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Carotenoids of Wild Type and Mutant Strains of the Green Aiga, Chlamydomonas reinhardi [1, 2]

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Chlamydomonas reinhardi is an excellent organism for research in photosynthesis for there are several mutant strains with impaired photosynthesis (21), as well as a number of mutant strains in which the pigments are visibly altered (25). In view of the recent investigations on the photosynthetic competence of these mutant strains (17, 19, 20) we felt it was desirable to obtain a complete description of their carotenoid pigments.

In a preliminary study of the carotenoid pigments of *C. reinhardi*, Sager and Zalokar (26) separated 15 carotenoid fractions from their light-grown wild type strain. They were able to identify α-carotene, β-carotene and lutein. When grown in the dark, the wild type strain produced only 6 fractions, including the 3 identified in the light. In contrast, a pale green mutant strain (no. 95) which dies when grown either photosynthetically or heterotrophically in the light, produced only α-carotene and β-carotene in the dark.

In the present study, 9 different carotenoid pigments could be identified in light-grown cultures of the wild type strain of *C. reinhardi*. Dark-grown wild type cultures contain 7 of these pigments, have less total pigment, and have a markedly decreased ratio of β-carotene/α-carotene. The latter 2 properties are also characteristic of the mutant strains described here.

Materials and Methods

Organisms. Six strains of *C. reinhardi* were used in the experiments reported; wild type (strain *137c*), a mutant strain of spontaneous origin, *y-2*, and 4 UV-induced mutant strains, *ac-16*, *ac-21*, *ac-115* and *ac-141* (18).

Wild type *C. reinhardi* is green when cultured in the light or dark, whereas *y-2* is green when cultured in the light but yellow when cultured in the dark. The mutant strain is, therefore, phenotypically similar to the yellow in the dark or yellow strain described by Sager (24). This yellow phenotype results from the inability of the mutant strain to synthesize chlorophyll in the dark. During the course of this investigation, it became apparent that we were working with mixed cultures of wild type and *y-2*. Cell population studies were therefore carried out on cultures grown

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under identical conditions to those used for the pigment analyses. Several wild type and *y-2* liquid cultures were grown in the light and in the dark and plated out. These plates yielded several hundred colonies from each original culture. Wild type cultures were found to contain 12 % *y-2* whereas the *y-2* cultures contained 3 % wild type. These results were the same whether the plates were cultured in the light or in the dark. Pigment analyses were carried out on similar liquid cultures and the following corrections applied: let w = pigment concentration in pure wild type cultures, x = pigment concentration found in wild type cultures, y = pigment concentration in pure *y-2* cultures and z = pigment concentration found in *y-2* cultures: then $x = (0.88)(w) + (0.12)(y)$ and $z = (0.03)(w) + (0.97)(y)$: therefore $w = (1.141)(x) - (0.141)(z)$ and $y = (1.035)(z) - (0.035)(x)$.

Growth Conditions. Cultures were grown on a shaking apparatus at 25° in high salt minimal medium supplemented with 2 % sodium acetate (30). They were either illuminated continuously at a light intensity of 4500 lux from daylight fluorescent lamps or cultured in the dark in flasks made light-tight with black masking tape. Pigment analyses of wild type and *y-2* were carried out on both light- and dark-grown cultures whereas analyses of the remaining strains were made on light-grown cultures.

Pigment Analysis. Cells were harvested during the logarithmic phase of growth by centrifugation at 4000 × *g* for 5 minutes, and were examined directly or after they had been stored at −20° for up to 48 hours. Packed cells (1.2–6.0 g wet wt) were suspended in 6 % KOH in methanol (5 ml/g wet wt) and saponified in the dark for 5 minutes at 40°. This mixture was rapidly chilled and the methanol decanted after a brief centrifugation. The cell residue was reextracted several times with methanol (5.0 ml/g wet wt) until it was colorless. All of the carotenoid pigments were transferred to diethyl ether (peroxide-free) following the addition of an equal volume of a 5 % sodium chloride solution to the pooled methanol extracts. Alkali was removed by washing the ether layer with water, and this extract was taken to dryness in vacuo at 40°. The pigments were redissolved in 10 % acetone in petroleum ether and were chromatographed on columns of weakened aluminum oxide, as described earlier (15). Four fractions (A, B, C, D) were eluted from these columns with 20 % acetone in petroleum ether, 40 % acetone in petroleum ether, acetone and absolute ethanol respectively, and these fractions were resolved into their individual carotenoid components by chromatography on magnesium oxide (Sea Sorb 43): Celite (1: 1).

