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>5760 ± 180.0</td>
<td>0</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>840 ± 23.0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean plus-or-minus the standard error of the mean. The values are the means of three analyses. Each analysis was carried out on a pool of 5 leaf samples.

**Pigment Content of Light-Grown Mutant and Normal Seedlings:** When grown in the light at 25°C 7-day-old mutant seedlings are pale-yellow in color and their normal siblings are deep-green in color. The deep-green color of the normal sibling masks the color due to the carotenoids, making it impossible to visually compare the carotenoid content of the mutant to that of the normal seedlings. However, quantitative, chromatographic pigment analyses of both normal and mutant seedlings reveal that the carotenoid content, as well as the chlorophyll content, of the mutant is different from that of the normal seedling. The pigment content of 7-day-old mutant and normal seedlings, grown in the light at 25°C, is shown in table 1. As in dark-grown plants, the flavoxanthin c, lutein, and violaxanthin b content of the mutant seedlings is less than that of the normal seedlings. However, in contrast to the dark-grown material, the light-grown mutant plants also contain less neo-beta carotene and beta carotene than do the light-grown normal seedlings. The normal seedlings also contain large amounts of chlorophyll a and chlorophyll b, while these compounds are not detectable in the light-grown, 7-day-old mutant plants. It is also noteworthy that the amounts of carotenols found in both mutant and normal seedlings is less in light-exposed than in dark-grown plants. This decrease in the carotenol content in the light-exposed plants is due mainly to a large decrease in violaxanthin content.

**Pigment Changes in Dark-Grown and Light-Exposed Mutant and Normal Seedlings:** Mutant seedlings grown in the light at 25°C begin to turn green in color when they are about 8 days of age and soon they become phenotypically indistinguishable from the normal seedlings. If accumulation of the carotenoids, as well as the chlorophylls, is controlled by the mutant gene it would seem reasonable to expect the carotenoid content of the mutant seedlings, as well as the chlorophyll content, to increase when the seedlings are about 8 days of age. To test this hypothesis two experiments were carried out.

In the first experiment mutant and normal seedlings were grown in darkness for 14 days and carotenoid determinations were made from samples taken every 24 hours, starting with 6-day-old seedlings. Protochlorophyll a analyses were also made on 6- and 10-day-old seedlings. In the second experiment, mutant and normal seedlings were grown in darkness at 25°C for 6 days and then exposed to continuous light. Carotenoid and chlorophyll determinations were made from samples taken 0 and 6 hours after exposure to light and thereafter at 24-hour intervals.

In each experiment the changes in the components within each class of pigments (carotenols, carotenes and chlorophylls) followed similar courses. Therefore, the data are shown in figures 1 and 2 as total carotenols (flavoxanthin c, violaxanthin b, and lutein), total carotenes (neo-beta carotene and beta carotene) and total chlorophylls (chlorophyll a and chlorophyll b).

The data from the first experiment are shown in figure 1. Six days after planting, the carotenol content of dark-grown mutant seedlings is 60% and the protochlorophyll a content 10% of that found in the normal seedlings, whereas the carotene content of the mutant seedlings does not differ from that of the normal seedlings. The carotenol content of the dark-grown normal seedlings increases between the sixth and eighth days after planting and thereafter it declines. On the other hand, the carotenol content of the dark-grown mutant seedlings remains the same between the 6th and 8th days after planting and then it slowly increases. In both dark-grown mutant and dark-grown normal seedlings the carotene content increases between the sixth and eighth days after planting and thereafter it slowly decreases. The protochlorophyll a content of dark-grown normal plants does not change between the sixth and tenth days after planting, whereas in dark-grown mutant seedlings it increases. Thus, in dark-grown mutant seedlings both carotenols and protochlorophyll a begin to increase at the time the mutant block is broken, however an increase in carotene content does not occur at this time. In fact, the pattern of carotene accumulation in the mutant seedlings is almost...
identical to that in the normal seedlings. This suggests that the mutant gene controls the formation of the protochlorophyll a and the carotenols, but not the carotenes.

