2nd ed. P. 336. John Wiley and Sons, Inc., New York 1953.
10. HURD-KARRER, A. M. Relation of sulphate to selenium absorption by plants. Amer. Jour. Bot. 25: 666–675. 1938.
11. KAHN, B. S. and LEIBOFF, S. L. Colorimetric determination of inorganic sulfate in small amounts of urine. Jour. Biol. Chem. 80: 623–629. 1928.
12. KYLIN, A. The uptake and metabolism of sulphate by deseeded wheat plants. Physiol. Plantarum 6: 775–795. 1953.
13. LINEWEAVER, H. and BURK, D. The determination of enzyme dissociation constants. Jour. Amer. Chem. Soc. 56: 658–666. 1934.
14. RICHARDS, L. A., ed. Diagnosis and improvement of alkali soils. P. 129. U. S. Dept. Agr., Handbook No. 60. 1954.
15. SHRIFT, A. Sulfur-selenium antagonism. I. Antimetabolite action of selenate on the growth of *Chlorella vulgaris*. Amer. Jour. Bot. 41: 223–230. 1954.
16. TANADA, T. Effects of ultraviolet radiation and calcium and their interaction on salt absorption by excised mung bean roots. Plant Physiol. 30: 221–225. 1955.
17. TRELEASE, S. F. and BEATH, O. A. Selenium. Pp. 1–292. Published by the authors, New York 1949.
18. VIETS, F. G. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466–480. 1944.
19. WEISSMAN, G. S. and TRELEASE, F. S. Influence of sulfur on the toxicity of selenium to Aspergillus. Amer. Jour. Bot. 42: 489–495. 1955.

PLASTID PIGMENT CHANGES IN THE EARLY SEEDLING LEAVES OF ZEA MAYS L.[1]

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Most publications dealing with the analyses and interrelationships of the plastid pigments of maize and Avena have been confined to groups of pigments rather than specific plastid pigments (3, 4, 5, 13). Since the changes in a group of pigments do not necessarily reflect the behavior of the individual pigments, knowledge about the specific pigment changes is desirable.

Moster et al (7, 8) have determined the kinds, as well as the amounts, of the major carotenoid pigments in young maize plants grown in the light and then exposed to different light intensities. However, information is lacking on the development of the specific carotenoid pigments in dark-grown corn seedlings and in etiolated corn seedlings exposed to light. This paper will present information on these subjects.

METHODS

All seedlings were planted in quartz sand and grown in a constant temperature chamber equipped with fluorescent lamps. The culture methods, as well as a description of the light source and constant temperature chamber, have been described (9).

Leaf samples for analyses were obtained in the following manner: the second seedling leaf was removed from 15 different plants and the 15 leaves divided into 3 samples of 5 leaves each. Using a steel die, a section 7 by 52 mm was punched from the distal end of each leaf. These 5 leaf punches were pooled to form one sample. The distal end of the second leaf was selected because it was found to be fully expanded at the time the analyses were started. Thus the pigment content is actually based upon a constant area. However, in order to make the units of concentration more easily comparable to values found in the literature, all pigment concentrations have been expressed as μgm/gm of initial dry weight of leaf. This is a valid method of expressing the pigment content, since the initial dry weights of the leaf sections in the various experiments were not significantly different and there was less than a 2 % difference between the dry weights of leaf sections taken over a 7-day period.

Determinations of initial dry weights were made from material collected 6 days after planting. Three collections of 5 leaf punches were made in the same manner as collections for pigment analyses. Each sample of 5 leaf punches was dried at 75° C for 24 hours, weighed and the weights averaged to give the initial dry weight.

