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# Paper-Chromatographic Separation of Chlorophylls and Carotenoids from Marine Algae

By S. W. JEFFREY
*C.S.I.R.O. Marine Laboratory, Cronulla, Sydney, Australia*

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Although many methods have been described for the partial separation of chloroplast pigments by paper chromatography (see review by Šesták, 1958) the species examined have been mainly higher plants and algae of the class Chlorophyceae, in which the main chloroplast pigments are chlorophylls *a* and *b*, with β-carotene and lutein as the major carotenoids. In the present work, a method was required for studying the pigment composition of planktonic algae which occur in the oceanic waters off Sydney. Representatives are found not only of the Chlorophyceae but also of the Bacillariophyceae, Dinophyceae and Chrysophyceae, and one would expect to find chlorophylls *a*, *b* and *c* together with a wide range of carotenoids (Strain, 1958). The method described below, which is a modification of the two-dimensional method of Lind, Lane & Gleason (1953), has enabled studies of the pigment composition of such diverse algal groups to be made, since it provides a complete separation of mixtures of chlorophylls *a*, *b* and *c*, carotenes and the xanthophylls lutein, violaxanthin, neoxanthin, fucoxanthin, peridinin and astaxanthin, as well as a number of xanthophyll pigments which occur in relatively small quantities.

## EXPERIMENTAL

### *Materials*

Representatives from four classes of marine algae were studied: green flagellates from the Chlorophyceae, diatoms from the Bacillariophyceae, naked dinoflagellates from the Dinophyceae and golden-brown flagellates from the Chrysophyceae (Fritsch, 1948). The material used was obtained either from uni-algal, but not bacteria-free, cultures of marine algae, or from mixed natural plankton in sea-water samples. The uni-algal cultures studied included four green flagellates [*Dunaliella tertiolecta* Butcher, *Nannochloris atomus* Butcher, *Chlorella stigmatophora* Butcher and *Tetraselmis suecica* (Kylin) Butcher], three diatoms [*Phaeodactylum tricornutum* Bohlen, *Nitzschia closterium* (Ehr.) and *Skeletonema costatum* (Grev.)], one dinoflagellate (*Gymnodinium* sp.) and two golden-brown flagellates (*Isochrysis galbana* Parke and *Sphaleromantis* sp.). *Phaeodactylum*, *Dunaliella* and *Isochrysis* were obtained from Dr Mary Parke of the Marine Laboratory, Plymouth. The other organisms were isolated from mixed plankton samples taken from the coastal waters off Sydney.

The organisms were grown in an Erdschreiber medium, consisting of sea water enriched with nitrates, phosphates and soil extract. The soil extract was prepared by autoclaving 1 kg. of soil with 1 l. of tap water at 15 lb./in.$^2$ for 2–3 hr., and filtering through an Eaton–Dikeman Paper 541. After diluting 1 vol. of the resulting extract with 2 vol. of tap water, concentrated stock nutrient media were prepared by adding 0·2 g. of $NaNO_3$ and 0·03 g. of $Na_2HPO_4,12H_2O$ to every 50 ml. of soil extract, and autoclaving at 5 lb./in.$^2$ for 1 hr. The final culture medium was prepared when needed by adding 50 ml. of this enriched extract to 1 l. of filtered sea water, and autoclaving at 5 lb./in.$^2$ for 1 hr. Stock cultures of algae were maintained in 200 ml. of culture medium in 250 ml. Erlenmeyer flasks at 18°, and illumination was from 40 w fluorescent tubes giving a light intensity of approx. 400 ft.-candles. Bulk cultures (4 l.) were continuously aerated in 5 l. Erlenmeyer or Haffkine flasks, and were used for analysis after 2–4 weeks' growth. The algae were harvested by continuous centrifuging at 5000*g* and the cells were washed several times by centrifuging at about 2000*g* for 10 min. This process removed most of the bacteria, as judged by microscopic examination under phase contrast.

In addition to these uni-algal cultures, mixed natural plankton from sea-water samples was used for pigment analysis. At least 35 l. of sea water was needed to obtain sufficient pigments for chromatography. The sea water was

collected in 3 gallon plastic jars and the algae were harvested and extracted within 2 hr. of collection of the water sample at sea.

## *Methods*

*Preparation of pigment extracts.* All reagents and solvents used for the extraction and chromatography were A.R. chemicals, and were used without further purification. Throughout the entire extraction period the tubes containing the pigments were kept in the dark as much as possible.