Pigment changes in plants exposed to light seven days after planting are shown in figure 2. Within 5 hours after exposure to light, appreciable quantities of chlorophyll are found in normal plants; whereas no chlorophyll is detectable in light-exposed mutant seedlings until after 44 hours of exposure to light (approximately 8 days after planting). However, once chlorophyll is formed in the mutant its accumulation follows a pattern similar to that of the normal, and the maximum chlorophyll content is comparable to the maximum amounts found in the normal seedlings. This was true for both chlorophyll a and chlorophyll b. Upon exposure of the dark-grown

![Graph showing pigment changes in light-exposed mutant and normal corn seedlings.](https://example.com/graph.png)

**Fig. 2.** Carotenol, carotene and chlorophyll accumulation in light-exposed mutant and normal corn seedlings. Seedlings were grown in darkness for 6 days and then exposed to continuous light. Determinations were started 6 days after planting. The carotenol content is the sum of flavoxanthin c, violaxanthin b and lutein. The carotene content is the sum of beta and neo-beta carotene and the chlorophyll content is the sum of chlorophyll a and chlorophyll b.

plants to light there is a decrease in the carotenol content of both mutant and normal seedlings. As indicated earlier this decrease in carotenol content is mainly due to a large decrease in the concentration of violaxanthin b. The carotenol content of the normal seedlings decreases for about 98 hours and then slowly increases. In the mutant seedlings the carotenol content decreases for the first 47 hours after exposure to light and then it increases. The carotenol content of normal seedlings increases within 6 hours following exposure to light and continues to increase in a manner paralleling chlorophyll formation for the next 66 hours, thereafter it remains relatively constant. On the other hand, the carotenol content of the mutant seedlings decreases during the first 29 hours after exposure to light and no increase is evident until after 47 hours of exposure to light (8 days after planting). Once the carotenol content of the mutant begins to increase, its accumulation, as in the normal seedlings, parallels that of chlorophyll and the carotenol content becomes comparable to that of the normal seedlings.

**Fig. 1.** Carotenol, carotene and protochlorophyll a accumulation in dark-grown mutant and normal corn seedlings. Determinations were started 6 days after planting. Carotenol content is the sum of lutein, flavoxanthin c and violaxanthin b. Carotene content is the sum of beta and neo-beta carotene.
It thus appears that the light-exposed mutant plants cannot begin to accumulate carotenols, carotenoids or chlorophylls in amounts comparable to those found in light-exposed normal seedlings until approximately 8 days after planting. This implies that in light-exposed mutant seedlings the mutant gene controls the formation of the carotenols, carotenoids and chlorophylls. However, if this is the case, carotenols and carotenoids should not be present in the mutant leaves prior to 8 days after planting. It seemed possible that the endosperm might provide precursors for carotenoid and chlorophyll formation, which lie beyond the mutant block. Such precursors could readily give rise to the amounts of carotenoids and protochlorophyll a found in the mutant prior to 8 days after planting.

**Pigment Content of Endospermless Dark-Grown Mutant and Normal Seedlings:** Embryos were dissected from corn seeds and grown in the dark at 25°C on a sterile mineral solution (6) to which 0.15 mole of glucose per liter and 0.75% agar were added before sterilization (10, 15). Intact seeds were germinated on the same medium to serve as controls. Plants developing from the excised embryos were small and had elongated leaves. In general, they resembled etiolated oat seedlings. The mutant plants were cream-white in color, while the normal plants were pale-yellow in color. Eight days after transfer of the embryo to the culture medium, the entire first and second leaf blades were analyzed for carotenols, carotenoids and protochlorophyll a.

The results of the analyses are given in Table II.

Protochlorophyll a was not detected in the leaves of endospermless mutant seedlings, but it was present in unreduced amounts in the leaves of the endospermless normal seedlings. The carotene content of the endospermless mutant and normal seedlings was less than that of plants grown from intact seeds, and on a percentage and absolute basis the reduction was the same in mutant and normal seedlings. The carotenol content of the endospermless mutant and normal seedlings was also less than that of plants grown from intact seeds and on an absolute basis the reduction was the same in both mutant and normal plants. However, on a percentage basis the reduction in the mutant seedlings was 92%, while in the normal it was only 48%. These data indicate that there is an endosperm factor that contributes to carotenoid and protochlorophyll a production in maize seedlings. Apparently, in 8-day-old mutant seedlings, carotene production is almost completely dependent upon this endosperm factor and protochlorophyll a production is completely dependent upon it. In contrast, carotene production in the mutant, as in the normal, is only partially dependent upon this endosperm factor.

**DISCUSSION**

In this discussion the carotenols (flavoxanthin c, violaxanthin b and lutein) will be considered as a group, since the changes in the individual carotenols paralleled each other and they are all hydroxylated carotenes (16). Beta carotene and neo-beta carotene will also be considered as a group, as the changes in neo-beta carotene and beta carotene followed the same pattern and they are isomeric hydrocarbons.

It is evident from the pigment analyses that the mutant seedling is different in color from the normal seedlings because of a deficiency in certain plastid pigments. When grown in darkness mutant seedlings are pale-yellow, whereas normal seedlings are bright-yellow. This is due to the decreased carotenol content of the mutant seedlings. Mutant seedlings grown in the light lack chlorophyll and contain less carotenols and carotenes than do normal seedlings. Thus they are pale-yellow in color while normal seedlings are green in color. Dark-grown, endospermless mutant seedlings are cream-white in color as contrasted to pale-yellow endospermless normal seedlings. This is because the endospermless mutant seedlings contain almost no carotenols.