The carotenoid pigments were extracted and chromatographically separated on magnesia columns using methods similar to those employed by Strain (11). The leaf samples were immersed in hot water (90 to 100° C) for 3 minutes and ground in a mortar containing sand, 15 ml of methanol and 0.1 gm $CaCO_3$. The resulting brei was filtered through a medium sintered glass filter and the filtrate was transferred to a separatory funnel. The chlorophylls were saponified by treating the filtrate with 15 ml of methanol containing KOH (35 gm/100 ml). Thirty ml of a 1 : 1 (v/v) mixture of petroleum ether and ethyl ether was then added to the methanol solution and the mixture was shaken gently. The carotenoids were transferred to the petroleum ether–ethyl ether solution by the addition of 25 ml of saturated salt water accompanied by gentle shaking, followed by the addition of 10 to 20 ml of water with further gentle shaking. The methanol-water solution was drained into a second separatory funnel, further diluted with saturated salt water, and again extracted with 15 ml of a 1 : 1 mix-

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ture of petroleum ether and ethyl ether. The petroleum ether–ethyl ether extracts were combined and washed 4 times with water. These washings removed all traces of methanol and most of the ethyl ether leaving the carotenoids dissolved in petroleum ether containing a little ethyl ether. The carotenoid pigments were chromatographically separated by pouring the petroleum ether solution over a column (130 mm long and 11 mm in diameter) composed of a 1 : 1 (w/w) mixture of magnesia and Hyflo Supercel. A vacuum (about 26 cm of Hg pressure) was used to draw the solvent through the adsorbent. Within 10 minutes after the petroleum ether solution was poured over the column, two well separated colored zones appeared. The upper yellow zone consisted of a mixture of carotenols, while the lower orange zone was composed of a mixture of carotenes. Coincident with the time that the last of the petroleum ether passed into the column, a 2 : 1 (v/v) mixture of petroleum ether and ethyl ether was poured over the column. The carotenes passed rapidly (in about 10 min) through the column and were collected in the perculate. The perculate, containing the carotenes, was evaporated under reduced pressure to dryness and the carotenes redissolved in hexane. After prolonged washing (90 min) with the petroleum ether–ethyl ether solvent the yellow band of carotenols was resolved into 5 distinct bands: a yellow band, which extended 2 mm beneath the surface of the absorbent, a lemon yellow band 2 mm in width located about 5 mm beneath the top band, a yellow band 6 mm in width 10 mm beneath the second band, an orange-yellow band 20 mm in width 10 mm beneath the third band and a yellow band 12 mm in width 5 mm beneath the fourth band. Each colored band was cut from the extruded column and eluted with 95 % ethyl alcohol over a sintered glass filter. The filtrates were washed into 10-ml volumetric flasks, made to volume with 95 % ethanol and the absorption spectrum of each pigment determined. The absorption spectrum of the pigment component eluted from the top yellow band had no definite absorption peaks and when this pigment component was readsorbed on a calcium hydroxide column and washed with ethyl ether it was resolved into 5 distinct components. These pigments made up a very small part of the total carotenoids and were not further investigated. The 4 remaining colored bands proved to be single pigments which have been characterized by Strain (11). These 4 bands in the order of their position on the column, starting from the top, had properties which were the same as the carotenoids neoxanthin, flavoxanthin c, lutein and violaxanthin b, respectively. Readsorption of these pigments upon $CaCO_3$, $Ca(OH)_2$ and/or alumina columns followed by washing with suitable solvent systems failed to reveal any other colored contaminates.

The orange pigment component that passed completely through the magnesia column, and was subsequently dissolved in hexane, exhibited an absorption spectrum very much like that of β-carotene. However, when this pigment component was adsorbed on an alumina column and washed with hexane, it was resolved into two orange bands. The absorption spectra of the upper and lower bands, respectively, were found to be identical to the absorption spectra reported by Beadle and Zscheile (2) for β-carotene and neo-β-carotene isolated from spinach leaves. A β-carotene fraction was isolated from fresh spinach leaves by the method of Beadle and Zscheile (2) and mixed with the carotene fraction obtained from corn leaves. When this mixture was chromatographed on magnesia and alumina columns, one band was formed on the magnesia column and two bands on the alumina column. Thus it appears that, in the corn leaves studied, the carotene fraction consisted only of β-carotene and neo-β-carotene. Alpha-carotene was assumed not to be present since repeated chromatography of the carotene fraction on magnesia, an adsorbent on which alpha and beta carotene are easily separated, revealed only one band.