Fraction A. This fraction contained the hydrocarbon pigments and could be separated on a magnesia column by development with petroleum ether.

Fraction B. Using 2 % ethanol in petroleum ether on a magnesia column this fraction resolved into cryptoxanthin (when present), lutein, zeaxanthin, and luteoxanthin (zeaxanthin monoepoxide monofuranoid).

Fraction C. Development with 6 % ethanol on a magnesia column resulted in a separation of 2 pigments, violaxanthin and trollein.

Fraction D. This fraction contained only a single pigment, neoxanthin, when developed with 10 % ethanol on a magnesia column.

Each pigment was identified by a number of procedures, which included spectrophotometry, mixed chromatography with authentic samples and color reactions with concentrated hydrochloric acid. In addition, quantitative partition coefficients were obtained, as described by Petracek and Zechmeister (23), and from these, M_{50} values were obtained, as described by Krinsky (14). M_{50} values represent the percentage methanol, in a solvent system petroleum ether-aqueous methanol which are required to give a partition coefficient (concentration in petroleum ether/concentration in methanol) of 50: 50 and are directly proportional to the number and types of polar substitutes in carotenoid molecules. Epoxides were detected by their characteristic hypsochromic spectral shift in the presence of dilute acid (12).

The results are expressed as micrograms carotenoid per gram wet weight of packed cells. The $E^{1\%}_{1\,cm}$ values are listed in table I, along with the λ_{max} values of the isolated pigments.

Table I. *Spectral Characteristics of the Carotenoids of Chlamydomonas reinhardi*

Carotenoid	Solvent	λ_{max} (mμ)	$E^{1\%}_{1\,cm}$ at λ_{max}	Reference
α-Carotene	Petroleum ether	445.5	2735	32
β-Carotene	" "	451.0	2580	32
P-460	" "	460.0	2720*	
Cryptoxanthin	Ethanol	451.0	2470	27
Lutein	"	445.5	2550	27
Luteoxanthin	"	423.0	2280	27
Zeaxanthin	"	452.5	2490	27
Violaxanthin	"	442.5	2550	13
Trollein	"	445.5	2350*	
Neoxanthin	"	437.5	2270	27

* Estimated value.

Table II. *The Carotenoids of Wild Type Chlamydomonas reinhardi*

Pigment	Light-grown		Dark-grown	
	μg/g wet wt	%	μg/g wet wt	%
α-Carotene	11.6	0.9	28.6	3.1
β-Carotene	374.1	29.2	387.7	41.8
P-460	51.6	4.0	19.5	2.1
Lutein	210.9	16.5	128.6	13.9
Luteoxanthin	42.8	3.3	...	
Zeaxanthin	26.8	2.1	...	
Violaxanthin	274.2	21.4	150.8	16.3
Trollein	120.3	9.4	115.6	12.5
Neoxanthin	148.5	11.6	95.3	10.3
Unknowns	18.2	1.4	...	
Total (range of 4 cultures)	1279.0 (996–1424)		926.1 (908–943)	
β-carotene / α-carotene	32.3		13.6	
xanthophyll / carotene	1.93		1.1	

Results

Wild Type. Results of the pigment analyses carried out on both light-grown and dark-grown cultures of wild type *C. reinhardi* are given in table II. Most of the carotenoid pigments found have been reported previously in the Chlorophyceae (10, 22, 29). The identifications of α-carotene, β-carotene, lutein, zeaxanthin, violaxanthin, and neoxanthin were made on the basis of their spectral properties, M_{50} values (14), and cochromatography with authentic samples from natural sources. In addition, a number of pigments were found which had not been observed previously in the Chlorophyceae.

