FORMATION OF CAROTENOIDS AND CHLOROPHYLLS IN ETIOLATED BARLEY SEEDLINGS EXPOSED TO RED LIGHT

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(WITH ONE FIGURE)

Introduction

For the investigation of many of the problems pertaining to the metabolism and function of the leaf pigments, it would be desirable to vary the relative proportions of the several leaf pigments in any given plant through changes in the environment. Average pigment determinations reported by RUDOLPH (**1**) have already indicated that red light, which promotes the formation of chlorophyll, does not stimulate the formation of the yellow pigments in etiolated bean leaves, but the individual determinations were subject to great variation. Moreover, etiolated bean leaves contain relatively large quantities of carotene and xanthophylls in nearly the same proportions found in green leaves, so that, for demonstration of the effect of red light on the formation of carotenoids in these seedlings, extremely precise determinations of the pigments were required.

Investigations in this laboratory have shown that etiolated barley leaves, in contrast with the etiolated bean seedlings, contain very small quantities of carotene relative to the xanthophylls. These investigations also made possible the precise determination of the carotenoid pigments, because it was found that the rapid oxidation of the yellow pigments which takes place when the etiolated leaves are ground with sand and acetone during the extraction of the pigments may be prevented by treatment of the leaves with hot water (**4**). By the use of the improved methods of analysis, it was possible to determine accurately the effect of red light on the formation of the carotenoids in etiolated barley plants.

When etiolated barley seedlings are exposed to red light, both carotene and chlorophylls increase rapidly. The xanthophylls, comparatively large quantities of which are present in the etiolated barley seedlings, increase more slowly. Investigation of the pigments of etiolated barley seedlings, which had been impregnated with sucrose and kept in the dark, demonstrated that the presence of sucrose did not promote the formation of carotene or chlorophyll. Determination of the absorption spectra of lutein acetate and of beta-carotene demonstrated that lutein and beta-carotene, the principal constituents of the leaf carotenoids, absorb considerable light in the red region of the spectrum in which chlorophyll exhibits maximum absorption. Since etiolated barley seedlings contain extremely little green

pigment, the red light absorbed by the yellow pigments in these plants must represent a large proportion of that absorbed by all the pigments that are present. Whether the formation of the carotene in red light is dependent upon the absorption of light by the yellow pigments or upon other factors remains to be established.

Experimentation

Plant material

Etiolated barley seedlings were obtained by germinating barley seeds in 6-inch square boxes in the dark room. All the seedlings were grown in the same soil in order to insure comparable nutritional conditions.

Apparatus

The apparatus for the illumination of the seedlings was constructed from a metal can $13 \times 9 \times 9$ inches. Light of the desired wave length was admitted to the can through two $6\frac{1}{2}$-inch square glass filters set in modeling clay over openings in the top of the can. A 100-watt, inside-frosted tungsten lamp was placed 3 inches above each of the glass filters. Ventilation for the light chamber was provided by means of flexible metal tubing $1\frac{1}{2}$ inches in diameter and $3\frac{1}{2}$ feet long soldered to openings in each end of the can. One tube was bent upward in the shape of an S; the other was bent downward

TABLE I

Transmission of light by red glass filters

Filter I		Filter II	
Wave length	Transmission	Wave length	Transmission
mμ	%	mμ	%
590	0.00	627.5	0.00
595	0.17	630	0.14
600	1.8	632.5	0.27
605	9.7	635	0.87
610	24.4	637.5	2.28
615	39.7	640	6.1
620	48.9	642.5	12.5
625	55.4	645	21.7
630	58.8	647.5	32.4
640	61.6	650	43.8
650	63.8	655	58.3
660	65.8	660	64.8
670	65.3	670	71.5
680	67.2	680	72.4
		690	73.3
		700	74.4

in the shape of an *S*. In order to prevent direct admission of light to the tubing, black baffles, 6 inches square, were placed 3 inches from the open ends. Temperature within the light chamber varied from 21 to 26.6°.

Typical spectral transmissions of the two types of glass filters used to obtain red light are shown in table I. These values were subject to some variation due to imperfections in the surface and to variation in the thickness of the glass. The transmission curves and the absorption of light by solutions of the pigments extracted from the leaves were determined with the photoelectric spectrophotometer described by SMITH (**2**).