Quantitative spectrophotometric determinations for the carotenoid pigments were made immediately following the isolation of each pigment. A model DU Beckman spectrophotometer, supplied with cells having a light path of 1 cm, was used to determine the absorbance of the pigment solutions. The isolated carotenes were made to a volume of 10 ml with hexane and analyzed as a binary mixture by the method of Beadle and Zscheile (2). The other carotenoids were each made to a volume of 10 ml with 95 % ethanol and the absorbance of each carotenol solution was determined at its respective maximum. Strain's (11) specific absorption coefficients for carotenoids isolated from Avena seedlings were used to convert the absorbance values to pigment concentration. The observed maxima were: lutein 4460 Å, flavoxanthin 4500 Å, and violaxanthin 4420 Å. Strain's (11) specific absorption coefficients at these maxima are: lutein 245.5; flavoxanthin 234.5, and violaxanthin 231.7. Although neoxanthin was isolated, it was difficult to elute from the column and, therefore, was not determined. It was usually present in about the same amount as violaxanthin b.

The analytical methods used to extract and separate the carotenoid pigments were evaluated by determining the percentage recovery of pigments added to leaf samples before extraction. The percentage recoveries of the added pigments were: carotenes 96 %, lutein 95 %, flavoxanthin 92 % and violaxanthin 93 %.

A modification of the method of Zscheile and Comar (15) was used to extract the chlorophyll pigments from the leaf material. The sample to be analyzed was ground in a mortar containing sand, 0.1 gm of calcium carbonate, and 20 ml of a mixture of petroleum ether and methanol (75 to 90 % petroleum ether). The resulting brei was filtered through a medium sintered glass filter and the residue washed with methanol until it was white. The filtrate was transferred to a separatory funnel and the methanol washed out with water. The remaining petroleum ether solution contained the cholorphylls. The entire extraction was carried out in diffuse light of 1 fc in-

tensity. The chlorophylls were readily separated from the carotenoids and resolved into chlorophyll a and chlorophyll b by chromatographing the petroleum ether solution on a starch column. Chromatographic tubes similar to those used by Zechmeister and Cholnoky (14) were employed in the preparation of the columns and the chromatograms were developed with petroleum ether containing 4 % ethyl ether. After the chlorophylls were separated, the adsorbent was removed from the tube and the two chlorophyll bands cut out with a sharp scalpel. Each pigment was eluted with ethyl ether, the eluate collected in a flask and made to a volume of either 10 or 25 ml. The absorption spectra of the pigments eluted from the two bands were found to correspond very closely to those for chlorophyll a, obtained from corn leaves, and chlorophyll b, obtained from barley by Zscheile and Comar (15). The main absorption peaks for chlorophyll a were located at 4300 Å and 6650 Å; for chlorophyll b at 4530 Å and 6500 Å.

Quantitative spectrophotometric determinations for the chlorophyll pigments were made immediately following the isolation of each pigment. The concentrations of the chlorophyll pigments were calculated from the absorbance and Zscheile and Comar's (15) specific absorption coefficients. The absorbance of the chlorophyll a solution was determined at 4300 Å and that of the chlorophyll b solution at 4530 Å. Zscheile and Comar's (15) specific absorption coefficient for chlorophyll a at 4300 Å is 147 and for chlorophyll b at 4530 Å is 173.

Experimental

The changes in the carotenoid pigment content of corn seedlings grown in darkness at 25° C were determined as a function of time. Daily samples of the second seedling leaf were analyzed for carotenoid pigments from the 6th through the 14th day after planting. Three samples, each consisting of 5 leaf punches having a total fresh weight of about 0.27 gm, were used in each analysis. The data from these analyses are shown in figure 1.