Cells were harvested by centrifuging, and with bulk cultures or large volumes of sea water continuous centrifuging at 5000*g* was used (Davis, 1957). The sedimented cells (between 0·1 and 0·5 ml. of wet packed cells) were extracted several times for 1–2 min. with small volumes (5–10 ml.) of 90% acetone until the residue was colourless. The combined acetone extracts were mixed with an equal volume of diethyl ether in a separating funnel, and washed thoroughly with at least 5 vol. of 10% (w/v) NaCl. It is necessary to use diethyl ether instead of light petroleum in this purification step, since chlorophyll *c* is insoluble in the latter solvent. The ether separated the pigments quantitatively from the acetone- and water-soluble impurities. After several washings with NaCl, the ether layer containing the pigments was collected, centrifuged briefly to remove suspended water droplets, transferred to a Petri dish and evaporated to dryness by blowing a stream of cool air over the solution. After evaporation of the ether, the pigment residue was freed from the remaining water droplets by drying in a current of air. This procedure was found more satisfactory than drying under a vacuum, or by the use of anhydrous sodium sulphate, which adsorbed the pigments. The pigment residue was then redissolved in the minimum quantity of diethyl ether and sometimes a trace of acetone was necessary to facilitate solution. Any remaining water droplets were removed by centrifuging before chromatography, since for good resolution of the pigments on the chromatogram it was essential that the extract be completely dry.

Pigments were not extracted with equal ease from all species of algae. The diatoms and $\mu$-flagellates, which were particularly resistant, were first suspended in water (for 1–3 min. for diatoms and 30 min. for the $\mu$-flagellates). This caused the cells to swell, and extraction then proceeded with little difficulty.

Since, when plankton from sea-water samples was left overnight in 90% acetone (Humphrey, 1960), there was always some breakdown (about 5–10%) of the chlorophylls to pheophytins, the cells were suspended in water for about 30 min. before acetone extraction, and 98% of the pigments were released by this treatment. If the residue was then left overnight in acetone, only a trace (less than 2% of the total) of material absorbing at 665 m$\mu$ was obtained. Chromatograms of extracts obtained by the water treatment were free of pheophytin components, except when large amounts of decomposing cells were seen microscopically before extraction. In these cases the pheophytins were presumably present in the original plankton sample.

*Chromatography.* Two-dimensional ascending chromatography was carried out at room temperature (18–23°) in the dark. Whatman 3MM chromatography paper was cut into 22 cm. squares, the extract, containing about 20–30 $\mu$g. of pigment, was applied at the origin, the paper was clipped together to form a cylinder and placed in a jar previously equilibrated with fresh solvent mixture.

*Solvents.* Methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol and *iso*pentanol in concentrations up to 4% (v/v) in light petroleum, b.p. 60–80°, were tested, and 4% propan-1-ol in light petroleum was chosen as giving the best resolution of carotenes, pheophytins and chlorophylls *a*, *b* and *c*. Because the xanthophylls overlay chlorophylls *a* and *b*, development of the chromatogram at right angles to the first dimension was necessary for the separation of these pigments.

This was achieved by using, as the second solvent, 30% (v/v) chloroform in light petroleum, b.p. 60–80° (cf. Lind *et al.* 1953). The chromatograms were developed for about 30 min. in each solvent, and allowed to dry in the dark for a few minutes after each run. Since the composition of both solvent mixtures was critical for good resolution of the pigments, the solvents were made up freshly each day.

If pigment extraction and chromatography were completed in 2 hr., no breakdown of the labile pigments resulted, and chromatograms of young cultures of algae were free of pheophytin spots.

For quantitative measurements, portions of pigment extracts were chromatographed and the spots corresponding to the various pigment fractions were eluted with ethyl ether for the carotenoids and with acetone for the chlorophylls. The extinction of the eluate was read in a Unicam SP. 500 spectrophotometer, and the results are expressed as mg. of pigment/g. dry wt. of cells.

*Identification of pigments.* Pigments were characterized after elution from the chromatogram by their absorption spectra as described by Strain, Manning & Hardin (1944), Goodwin (1955), Smith & Benitez (1955), Strain (1958) and Anderson (1959). Good agreement with the published data was obtained. Table 1 shows the absorption maxima obtained for the pigments studied. Examples from only a few organisms are given, but in all cases similar agreement with the published data was obtained. It must be noted, however, that contamination of any one xanthophyll fraction with small quantities of similar pigments would not be detected spectrophotometrically.