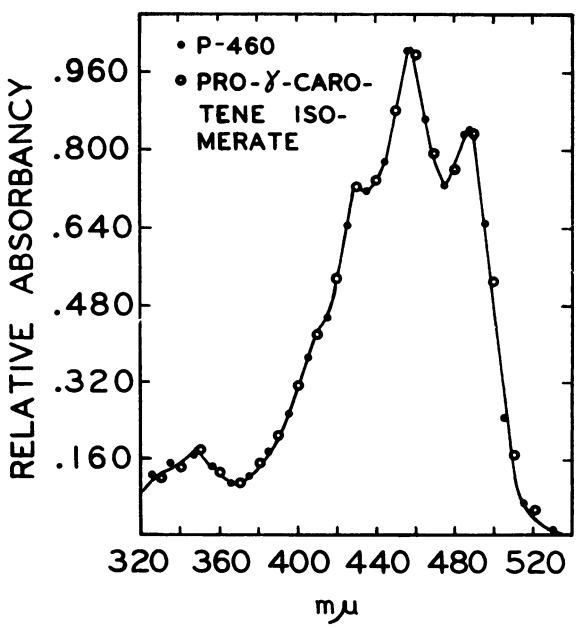

FIG. 1. The absorption spectra of P-460 (○) and of the iodine isomerate of *pro*-γ-carotene (●) (31) in petroleum ether.

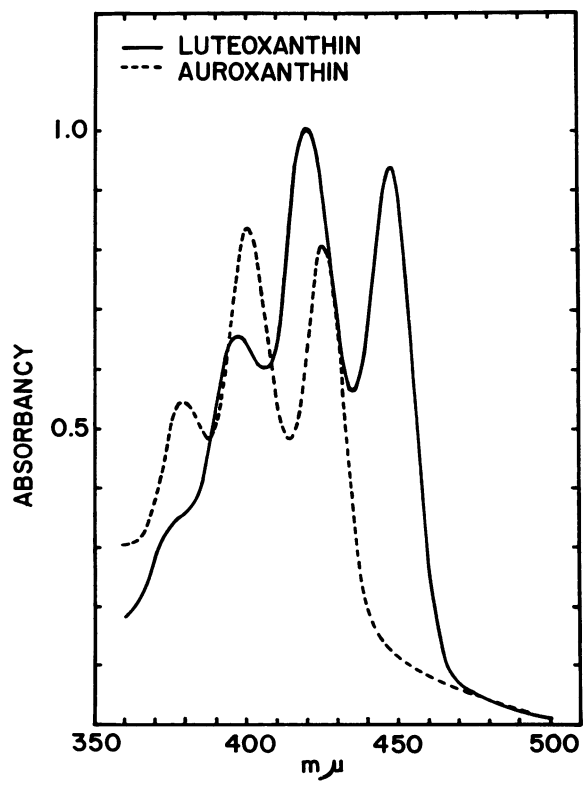

FIG. 2. The absorption spectrum of luteoxanthin (——) in absolute ethanol. Also plotted is the spectrum of the auoxanthin (- - -) formed from luteoxanthin by the addition of hydrochloric acid at a final concentration of 0.006 N.

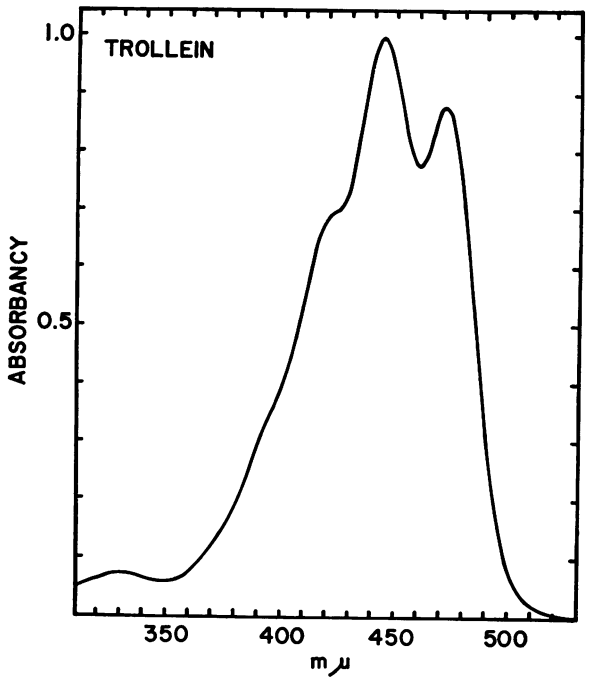

FIG. 3. The absorption spectrum of trollein in absolute ethanol.

