needs little attention. A lower rate is possible if a simple ethanol trap is connected to the exit pipe to prevent back-contamination with air.

The counter has a plateau of 1275–1775 v with a slope of 1.2 % per 100 v. With a working potential of 1400 v the counter is stable. The background is stable over long periods (about 50 counts/min. in a lead castle 1 in. thick).

The counter can be connected to a standard or log rate meter coupled to a pen recorder. A relay on the fraction-cutter siphon records each fraction on paper. The maximum rate of flow of the liquid depends on the radioactivity and the integrating time of the rate meter. With the geometry used and with ^{14}C in a liquid of specific gravity 1.0, approximately 1 % of disintegrations are recorded. This means that the counter gives the same number of counts as 0.2 ml. of the liquid evaporated and counted under an end-window Geiger-Müller tube. The counter must be calibrated each time it is reassembled, because the geometry of the liquid

film may be altered. The pressure of the liquid should be kept constant.

The counter provides a continuous, immediately available record of radioactivity coming off a column, and it obviates a large number of tedious sampling functions. It is not intended as an instrument for accurate measurements.

SUMMARY

A simple helium-ethanol flow counter suitable for indicating radioactivity due to weak β -emitting isotopes in the effluent from a chromatographic column is described.

I wish to thank Dr J. E. S. Bradley and Mr R. C. Holloway for their advice, Mr C. Doré for constructing the counter, and Mr K. R. Hobbs for his technical assistance. This counter was exhibited at the Second International Isotope Conference at Oxford, 1954.

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Studies in Carotenogenesis

20. CAROTENOIDS OF SOME SPECIES OF *CHLOROBIVM**

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No unequivocal information about the presence or absence of carotenoids in the green sulphur bacteria is available. van Niel & Muller (1931) failed to detect carotenoids in their cultures, but Katz & Wassink (1939) reported their presence in a different strain. It has now been possible to examine three species of *Chlorobium*; carotenoids were found in all and they have been separated and identified. A preliminary report of this work has already been given (Goodwin & Land, 1955). Barer (1955) has also recently mentioned the presence of γ -carotene in species of *Chlorobium*.

EXPERIMENTAL

Cultures. The three species of *Chlorobium* examined were *C. thiosulphatophilum* (kindly supplied by Dr June Lascelles, Dept. of Biochemistry, The University of Oxford), *C. limicola*, and an unidentified halophilic species, both kindly supplied by Mr K. R. Butlin, Chemical Research Laboratory, D.S.I.R., Teddington, Middlesex.

Culture medium. The medium used was that recommended by Larsen (1952). For culturing the halophilic

strain the NaCl concentration was increased from 1.0 to 2.5 % (w/v).

Diphenylamine media were made up from a quarter-saturated solution (1/140 000) of diphenylamine as the solvent instead of pure water.

Cultural conditions. The bacteria were grown anaerobically in completely filled glass-stoppered bottles at 30° in a glass incubator illuminated by two banks of four 60 w tungsten-filament lamps (Garton, Goodwin & Lijinsky, 1951). *Phycomyces blakesleeana*, required as a source of γ -carotene, was grown under conditions described by Garton *et al.* (1951).

Extraction and separation of pigments. The centrifuged cells were first extracted twice with methanol to remove chlorophyll (Polgár, van Niel & Zechmeister, 1944; Goodwin & Osman, 1953) and then twice with ethyl ether. The combined methanol fractions were saponified by the method of Goodwin & Morton (1946) to remove chlorophyll and lipids that might interfere with chromatography. The unsaponifiable matter was extracted, combined with the original ethereal extract, dried by standing over anhydrous Na_2SO_4 , evaporated to dryness *in vacuo* at 30° and redissolved in light petroleum (b.p. 40–60°) ready for chromatography (throughout this work the term light petroleum refers to a fraction with boiling point 40–60°).

The pigments were separated by chromatography on activated alumina (P. Spence and Co., Widnes, grade O),

* Part 19: Goodwin (1956).

grade O deactivated with methanol (Goodwin & Srisukh, 1949), an appropriate mixture of the two, or a MgO/Celite (2:1) mixture (Starr & Saperstein, 1953).

Isomerization of pigments. This was carried out by treatment with I_2 , according to the method of Zechmeister (1944).

Rubixanthin. A chromatographically homogeneous specimen of this pigment was obtained from ripe rose hips (Kuhn & Grundmann, 1934) by the method of Goodwin (1956).