In order to make certain that the etiolated plants were exposed only to light of the wave lengths transmitted by the red filter, blue glass filters were placed over the red glass filters, and etiolated plants were grown in the light chamber while the tungsten lamps were allowed to burn continuously. After several days, the plants did not contain increased quantities of carotene or green pigments. This indicated that stray light was not entering the light chamber and that blue light was not transmitted by the red glass filters.

DETERMINATION OF PIGMENTS

Samples of the leaves (5 gm.) were placed in water at 95–100° for 1 minute. The water was separated by decantation and the leaves were ground with sand (12.5 gm.) and a little acetone. The ground mass was placed on a filter consisting of heat-treated siliceous earth supported on cotton, and the solvent was removed from the leaf material with suction. The residue was then washed with acetone until all the pigments had been removed. After the addition of ether (75 ml.) to the filtrate containing the pigments (75 to 100 ml.), the pigments were transferred to the ether by further dilution of the solution with water (about 200 ml.). The aqueous layer was separated from the ether and re-extracted with fresh ether (40 ml.). The combined ether layers were washed well with water and rinsed into a volumetric flask (100-ml.) with ethanol. An aliquot portion of this solution was diluted several times with ethanol, and the absorption of the solution was determined. Another aliquot portion of the solution of pigments was treated with a solution of potassium hydroxide in methanol in order to saponify the chlorophylls. The saponified chlorophylls were separated from the yellow pigments by washing the solution with water after the addition of some ether. The ether solution of the carotenoids was transferred to a volumetric flask and diluted to volume with ethanol. An aliquot portion of this solution was diluted with ethanol and the absorption was determined. Another aliquot portion of the solution of the carotenoids was diluted with petroleum ether, and the pigments were transferred to the petroleum ether by the addition of water to the solution. The petroleum ether solution was then extracted successively with 60, 70, 80, and 90 per

cent. methanol in order to remove the ether and the xanthophylls. The petroleum ether solution of the carotenes was diluted to a definite volume with ethanol, and the absorption was determined.

Experience with the leaf xanthophylls had demonstrated that leaves contain a mixture of these pigments which differ from one another with respect to their spectral absorption properties (3). In order to compare the xanthophyll, carotene, and chlorophyll content of leaves which had been permitted to develop under various conditions, it was necessary to calculate the spectral absorption coefficients of the pigments per gram of leaf material per liter of extract. In order to prevent confusion with E, the symbol for the absorption coefficient per gram of pigment per liter of solution, the following symbols have been used:

$$s = (\log I_o - \log I)\ (1/Lc)$$

where I_o and I are respectively the transmission of L cm. (2 cm.) of solvent and solution. The concentration, c, is expressed in grams of leaf material per liter of extract. Since s(total pigments), s(total carotenoids), and s(carotene) were determined experimentally,

$$s(\text{chlorophyll}) = s(\text{total pigments}) - s(\text{total carotenoids}),$$

and

$$s(\text{xanthophyll}) = s(\text{total carotenoids}) - s(\text{carotene}).$$

Results

The results of several experiments are summarized in table II. In experiment I, the pigments of etiolated barley seedlings 12 days old were determined. In experiment II, etiolated barley seedlings 5 days old were exposed continuously for 89 hours to light from filter I. For experiment III, the seedlings were exposed continuously for 168 hours to light from filter I. The plants for experiment IV were exposed continuously for 48 hours to light which passed through two glasses of filter II. In experiment V, the etiolated seedlings were exposed continuously for 96 hours to light which passed through two glasses of filter II.

The pigment content of etiolated barley seedlings which had been impregnated with 7.5 per cent. sucrose solution is recorded under experiment VI in table II. The etiolated seedlings, 9 days old, were cut so that the leaves were about 3 inches long. These leaves were impregnated with the sucrose solution by alternately exposing the leaves to vacuum in the presence of the sugar solution and then releasing the vacuum. The impregnated leaves were permitted to stand in the dark with their cut ends in a little of the sucrose solution. After 15 hours the leaves were removed from the solution, killed in hot water, and the relative proportions of the carotenes and xanthophylls were determined.