Pigment P-460 was observed as a pinkish-orange band moving behind β-carotene on magnesium oxide-celite columns when developed with petroleum ether. It has a partition coefficient of 100: 0 in a 95% methanol: petroleum ether system, which remained unchanged after saponification. The spectrum of this pigment, plotted in figure 1, was virtually unchanged when exposed to light in the presence of iodine.

Figure 2 represents the absorption spectra, in absolute ethanol, of luteoxanthin and the product formed by the addition of a trace of hydrochloric acid. Luteoxanthin can be characterized by its M_{50} value of 64.3, characteristic of a compound containing 2 hydroxyl groups and 2 oxide groups (14), by the hypsochromic displacement of 19 mμ when it is converted to auroxanthin following the addition of a trace of hydrochloric acid, and by cochromatography with an authentic sample of luteoxanthin isolated from acetic acid-treated violaxanthin (28). This pigment is found in the light-grown cultures, but not in dark-grown cultures of the wild type strain (table II).

The absorption spectrum of trollein, present in both light-grown and dark-grown cultures of wild type *C. reinhardi*, is shown in figure 3. Epoxide groups are absent, for when the pigment is treated with dilute hydrochloric acid, there is a hypsochromic displacement of only 1 to 2 mμ, apparently due to the formation of *cis*-isomers. When an ethereal solution of the pigment is treated with concentrated hydrochloric acid, there is no color development, which indicates a lack of a furanoid group. Quantitative partition coefficients yield an M_{50} value of 53.3, indicative of 3 hydroxyl groups.

On occasions, small quantities of pigments with properties similar to mutatochrome, flavoxanthin, and auroxanthin were observed, but they were not present in sufficient quantities for positive identification. An $E\,{}^{1\%}_{1\,cm}$ value of 2250 at 440 mμ was used to determine the concentration of the unknown pigments.

Dark-grown wild type cultures contain about 70% of the carotenoid pigment present in the light-grown cultures. This decrease is due to a lower concentration of xanthophylls in the dark-grown cultures, for the total carotene level does not differ when wild type cultures are grown in the light or in the dark. There is, however, a change in the β-carotene/α-carotene ratio, which drops from a value of 32.3 in the light-grown cultures to 13.6 in the dark-grown cultures. In addition, we were unable to detect zeaxanthin or luteoxanthin in these dark-grown cultures.

Table III. *The Carotenoids of Mutant Strain y-2*

Pigment	Light-grown		Dark-grown	
	μg/g wet wt	%	μg/g wet wt	%
α-Carotene	18.8	2.3	15.6	5.2
β-Carotene	193.8	23.9	140.7	47.3
P-450	5.9	0.7	3.7	1.2
Cryptoxanthin	7.2	0.9	4.5	1.5
Lutein	202.1	24.9	71.8	24.2
Zeaxanthin	2.5	0.3	...	
Violaxanthin	149.6	18.4	31.1	10.5
Trollein	151.3	18.6	22.9	7.7
Neoxanthin	79.8	9.8	6.8	2.3
Total (range of 2 cultures)	811.0 (762–860)		297.1 (290–304)	
β-carotene / α-carotene	10.3		9.0	
xanthophyll / carotene	2.71		0.86	

Table IV. *The Carotenoids of Photosynthetic Mutants of Chlamydomonas reinhardi*

Pigment	*ac-16*		*ac-21*		*ac-115*		*ac-141*	
	μg/g wet wt	%	μg/g wet wt	%	μg/g wet wt	%	μg/g wet wt	%
α-Carotene	6.7	1.3	22.9	4.8	35.9	3.8	23.2	4.4
β-Carotene	109.9	21.3	66.3	13.9	186.2	19.7	129.0	24.5
P-460	...		3.4	0.7	5.7	0.6	8.4	1.6
Cryptoxanthin	...		3.3	0.7	6.6	0.7	...	
Lutein	112.5	21.8	147.9	31.0	258.1	27.3	194.9	37.0
Luteoxanthin	42.3	8.2	5.2	1.1	11.4	1.2	3.2	0.6
Zeaxanthin	...		25.8	5.4	21.7	2.3	7.4	1.4
Violaxanthin	58.8	11.4	89.2	18.7	154.2	16.3	68.5	13.0
Trollein	92.4	17.9	51.5	10.8	127.6	13.5	32.6	6.2
Neoxanthin	80.0	15.5	61.6	12.9	125.7	13.3	59.0	11.2
Unknowns	14.0	2.7	...		11.3	1.7	...	
Total	516.6		477.1		944.4		526.2	
% of light-grown wild type	40.4		37.3		73.8		41.1	
β-carotene / α-carotene	16.4		2.9		5.2		5.6	
xanthophyll / carotene	3.43		4.15		3.15		2.28	

y-2. The carotenoid pigments found in both light-grown and dark-grown cultures of the *y-2* strain are listed in table III. In general, the pigment distribution was similar to that observed with the wild type strain (table II), but *y-2* was found to contain small amounts of cryptoxanthin and to lack luteoxanthin.