In a second experiment, seedlings were grown in the dark at 25° C for 6 days after the time of planting the seed. Following the 6-day dark period, the seedlings were exposed to continuous light of an average intensity of 700 fc. At 0 and 6 hours after exposure to light and thereafter at daily intervals, 3 leaf samples were collected and analyzed for plastid pigments. Duplicate collections were made. From the first collection chlorophyll concentrations were determined and from the second carotenoid concentrations. The results of the carotenoid analyses are shown in figure 2 and those of the chlorophyll analyses in figure 3.

Results

The data in figure 1 show that lutein is the most abundant carotenoid pigment in dark-grown corn seedlings and that violaxanthin, flavoxanthin, β-carotene and neo-β-carotene are present in lesser amounts in the order listed. In the dark-grown seedlings the

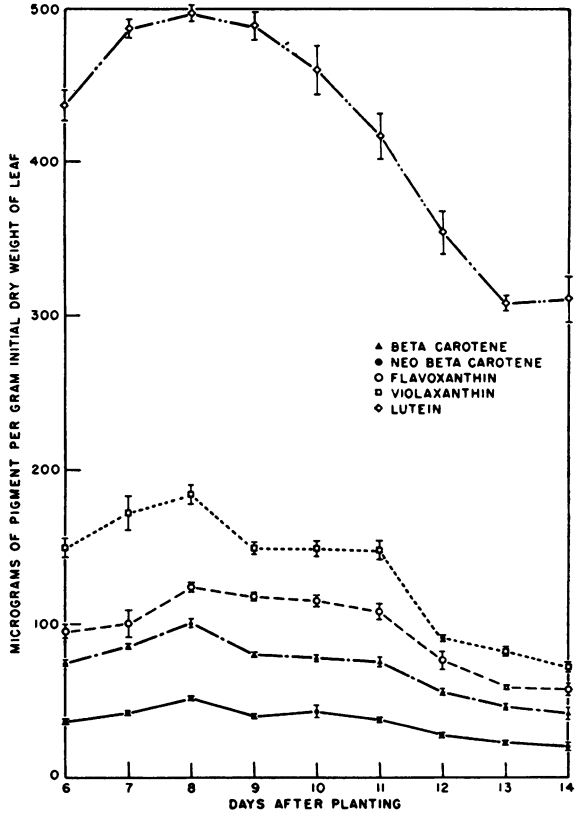

Fig. 1. Carotenoid content of dark-grown corn seedlings as a function of time after planting of seed. Corn seedlings were germinated and grown in darkness and the concentrations of the carotenoid pigments in the second leaf were determined daily from the 6th through the 14th day after planting the seed. The means and standard errors of the means are plotted.

concentration of each carotenoid pigment increased slightly during the first 3 days of analyses. Thereafter the concentrations decreased, until the final two days of the analyses. On the final two days of analyses the distal portion of the second leaf was brown, indicating the leaf had begun to deteriorate.

Since all of the pigments followed similar courses of accumulation and loss, the relative concentrations of pigments in the dark-grown seedlings did not change. However, this was not the case for the pigments in dark-grown corn seedlings exposed to continuous light. As the data in figure 2 show, the exposure of dark-grown corn seedlings to light resulted in an immediate decrease in the concentrations of flavoxanthin, violaxanthin and lutein and a rapid increase in the concentrations of β-carotene and neo-β-carotene. The decreases in the concentrations of violaxanthin and lutein were virtually complete by the end of the first day, while the decrease in flavoxanthin extended over a period of 3 days. The increase in the concentration of β-carotene continued for a period of 4 days, while the accumulation of neo-β-carotene was

complete within 3 days. Initially the concentrations of pigments in the dark-grown seedlings in order of decreasing amounts were: lutein, violaxanthin, flavoxanthin, β-carotene and neo-β-carotene. However, exposure to light altered the order to: β-carotene and lutein, neo-β-carotene, flavoxanthin and violaxanthin.