RESULTS

Chlorobium limicola

A typical chromatogram for the separation of the polyenes from *C. limicola* is given in Table 1. It will be seen that the pigment system consists virtually of two zones, 3 and 7. Zone 3 has been identified as γ -carotene by (a) its absorption spectrum with maxima at 435, 460 and 490.5 $m\mu$. in light petroleum and 450, 473 and 504.5 $m\mu$. in benzene and characteristic shape (Fig. 1); (b) the

behaviour (hypophasic in the light petroleum/95% v/v, aqueous-methanol system) indicate that it is rubixanthin (monohydroxy- γ -carotene) (Kuhn & Grundmann, 1934). This has been confirmed by chromatographic comparison with a chromatographically homogeneous specimen of rubixanthin obtained from rose hips. When mixed, the two pigments could not be separated on columns of either weakened alumina or a mixture of MgO/Celite (2:1).

Zone 1, from its green fluorescence in ultraviolet light and absorption maxima [at 330 (inflexion), 348 and 368 $m\mu$.] contains traces of phytofluene. It occurs in such small quantities that it has not been possible to separate it completely from traces of a yellow material which run very closely with it on chromatography.

Zone 2 represents small amounts of pro- γ -carotene. Its absorption spectrum (Fig. 2) does not correspond exactly in shape with that of pro- γ -

Table 1. *Separation of polyenes of Chlorobium limicola*

Zones in order of increasing adsorptive affinity; adsorbent deactivated alumina; developer light petroleum containing varying amounts of ethyl ether.

Zone no.	Description	Absorption maxima in light petroleum ($m\mu$)	Concn. of ether (v/v) required for elution (%)	Relative amount of total polyene present (%)	Provisional identification
1	Very pale-yellow, slight green fluorescence	348, 368, 430	0-1	Trace	Mainly phytofluene
2	Pale orange	—	1	Trace	Pro- γ -carotene
3	Yellow-orange	435, 460, 490.5	2-3	85	γ -Carotene
4	Orange	425, 448 (indefinite)	6	Trace	Indefinite
5	Yellow	428	8	Trace	Indefinite
6	Orange-pink	446, 470, 500	10	Trace	Indefinite
7	Orange	435, 458, 487	12-15	12-15	Rubixanthin

inability to separate it chromatographically from an authentic specimen of γ -carotene from *Phycomyces blakesleeana* (Goodwin, 1952a); (c) its very characteristic spreading on a column owing to ease of isomerization (Zechmeister & Schroeder, 1942); (d) its partition behaviour: it is epiphasic when partitioned between light petroleum and 95% (v/v) aqueous methanol; (e) on isomerization by iodine changes characteristic of an all-*trans* carotenoid occur: the extinction, definition and position of the maxima all drop. The resulting absorption spectrum (Fig. 1) is very similar to that given by Zechmeister (1944) for isomerized γ -carotene, except that although the extinction of the isomerized pigment in the region 350-360 $m\mu$. is higher than that of the all-*trans* pigment, it has never been possible to obtain a marked *cis* peak at about 355 $m\mu$.

Zone 7 has the same absorption spectrum as γ -carotene, but its adsorptive affinity and partition

Fig. 1. Absorption spectrum of γ -carotene (fraction 3, Table 1) in light petroleum. Before isomerization, —; after isomerization, — — —.

carotene described by Zechmeister & Schroeder (1942), mainly owing to contamination with traces of a pigment absorbing at slightly lower wavelengths. The minute traces available precluded complete purification. The reaction of this pigment with iodine is, however, very characteristic of pro- γ -carotene. Immediately on adding iodine there is a marked darkening in colour; the absorption spectrum increases in intensity, shifts very considerably to longer wavelengths and resembles closely the absorption spectrum of isomerized γ -carotene (Fig. 2). Isomerization of γ -carotene or

Fig. 2. Absorption spectrum of pro- γ -carotene (fraction 2, Table 1) in light petroleum. Before isomerization, —; after isomerization, — — —.

lycopene with iodine does not yield any pro- γ -carotenes or prolycopene, but treatment of these procarotenes with iodine results in the production of the usual equilibrium mixture of these pigments (Zechmeister & Petracek, 1952). The extinction of the maximum at 435 m μ is slightly too high for isomerized γ -carotene (see Fig. 1), because of the presence of traces of the impurity just mentioned.

Zones 4–6 represent small traces of materials with indeterminate absorption spectra. They may be artifacts, and have not been examined in detail.

Chlorobium thiosulphatophilum and the halophilic Chlorobium species

The pigments obtained from these other species examined were identical with those obtained from *C. limicola*, except that, in the halophilic strain, rubixanthin was either absent or present in much reduced quantities.