Light-grown cultures of *y-2* contain about 63 % of the total pigment found in comparable wild type cultures. However, the dark-grown cultures of *y-2* contain only 32 % of the carotenoid pigment found in dark-grown cultures of wild type. Both light- and dark-grown cultures of *y-2* have β-carotene/α-carotene ratios close to the value found for the dark-grown cultures of wild type.

ac-16. This mutant strain (table IV) contains only 40 % of the carotenoids found in the wild type. It lacks P-460 and zeaxanthin, and contains relatively less β-carotene and violaxanthin and relatively more trollein, neoxanthin, and luteoxanthin. The β-carotene/α-carotene ratio is similar to the value found for wild type cultures grown in the dark.

ac-21. While this mutant strain contains approximately the same amount of carotenoid pigment as does *ac-16* (37 % of the light-grown wild type value, table IV), there is a very marked alteration in the carotene fraction. The mutant contains only 17 to 18 % of the β-carotene found in both light- and dark-grown cultures of wild type strain, and the β-carotene/α-carotene ratio is 2.9, the lowest value recorded for these strains of *C. reinhardi*.

ac-115. With respect to its total carotenoid content, this mutant approaches the level found in light-grown wild type (74 %) and actually contains more than the dark-grown wild type (102 %). However, dark-grown wild type contains twice as much carotene as does *ac-115*. The distribution of pigments in *ac-115* is very similar to light-grown cultures of *y-2*, with the single exception that *ac-115* contains luteoxanthin.

ac-141. Although this mutant contains only 41 % of the total carotenoid found in light-grown wild type cultures (table IV), there is essentially no decrease in the amount of lutein. Thus, the mutant strain is highly enriched with respect to this pigment. There is also an enrichment in the relative amount of α-carotene, resulting in a β-carotene/α-carotene ratio of 5.6.

Discussion

The distribution of carotenoid pigments among the various classes of algae has been reviewed recently (10, 22, 29). In general, it appears that the Chlorophyceae are very similar to green plants, for both groups contain α-carotene, β-carotene, lutein, violaxanthin, and neoxanthin. This pattern can be altered by mutation. Claes described 4 mutant strains of *Chlorella vulgaris* in which the synthesis of the carotenoid pigments was altered (2–6). Allen, Goodwin, and Phagpolngarm described 3 mutant strains of *Chlorella pyrenoidosa* which also had altered carotenoid patterns (1).

The carotenoids of *C. reinhardi* have been studied by Sager and Zaloker (26), and they identified α-carotene, β-carotene, and lutein in their pigment fractions from light-grown wild type cultures. Our results confirm the presence of these 3 well-known Chlorophyceae pigments, and we have also identified the epoxide carotenoids, violaxanthin and neoxanthin, in similar cultures. In addition, we have isolated and identified 3 pigments not previously reported in *C. reinhardi*.

One of these has been designated P-460 from the wave length of the major absorption peak in petroleum ether. From its position on magnesium oxide-celite columns and its partition coefficient, which remains constant with saponification, we conclude that P-460 is a hydrocarbon. As seen in figure 1, the spectrum of P-460 is almost identical to the spectrum obtained by isomerizing pro-γ-carotene (31), and it would therefore appear that P-460 is a naturally occurring *cis*-isomer of γ-carotene. Haxo and Clendenning have reported large amounts of γ-carotene in the gametes of the green alga, Ulva (11).