From the data in figure 3 it is evident that the exposure of dark-grown seedlings to light resulted in the accumulation of chlorophyll a and chlorophyll b. The accumulation of chlorophyll a was very rapid and complete within 4 days, while that of chlorophyll b was gradual, extending over a period of 5 days. At the end of the first 6 hours of exposure to light, the ratio of chlorophyll a to chlorophyll b was 37 : 1. Thereafter the ratio decreased and at the end of the fourth day was 6 : 1, a ratio close to that found in non-starch forming monocotyledons (10).

Discussion

While much information can be obtained about the carotenoids by studying them as a group, it is important that the individual components also be considered. The data in the present study indicate that an environmental change can affect each component of the carotenoids in a different way or to a different degree. Thus it is apparent that caution should be used in interpreting data obtained from analyzing the carotenoids as a group.

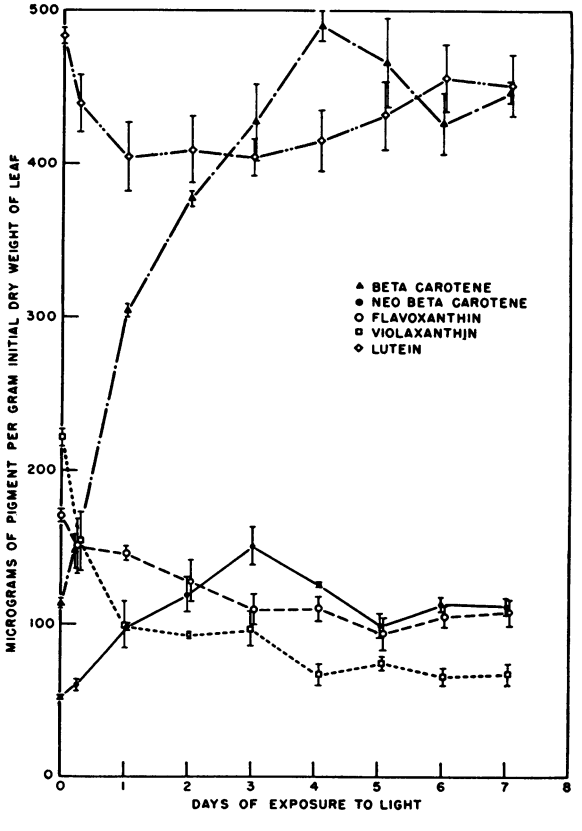

Fig. 2. Carotenoid content of corn seedlings as a function of days of exposure to light. Corn seedlings were germinated and grown in the dark. Six days after planting the seedlings were exposed to continuous light and the concentrations of carotenoid pigments in the second leaf were determined at intervals over a 7-day period. The means and standard errors of the means are plotted.