Action of diphenylamine

Diphenylamine inhibits the synthesis of the normal carotenoid components in some members of the Thiorhodaceae and Athiorhodaceae (Goodwin & Osman, 1953, 1954; Goodwin & Land, unpublished observations); the synthesis of the more saturated polyenes—phytoene, phytofluene, ζ -carotene and neurosporene—is, however, stimulated. At a level of 1/70 000 diphenylamine inhibits growth in all three species of *Chlorobium*. At a level of 1:140 000, however, it has qualitatively a similar effect to that just described. The synthesis of γ -carotene and rubixanthin is virtually completely suppressed. Phytoene and ζ -carotene appear in diphenylamine cultures, but only in very small amounts; the amount of phytofluene is also slightly increased. This stimulation is quantitatively much less than that observed in the purple bacteria. Neurosporene has never been observed in diphenylamine cultures of *Chlorobium*, and this also applies to phytoflueneol, hydroxy- ζ -carotene and hydroxyneurosporene, which have recently been found in diphenylamine cultures of some purple bacteria (Goodwin & Land, unpublished observations).

Two unidentified colourless materials have also been observed in small amounts in diphenylamine cultures. One, non-fluorescent ($\lambda_{\max}$ 257 m μ in light petroleum), running very close to phytofluene, is probably the same as that observed in diphenylamine cultures of *Phycomyces blakesleeanus* (Goodwin, 1952a); the other, fluorescing blue ($\lambda_{\max}$ 274 m μ in light petroleum) is adsorbed between phytofluene and ζ -carotene. These materials have not been investigated further.

DISCUSSION

The occurrence of γ -carotene and its derivatives as the major carotenoids in *Chlorobium* is unique. These pigments do not occur in any other photosynthetic bacterium yet examined (Goodwin & Land, unpublished observations) and, with one exception, do not exist in the photosynthetic tissues of higher plants (Goodwin, 1952b). The exception is the leaf of the marsh dodder (*Cuscuta salina*), in which γ -carotene is the major carotenoid component (Mackinney, 1935). γ -Carotene was also thought to be absent from the Algae, but recently Strain (1951) has shown that it is present in trace amounts in a number of green algae (Chlorophyceae).

In general, however, γ -carotene can be considered to be a pigment much more characteristic of non-photosynthetic than of photosynthetic tissues, being widely distributed in fruit, flower petals and fungi, especially the last (Goodwin, 1952b). In non-

photosynthetic bacteria, however, its existence appears to be confined to the Mycobacteriaceae (Goodwin & Jamikorn, 1956).

The effect of diphenylamine on species of *Chlorobium* is qualitatively similar to its effect on *Rhodospirillum rubrum* (Goodwin & Osman, 1954) and a species of *Chromatium* (Goodwin & Land, unpublished observations), and different from its effect on *Rhodopseudomonas spheroides* (Goodwin, Land & Osman, 1955) and the algae *Chlorella vulgaris* (Goodwin, 1954) and *Euglena gracilis* v. *bacillaris* (Goodwin & Jamikorn, 1954), where growth and carotenoid synthesis are equally inhibited and there is no stimulation of synthesis of phytofluene etc.

SUMMARY

1. *Chlorobium limicola*, *Chlorobium thiosulphatophilum* and an unnamed halophilic species produce γ -carotene as the main carotenoid pigment (85 % of the total). Rubixanthin also occurs in the first two species (10–15 % of the total). Traces of pro- γ -carotene and three unidentified pigments also exist; the last may be artifacts.

2. Diphenylamine strongly inhibits the synthesis of γ -carotene and its derivatives, but only slightly stimulates the production of phytoene, phytofluene and ζ -carotene.

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Studies on Cholinesterase

10. RETURN OF CHOLINESTERASE ACTIVITY IN THE RAT AFTER INHIBITION BY CARBAMOYL FLUORIDES*

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Eserine, prostigmine and analogous carbamates have, in contrast to the organophosphorus derivatives, generally been regarded as reversible inhibitors of the cholinesterases. However, a detailed study of the mechanism of inhibition of the pseudo-cholinesterase by a compound of this type (Myers, 1952) showed that the reaction was similar to that

proposed for the inhibition by the organophosphorus derivatives (cf. Burgen, 1949; Burgen & Hobbiger, 1951; Wilson, 1951, 1952; Aldridge, 1950, 1953; Aldridge & Davison, 1952a, 1953). The inhibited enzyme is considered to be a dimethyl-carbamoyl derivative for an inhibitor such as prostigmine (cf. Myers, 1954) and therefore analogous to the proposed dialkyl phosphoryl derivatives of the esterases. This conclusion was

* Part 9: Myers (1953).