An additional pigment has been identified as trollein. This was first described by Curl and Bailey (8) as a polyhydroxy xanthophyll present in orange juice. This pigment appears identical to a xanthophyll isolated from green algae by Strain (29). When chromatographed on sugar columns, and developed with 0.5 % *n*-propanol in petroleum ether, the unidentified xanthophyll described by Strain adsorbed between violaxanthin and neoxanthin. We find that trollein behaves similarly when chromatographed with violaxanthin and neoxanthin on a sugar column, forming the middle zone. Krinsky (14) has suggested that this compound is a tri-hydroxy xanthophyll, and Krinsky et al. (16) have also isolated this pigment from another alga, *Euglena gracilis*.

The third new pigment which we have characterized from *C. reinhardi* does not appear to have been observed previously in any algal species. This pigment is luteoxanthin, a monoepoxide monofuranoid xanthophyll first isolated by Curl and Bailey (7) from a number of fruits. Luteoxanthin can be formed in vitro during the acid-catalyzed conversion of violaxanthin to auroxanthin (28). Inasmuch as traces of an auorxanthin-like pigment were occasionally found in the light-grown cultures along with luteoxanthin, there was the possibility that both of these pigments arose from violaxanthin during the extraction process. This seems unlikely, in view of the fact that we found luteoxanthin in all of the light-grown wild type cultures that we examined (both fresh and frozen material), but found none of this pigment in dark-grown cultures of the wild type strain which received identical treatments (table II). In addition, if conditions during the extraction permitted the conversion of violaxanthin to luteoxanthin, one would expect to find neoxanthin converted to its corresponding furanoid, neochrome (9), but this latter pigment was never observed in these extracts. Thus, it appears that the presence of luteoxanthin specifically differentiates light-grown cultures of the wild type from dark-grown cultures.

Zeaxanthin, found frequently in higher plants, has not been previously reported in the Chlorophyceae. This pigment is present in all of the light-grown cultures of *C. reinhardi* reported here except *ac-16*.

There are, therefore, 3 major differences between the light-grown and dark-grown cultures of the wild type strain of *C. reinhardi* with respect to their carotenoid content. (A) Dark-grown cultures contain 28 % less carotenoids than do the light-grown cultures. This decrease in total carotenoid occurs completely at the expense of the xanthophyll fraction, for the total carotenes remain constant in both the light and dark. A consequence of this is the fact that the xanthophyll/carotene ratio falls from a value of 1.9 in the light-grown cultures to 1.1 in the dark-grown cultures. (B) The β-carotene/α-carotene ratio also decreases, from a value of 32.3 in the light-grown cultures to a value of 13.6 in the dark-grown cultures. (C) Zeaxanthin and luteoxanthin are absent from dark-grown cells. Although these 2 pigments account for only about 5 % of the total carotenoid in the light-grown cultures, they should have been readily detectable in the dark-grown cultures, if present.

The full significance of these observations is as yet unknown. It has been shown, however, that dark-grown cultures of the wild type strain cannot carry out a Hill reaction using either *p*-benzoquinone or ferricyanide as an electron acceptor until they have been exposed to light for 15 minutes (R. P. Levine and D. Graham, unpublished observations).

Our results differ from those presented by Sager and Zalokar (26), particularly with respect to the amount of xanthophylls present. We find 12 times as much xanthophyll present in light-grown wild type cultures and twice as much in dark-grown wild type cultures. One factor which might account for some of these differences is the age of the cultures at harvesting. Sager and Zalokar reported using cells harvested at the end of the logarithmic phase of growth, whereas we harvested cells during the logarithmic phase of growth. In addition, they did not report the light intensity at which their cultures were grown.

The mutant strain, *y-2*, appears very similar to wild type when grown in the light, with the exceptions that it contains a small amount of cryptoxanthin, and luteoxanthin is absent. In the dark, when *y-2* is no longer able to synthesize chlorophyll, there is a very marked decrease in the total carotenoids, and this decrease is seen in both the carotene and in the xanthophyll fractions.

In comparison, the pigment mutant described by Sager and Zalokar (26) as a pale green mutant (no. 95) lacks all xanthophylls and contains only α- and β-carotene. The β-carotene/α-carotene ratio is 2.9. This mutant strain was grown in the dark, for it dies when grown aerobically in the light. In this respect, it resembles some of the mutant strains of *Chlorella vulgaris* (2–6) and *Chlorella pyrenoidosa* (1) which will also be killed when exposed to light and to oxygen. The Chlorella mutants lack xanthophylls, but contain colorless polyenes, such as phytofluene and phytoene, which are absent in the wild type strains. These colorless polyenes were not reported in the pale green mutant (26), and they are also absent from strain *y-2*.