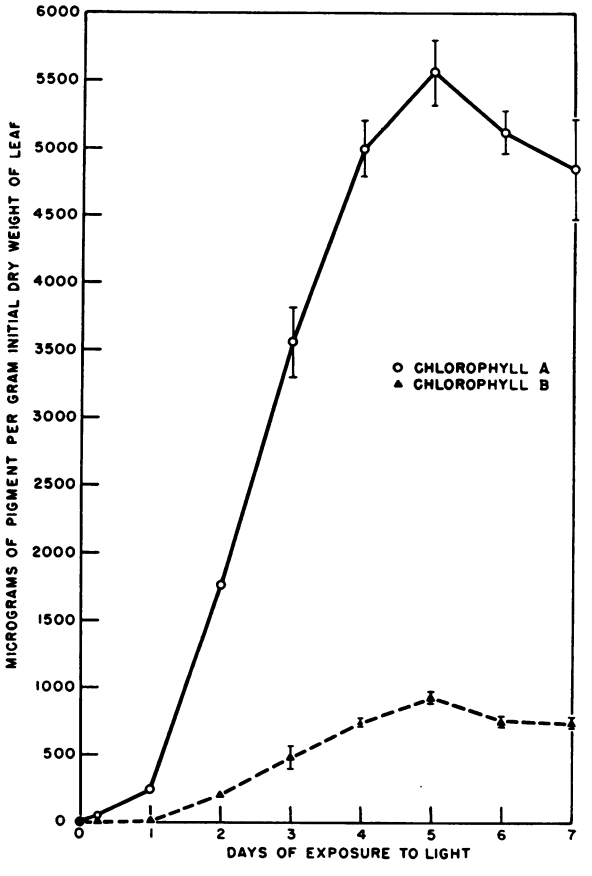

Fig. 3. Chlorophyll content of corn seedlings as a function of days of exposure to light. Corn seedlings were germinated and grown in the dark. Six days after the time of planting, the seedlings were exposed to continuous light and the concentrations of chlorophyll a and chlorophyll b were determined at intervals over a 7-day period. The means and standard errors of the means are plotted. The standard error is not shown when the value is less than the vertical dimension of the symbol used to indicate the mean value.

Frank (3, 4) has observed that the exposure of dark-grown seedlings to light results in a reduction in total carotenoids and the formation of chlorophyll. She has suggested that this reduction in carotenoids is due to the diversion of carotenoid precursors into phytol groups. However, in the present study exposure of dark-grown seedlings to light did not affect all of the carotenoids in the same way. The concentration of the carotenes increased while the concentration of the carotenols (flavoxanthin, lutein and violaxanthin) decreased. Therefore, if a reduction in carote-

noids concomitant with an increase in chlorophyll is considered to be the result of a diversion of carotenoid precursors to phytol groups, it appears that only the carotenol (flavoxanthin, lutein and violaxanthin) precursors are diverted into phytol groups. Thus it might be more profitable to study the relationship of the chlorophylls to the carotenols rather than to the total carotenoids (3, 4, 5).

In a study designed to determine the nature of the increase in carotenes induced by exposure of dark-grown plants to light, Bandurski (1) isolated the carotenes from the other carotenoids. From his work on the isolated carotene fraction he concluded that the rapid synthesis of carotenes is dependent upon the availability of photosynthetic products. On the other hand, Withrow's (13) data are at variance with this conclusion since they indicate that photosynthetic products are not necessary for the production of large amounts of carotene. In experiments with bean and corn seedlings he found that large amounts of carotene accumulated when plants were exposed to low irradiance at 725 mμ, a wave length at which no chlorophyll was formed and therefore no photosynthesis possible. However, an examination of Withrow's method of extracting carotenes indicates that his procedure extracts carotenols as well as carotenes. Therefore, his spectrophotometric method of analysis measured the carotenoid concentration (carotenols plus carotenes) rather than carotene concentration. Thus the "carotene" content of his dark-grown corn seedlings is 4 times that of our dark-grown plants. Since Withrow actually measured carotenoid content, his data does not necessarily show that large amounts of carotenes are produced in the absence of photosynthetic products.

The absence of α-carotene from corn leaves agrees with the finding of MacKinney (6), but it does not agree with that of Moster (8). The conditions under which the plants were grown may be responsible for the different results. The environment in which plants are grown is undoubtedly responsible for the absence of zeaxanthin from corn leaves. In the present study seedlings were grown under a light intensity of 700 fc and at a temperature of 25° C and no zeaxanthin was detected. However, the absence of zeaxanthin was not unexpected since Moster (7) has shown that each of these conditions is extremely unfavorable for the accumulation of zeaxanthin.

It should be noted that the ratios of carotenoids we have found in light-grown corn seedlings, as contrasted to dark-grown corn seedlings, are similar to those reported for other monocotyledonous seedlings such as barley (12).