TABLE II

FORMATION OF PIGMENTS IN ETIOLATED BARLEY SEEDLINGS EXPOSED TO VARIOUS CONDITIONS

WAVE LENGTHS	PIGMENTS	ABSORPTION COEFFICIENTS IN EXPERIMENTS					
		I	II	III	IV	V	VI
mμ		s	s	s	s	s	s
	Carotene	0.0010	0.0085	0.0078	0.0068	0.0071	0.0016
440	Xanthophyll	0.0177	0.0260	0.0239	0.0199	0.0196	0.0217
	Chlorophyll	0.0003	0.0415	0.0360	0.0248	0.0311	.....
	Carotene	0.0010	0.0096	0.0087	0.0078	0.0081	0.0016
455	Xanthophyll	0.0139	0.0209	0.0188	0.0157	0.0158	0.0177
	Chlorophyll	0.0001	0.0195	0.0182	0.0101	0.0155	.....
	Carotene	0.0009	0.0083	0.0076	0.0066	0.0068	0.0013
465	Xanthophyll	0.0138	0.0214	0.0189	0.0166	0.0159	0.0174
	Chlorophyll	0.0000	0.0208	0.0192	0.0110	0.0161	.....
	Carotene	.....	0.0087	0.0077	0.0069	0.0071	0.0014
475	Xanthophyll	.....	0.0217	0.0196	0.0171	0.0163	0.0196
	Chlorophyll	.....	0.0154	0.0130	0.0070	0.0118	.....
662	Chlorophyll	.....	0.0467	0.0437	0.0301	0.0373	.....
664	Chlorophyll	0.0000	0.0473	0.0447	0.0302	0.0375	0.0000
666	Chlorophyll	.....	0.0453	0.0422	0.0295	0.0356	.....

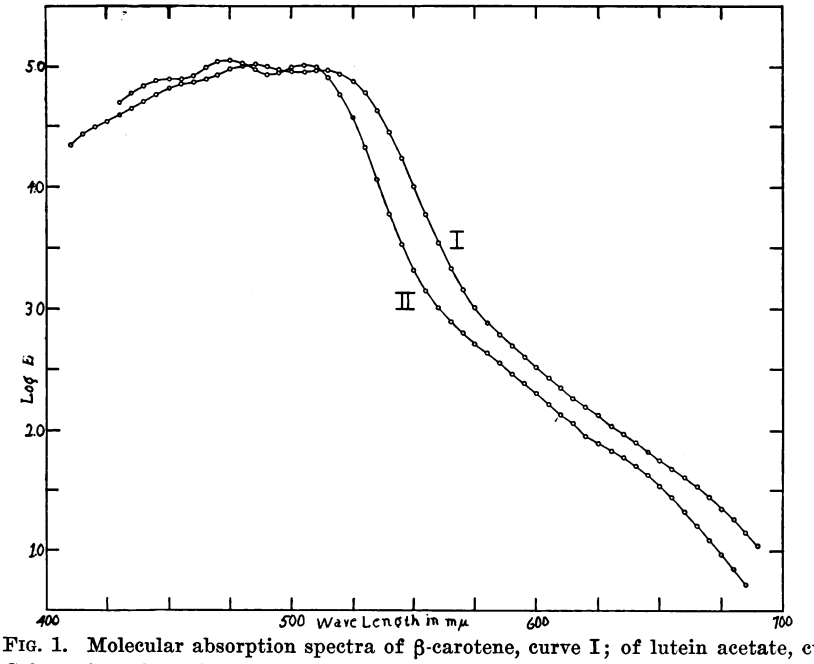

FIG. 1. Molecular absorption spectra of β-carotene, curve I; of lutein acetate, curve II. Solvent is carbon disulphide.

Molecular, spectral absorption curves of beta-carotene and of lutein acetate in solution in carbon disulphide are shown in figure 1. Since it was impossible to obtain a sufficiently concentrated solution of lutein in carbon disulphide for the determination of the absorption coefficients in the red region of the spectrum, it was necessary to use the more soluble acetate. This ester of lutein exhibits molecular absorption coefficients identical with those of lutein in the spectral region from 400 to 530 mμ. The molecular absorption coefficients were calculated according to the formula:

$$\log E = \log [(\log I_o - \log I)\ (1/Lc)]$$

where the concentration, c, is expressed in moles per liter of solution. Even considering the shift in the absorption spectra of the carotenoids caused by carbon disulphide (**2**), these pigments absorb considerable light in the red region of the spectrum in which chlorophyll exhibits maximum absorption.

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