The 4 remaining mutant strains of *C. reinhardi* under consideration have impaired photosynthesis. They are unable to fix CO_2 in the light at a rate comparable to the wild type strain (21) and each is pre-

sumed, therefore, to be blocked in some step of photosynthesis. These steps have been partially elucidated. Whole cells or isolated chloroplast fragments of *ac-16* and *ac-21* give a Hill reaction whereas similar preparations of *ac-115* and *ac-141* do not (20, 21). It has also been shown that both *ac-115* and *ac-141* are deficient in plastoquinone (20).

The analysis of the carotenoid pigments in light-grown cells of these 4 mutant strains reveals similarities amongst the mutant types which tend to distinguish them from the wild type strain (table IV). All 4 of these mutant strains have a lower carotenoid content and a lower β-carotene/α-carotene ratio than the light-grown wild type strain. In this respect, these mutants resemble the dark-grown wild type strain, which has similar characteristics. They differ from dark-grown wild type, however, for they have a higher xanthophyll/carotene ratio.

In spite of the similarities between the mutant strains and the dark-grown wild type strain, each strain has a characteristic pattern of types and amounts of carotenoids which distinguishes it from the other mutant strains and from both the dark- and light-grown wild type.

It must be concluded, therefore, that the carotenoid pigments found in these 4 mutants, while characteristic for each strain, do not appear to show sufficient differences from those present in the wild type strain to account for their inability to carry out normal photosynthesis.

Summary

Light-grown cultures of the wild type strain of *Chlamydomonas reinhardi* have been shown to contain the usual pigments of the Chlorophyceae: α- and β-carotene, lutein, violaxanthin, and neoxanthin. In addition, zeaxanthin, luteoxanthin, trollein, and a new carotene, P-460, which appears to be a *cis*-isomer of γ-carotene have been identified.

Dark-grown cultures of the wild type strain contain less total carotenoid, and this decrease occurs exclusively in the xanthophyll fraction, resulting in a lower xanthophyll/carotene ratio than that found in the light-grown cultures. The dark-grown cultures are also characterized by a lower β-carotene/α-carotene ratio, and 2 xanthophylls, zeaxanthin and luteoxanthin, are absent.

The mutant, *y-2*, when cultured in the light, is similar to the wild type strain, except that it lacks luteoxanthin and contains a small amount of cryptoxanthin. In the dark it shows a very marked decrease in total carotenoids.

Light-grown cultures of 4 mutant strains with impaired photosynthetic ability, *ac-16*, *ac-21*, *ac-115*, and *ac-141*, all have characteristic carotenoid patterns which distinguish them from the light-grown wild type strain. They share the properties of having higher xanthophyll-carotene ratios, lower total carotenoids, and lower β-carotene/α-carotene ratios than the corresponding wild type culture. The latter 2 properties are also characteristic of the wild type strain cultured in the dark.

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Determinations of the Heat Transfer Coefficient of a Leaf [1]

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In equilibrium conditions the net radiant energy flux R falling onto a leaf is divided mainly between the flow of latent heat for the evaporation of transpired moisture LE, and sensible heat passing into the ambient air H. The latter defines a heat-transfer coefficient h in terms of a temperature difference, thus

$$H = h(T_{leaf} - T_{air}). \qquad \text{I}$$

Where the heat transfer is the result of convection, the coefficient may be replaced (10, 14, 15) by an equivalent diffusion resistance r

$$r = c_p \rho / h = \frac{0.018 \text{ sec/cm}}{h}, \qquad \text{II}$$

approximately, where c_p is the specific heat of air at constant pressure (cal/g °C), ρ is the density of air (g/cm³), and h is the heat-transfer coefficient (cal/cm²min °C). However, it is convenient in the present note to retain the concept of the transfer coefficient. The aim is to review previous experimental methods of deriving the coefficient, and then to describe a modification.

Methods

Steady-State Methods. Brown and Escombe (2) used equation I to derive the coefficient, which was called the 'emissivity' in their paper. This term implies radiative transfer, which certainly occurs, but it accounts for only 0.008 cal/cm²min °C of the value of the heat-transfer coefficient (18). The flux H was reckoned by Brown and Escombe as the difference between the radiation flux R and the latent-heat flux

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