Summary

A strain of maize (single cross L 289 × L 205) was grown under a controlled environment of light and temperature. The changes in the concentrations of the major carotenoid pigments and in the concentration of chlorophylls have been followed for a 7-day period, using only the second seedling leaf for the pigment analyses. Beta carotene, neo-β-carotene, lutein, flavoxanthin, violaxanthin, chlorophyll a and chlorophyll b were isolated as separate pigments. In both dark-grown and light-grown material, neoxanthin was also identified and was present in amounts comparable to that of violaxanthin. However, no α-carotene or zeaxanthin was found in the preparations.

In dark-grown plants lutein was found to be the major leaf carotenoid with violaxanthin, flavoxanthin, β-carotene and neo-β-carotene present in lesser amounts in the order listed. In these plants the concentration of each carotenoid pigment increased during the first 3 days of observation and thereafter it decreased. On the other hand, within 6 hours following the illumination of dark-grown seedlings, the concentrations of flavoxanthin, lutein and violaxanthin decreased and the concentrations of β-carotene and neo-β-carotene increased. After 4 days of exposure to light β-carotene and lutein were the most abundant carotenoid pigments; while neo-β-carotene, flavoxanthin and violaxanthin were present in lesser amounts.

Literature Cited

1. Bandurski, R. S. Synthesis of carotenoid pigments in detached bean leaves. Bot. Gaz. 111: 95–109. 1949.
2. Beadle, B. W. and Zscheile, F. P. Studies on carotenoids. II. The isomerization of beta carotene and its relation to carotene analyses. Jour. Biol. Chem. 144: 21–33. 1942.
3. Frank, S. The relation between carotenoid and chlorophyll pigments in Avena coleoptiles. Arch. Biochem. 30: 52–61. 1951.
4. Frank, S. and Kenney, A. L. Destruction of chlorophyll and carotenoids in corn seedlings. Federation Proc. 13: 47–48. 1954.
5. Frank, S. and Kenney, A. L. Chlorophyll and carotenoid destruction in the absence of light in seedlings of *Zea Mays* L. Plant Physiol. 30: 413–418. 1955.
6. MacKinney, G. Leaf carotenes. Jour. Biol. Chem. 111: 75–84. 1935.
7. Moster, J. B. and Quackenbush, F. W. The effect of temperature and light on the carotenoids of seedlings grown from three corn hybrids. Arch. Biochem. Biophys. 38: 297–303. 1952.
8. Moster, J. B., Quackenbush, F. W. and Porter, J. W. The carotenoids of corn seedlings. Arch. Biochem. Biophys. 38: 287–297. 1952.
9. Phinney, B. O. and Kay, R. E. Interaction of environment and genotype in the expression of a virescent gene, pale-yellow-1, of maize. Hilgardia 23: 185–196. 1954.
10. Rabinowitch, E. Photosynthesis and Related Processes, vol. I. Pp. 1–599. Interscience Publishers, Inc., New York 1945.
11. Strain, H. H. Leaf Xanthophylls. Carnegie Inst. Wash. Publ. No. 490. 1938.
12. Strain, H. H. Formation of carotenoids and chlorophylls in etiolated barley seedlings exposed to red light. Plant Physiol. 13: 413–418. 1938.
13. Withrow, R. B., Klein, W. H., Price, L. and Elstad, V. Influence of visible and near infrared radiant energy on organ development and pig-

ment synthesis in bean and corn. Plant Physiol. 28: 1–14. 1953.
14. ZECHMEISTER, L. and CHOLNOKY, L. Principles and Practice of Chromatography. Pp. 1–362. John Wiley and Sons, New York 1948.
15. ZSCHEILE, F. P. and COMAR, C. L. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. Bot. Gaz. 102: 463–481. 1941.