Frank (8, 9) and others have suggested that the carotenoids and the phytol portion of the chlorophyll molecule probably arise from colorless precursors common to both. On the basis of the unitary action of the gene (3-5), genetical evidence also indicates that the carotenoids and chlorophylls are closely interrelated in their biosynthesis, since many single genes are known to affect the presence of the carotenoids as well as the chlorophylls.

**Table II**

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Normal With endosperm</th>
<th>Normal Without endosperm</th>
<th>Mutant With endosperm</th>
<th>Mutant Without endosperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µgm pigment/gm fresh wt of leaf</td>
<td></td>
<td>µgm pigment/gm fresh wt of leaf</td>
<td></td>
</tr>
<tr>
<td>Flavoxanthin c</td>
<td>6.4 ± 0.47*</td>
<td>3.0 ± 0.50</td>
<td>3.3 ± 0.20</td>
<td>0.30 ± 0.10</td>
</tr>
<tr>
<td>Violaxanthin b</td>
<td>8.5 ± 0.19</td>
<td>4.9 ± 0.65</td>
<td>4.5 ± 0.40</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Lutein</td>
<td>31.0 ± 0.90</td>
<td>16.0 ± 0.68</td>
<td>16.5 ± 0.40</td>
<td>1.2 ± 0.10</td>
</tr>
<tr>
<td>Beta Carotene</td>
<td>3.2 ± 0.14</td>
<td>1.0 ± 0.31</td>
<td>3.1 ± 0.11</td>
<td>1.2 ± 0.11</td>
</tr>
<tr>
<td>Neo-Beta Carotene</td>
<td>1.8 ± 0.10</td>
<td>0.45 ± 0.10</td>
<td>1.5 ± 0.01</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>Protochlorophyll a</td>
<td>2.4 ± 0.10</td>
<td>1.9 ± 0.20</td>
<td>&lt;0.50</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean plus-or-minus the standard error of the mean. The values are the means of three analyses.
In the present study the action of a single gene upon the accumulation of chlorophylls and carotenoids was investigated. Although the data do not indicate which step in the pathway of plastid pigment synthesis was affected by the mutant gene, they do provide information about the position of the mutant block relative to the production of carotenols, carotenes and chlorophylls.

During the first 8 days after planting, the mutant seedlings are devoid of chlorophyll when grown in the light, and when grown in darkness they contain only a very small amount of protochlorophyll a. This might imply that the mutant seedling is unable to accumulate chlorophyll because of a defective destruction of protochlorophyll a. However, when endospermless mutant seedlings are grown in darkness they are devoid of protochlorophyll a. Therefore, it appears more likely that the protochlorophyll a found in the dark-grown mutant is derived from an endosperm factor which is independent of the mutant gene and that there is a block in chlorophyll synthesis prior to the formation of protochlorophyll a. The pigment analyses also show that, during the first 8 days after planting, the carotenol content of the mutant is always less than that of the normal. Although 7-day-old dark-grown mutant seedlings contain a relatively large amount of carotenol, 8-day-old dark-grown endospermless mutant seedlings contain virtually no carotenols. This strongly suggests that the carotenols found in the mutant seedlings, during the first 8 days after planting, are derived from an endosperm factor. This endosperm factor appears to be independent of the mutant gene, since it contributes equally to carotenol synthesis in both the mutant and normal seedlings.

Additional evidence for the control of both chlorophyll and carotenol production by the mutant gene is found in the unique reversion of the mutant seedlings to a normal phenotype. About 8 days after planting, the mutant block is apparently broken and both dark-grown and light-exposed mutant seedlings begin to accumulate carotenols and chlorophylls (or protochlorophyll a). This would be expected if the mutant gene blocks carotenol and chlorophyll formation at a common point in their synthesis.

The accumulation of the carotenols does not appear to be under the control of the mutant gene. In dark-grown mutant plants the carotene content is equal to that of the normal seedlings and the pattern of carotene accumulation is the same as in the normal seedlings. Likewise dark-grown, endospermless mutant seedlings contain as much carotene as dark-grown, endospermless normal plants. Thus it appears that carotene is synthesized by the mutant seedlings. On the other hand, prior to 8 days after planting, normal seedlings exposed to light contain much more carotene than do mutant plants. This implies that in light-exposed mutant seedlings the synthesis of carotenols, as well as chlorophylls and carotenols, is controlled by the mutant gene. However, the control of carotene synthesis by the mutant gene in light-exposed mutant seedlings, may be an indirect effect. Bandurski (1) has indicated that photosynthetic products are necessary for the production of large amounts of carotene. The exposure of mutant seedlings to light, prior to eight days after planting, does not give rise to chlorophyll synthesis and thus the products of photosynthesis are not available for increased carotene production. Therefore it seems likely that the mutant gene blocks the synthesis of the chlorophylls (or protochlorophyll a) and the carotenols, while the synthesis of carotenols, in light-exposed mutant seedlings, is indirectly affected by a lack of photosynthetic products due to the inability of the mutant seedlings to synthesize chlorophyll.