STUDIES ON 3-INDOLEACETIC ACID METABOLISM. II. SOME PRODUCTS OF THE METABOLISM OF EXOGENOUS INDOLEACETIC ACID IN PLANT TISSUES[1,2]

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It has long been known that indoleacetic acid (IAA) is taken up by plants and translocated to various tissues, causing characteristic modifications in the pattern of growth. However the fate of IAA in vivo has received little attention. This is surprising in that IAA and its derivatives are virtually the only materials of known chemical constitution which both exhibit the formative effects of plant growth substances and occur naturally in plants. The fact that the concentrations of endogenous IAA are extremely low is no doubt in part responsible for the neglect of an important field of research. The authors of this paper have attempted to overcome the difficulty by supplying much larger amounts from an ambient solution. They are quite aware, however, that the reactions associated with relatively high concentrations of applied IAA may differ significantly from metabolic reactions as they occur in nature.

Exogenous IAA participates in several biological reactions. Plants are known to contain IAA oxidizing enzymes and part of the IAA administered to plants presumably undergoes oxidative degradation to products which are no longer growth active (8). Hemberg (3) has noted a rise in "bound auxin" in IAA treated corn kernels. Siegel and Galston (6) have reported that IAA administered to excised pea roots becomes attached to proteins. On the other hand we have recently found that IAA administered to pea stems is conjugated with aspartic acid (1). The present communication constitutes a report on the extention of our investigations to other plant species.

MATERIALS AND METHODS

A survey was carried out using 12 plant species belonging to 8 families. The following tissues were investigated: etiolated coleoptiles of oats, corn and barley all harvested just before the emergence of the primary leaf; etiolated epicotyls of peas 7 days old; etiolated hypocotyls of sunflower, cucumber and buckwheat, 7, 4, and 4 days old respectively; etiolated potato sprouts 3 to 5 inches long; stems and petioles of greenhouse grown pea, tomato, and cabbage plants. Immediately after harvesting, the tissue was weighed and cut into pieces aproximately 2 inches long. To each 100 gms of tissue were added 2 liters of M/60 sodium dihydrogen phosphate with or without 60 mg of IAA.[3] The floating tissue was incubated at 24° C for 24 hours in the dark with gentle shaking. After this period the tissue was removed, thoroughly washed, and frozen at −8° until it could be conveniently extracted. The residual IAA in the ambient solutions was measured by the acid-ferric chloride (Salkowski) method (8) and in some cases the solutions were ether extracted and chromatographically analyzed for other Salkowski-positive substances.

To each 150 gm of frozen tissue was added 65 ml of 0.3 *N* sodium bicarbonate solution. The tissue was then ground in a Waring blendor and filtered through cheese cloth. The resulting brei was saturated with ammonium sulfate, infusorial earth was added and the precipitated proteins were removed by filtration on a Buchner funnel. (The unwashed precipitate from pea contained from 10 to 20 % of the Salkowski-positive material. However, when the precipitate was thoroughly washed by redissolving it in water and reprecipitating it with ammonium sulphate, practically all the Salkowski-positive material was in solution. Since the washings contained the same substances as the filtrate and in about the same proportions only the first filtrates were regularly investigated in this survey.) The filtrate (pH about 7.5) was extracted once with 100 ml and twice with 50 ml of peroxide-free ether, acidified to pH 2.5 with phosphoric acid and extracted with ether as before. Then the acidified filtrate was extracted with 100, 50 and 20 ml portions of 1-butanol. The aqueous residue was by this time Salkowski-negative. The acidic extracts were made slightly alkaline either with sodium bicarbonate solution or with dilute ammonia and taken to dryness, the

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[2] Contribution No. 62, Canada Department of Agriculture, Science Service Laboratory, University Sub Post Office, London, Ontario, Canada.

[3] Some samples of indoleacetic acid from commercial sources are very impure and contain appreciable amounts of indoleacetamide and other Salkowski reactive substances. The following purification procedure, however, has been found to be effective: Commercial IAA is dissolved in water (about 100 ml/gm) containing an excess of sodium bicarbonate. The aqueous solution is extracted several times with 1-butanol, then several times with ether. The dissolved ether is removed by aeration and the solution is slowly acidified to pH 3.0 with phosphoric acid. About 70 % of the original IAA separates on standing as white, flake-like crystals.