If the phytol portion of the chlorophyll molecule and the carotenoids arise from common precursors it seems possible that the mutant gene may interfere with the hydroxylation of a common hydrocarbon chain from which arise phytol and the carotenols, whereas the carotenes could be formed from this hydrocarbon chain prior to its hydroxylation. Such a common metabolic pathway, in which the formation of carotene hydrocarbons proceeds hydroxylated carotenoids, would be in agreement with the suggested order for the synthesis of carotene and carotenols in the yeast, Rhodotorula (7).

The reversion of the mutant seedlings to the normal phenotype has been discussed in a previous paper (13).

**Summary**

1. It was shown by chromatographic pigment analyses that the seedling mutant, pale-yellow, 1, is devoid of chlorophyll a and chlorophyll b when grown in the light. When grown in darkness it contained only 10% of the amount of protochlorophyll a found in dark-grown normal seedlings. In addition, light-grown mutant seedlings were found to contain less carotenes and carotenols than were present in the normal seedlings. On the other hand, although dark-grown mutant seedlings contained less carotenols than normal seedlings, the same amounts of carotene were found in both mutant and normal seedlings.

2. Eight-day-old, endospermless mutant seedlings contained no protochlorophyll a and virtually no carotenols, while the carotene content was equal to that found in endospermless normal seedlings. This was interpreted to mean that the protochlorophyll a and the carotenols found in the mutant seedling, prior to 8 days after planting, are derived from an endosperm factor, while carotene production is not completely dependent upon this factor. This endosperm factor also contributed to the production of carotenols and carotenes in the normal seedlings.

3. It was found that, 8 days after planting, the mutant block is apparently broken. At this time the light-exposed mutant seedlings begin to accumulate chlorophyll, carotenols and carotenes, whereas the dark-grown mutant seedlings begin to accumulate protochlorophyll a and carotenols, but not the carotenes.

4. The interrelationship of the chlorophylls and the carotenoids were discussed in terms of the action
of the mutant gene. It was suggested that the gene may be operating on a common pathway for the synthesis of the chlorophylls and carotenoids, at a position after the synthesis of carotenes, but before the synthesis of the carotenols and the phytol portion of the chlorophyll molecule.

The authors wish to thank Dr. E. G. Anderson, Professor of Genetics, California Institute of Technology, Pasadena, California, for providing most of the seed used in these experiments.

LITERATURE CITED

OXIDATIVE PHOSPHORYLATION BY SWEET POTATO MITOCHONDRIA AND ITS INHIBITION BY POLYPHENOLS

M. Lieberman and J. B. Biale
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In a previous study (11) it was found that the rate of oxidation of ω-ketoglutarate by a mitochondrial preparation from sweet potato roots was considerably enhanced when diposphothiamine, diposphopyridine nucleotide, and coenzyme A were added to a basic reaction mixture of adenylate, magnesium, and glucose in a phosphate buffer at pH 7.0. It was of interest, therefore, to examine the phosphorylative capacity of this reconstituted ω-ketoglutarate oxidase. The specific purpose of this paper is to report the conditions that have been found for optimum oxidative phosphorylation and the effects of some inhibitors on oxygen and phosphorus uptakes by the particulate fraction of the sweet potato.

MATERIALS AND METHODS

Key West sweet potatoes (Ipomoea batatas Poir) were used in these experiments. The mitochondria were prepared by the methods outlined in another paper (11). The final mitochondrial pellet was suspended in 10 ml of 0.5 M sucrose instead of 6 ml as used for the studies on cofactor requirements. Assay of oxidative activity by Warburg techniques and nitrogen determinations were carried out as already described (11). Inorganic phosphorus was determined by the method of Bernhart and Wreath (2). Each experiment was conducted at least 3 times and the data reported are averages from these experiments.

RESULTS

 EFFECTS OF PHOSPHATE AND HEXOKINASE ON OXIDATIVE PHOSPHORYLATION: Rates of oxidation and phosphorylation were studied in increasing concentrations of phosphate in the presence and absence of hexokinase. Phosphorus was determined immediately after the addition of the mitochondria to