That the astaxanthin found in *Nannochloris atomus* was esterified was indicated by its chromatographic and spectrophotometric similarity with esterified astaxanthin prepared from eggs of *Artemia salina* (Gilchrist & Green, 1960). In addition, esterified astaxanthin from both sources was epiphasic when solutions of the pigment in light petroleum were shaken with 90% methanol (Goodwin & Srisukh, 1949).

The pigment fractions were further identified by co-chromatography with authentic samples in the two solvent systems (4% propan-1-ol in light petroleum and 30% chloroform in light petroleum). The pigments from two previously examined organisms were used as standards. *Dunaliella tertiolecta*, studied by Gilchrist & Green (1960), provided standard chlorophylls *a* and *b*, carotenes, lutein, violaxanthin and neoxanthin, and *Nitzschia closterium*, analysed by Strain *et al.* (1944), provided standard chlorophylls *a* and *c*, carotenes, diatoxanthin, diadinoxanthin, fucoxanthin and neofucoxanthins. Since no previously examined dinoflagellate cultures were available to us we had no authentic source of dinoxanthin or peridinin, and in these two cases provisional identification rested solely on absorption data. In every other case co-chromatography of

Table 1. *Absorption maxima of pigments in marine algae separated by paper chromatography*

References: 1, Smith & Benitez (1955); 2, Anderson (1959); 3, Strain, Manning & Hardin (1944); 4, Goodwin & Srisukh (1949).

| Pigment | Published maxima (mμ) | Reference | Maxima found (mμ) | Solvent | Organism |
|---|---|---|---|---|---|
| Chlorophyll *a* | 663, 615, 580, 535, 430, 410 | 1 | 665, 618, 580, 535, 430, 412 | Acetone | *Dunaliella tertiolecta* |
| Chlorophyll *b* | 645, 595, 455 | 1 | 645, 598, 455 | Acetone | *D. tertiolecta* |
| Chlorophyll *c* | 626, 577·5, 443·6 | 1 | 625, 580, 445 | Ethyl ether | *Isochrysis galbana* |
| Lutein | 422, 445, 476 | 2 | 422, 445, 474 | Ethyl ether | *D. tertiolecta* |
| Violaxanthin | 421, 440, 470 | 2 | 421, 441, 471 | Ethyl ether | *D. tertiolecta* |
| Neoxanthin | 414, 436, 466 | 2 | 414, 437, 466 | Ethyl ether | *D. tertiolecta* |
| Diatoxanthin | 453, 481 | 3 | 453, 481 | Ethanol | *I. galbana* |
| Diadinoxanthin | 448, 478 | 3 | 448, 478 | Ethanol | *I. galbana* |
| Fucoxanthin | 453 | 3 | 453 | Ethanol | *I. galbana* |
| Neofucoxanthin A<br>Neofucoxanthin B | 447<br>446 | 3 | 447* | Ethanol | *I. galbana* |
| Dinoxanthin<br>Diadinoxanthin | 441·5, 471<br>448, 478 | 3 | 446, 475* | Ethanol | *Gymnodinium* sp. |
| Peridinin<br>Neoperidinin | 475<br>464 | 3 | 470* | Ethanol | *Gymnodinium* sp. |
| Esterified astaxanthin | 503<br>488 | 4 | 501<br>486 | Carbon disulphide<br>Pyridine | *Nannochloris atomus* |
| α- and β-Carotene mixtures | 428, 452, 480 | 2 | 429, 450, 478 | Ethyl ether | *D. tertiolecta* |

* One spot obtained and assumed to be a mixture of these two components.

a standard pigment with the appropriate pigment fraction from a previously unexamined organism resulted in a single spot on the chromatogram in both solvent systems. This was taken as satisfactory evidence for the identification of the pigments.

## RESULTS

### *Separation of pigments*

Fig. 1 shows chromatograms obtained from typical representatives of the Chlorophyceae, Bacillariophyceae, Dinophyceae and Chrysophyceae. The chlorophylls and carotenes showed good separation and in uni-algal cultures the xanthophylls also gave good resolution. However, when extracts from different organisms were mixed and chromatographed together some overlapping of the xanthophyll zones occurred. In mixtures of diatoms and dinoflagellates peridinin appeared between fucoxanthin and neofucoxanthins A and B, and overlapped both components. In mixtures of green flagellates and diatoms, violaxanthin, diatoxanthin and diadinoxanthin appeared as one spot on the chromatogram. α- and β-Carotene did not separate. Esterified astaxanthin (e.g. in *Nannochloris atomus* or in sea-water samples containing a few zooplankton) appeared as a pink zone which ran closely behind the carotene fraction in both solvents. Good resolution of the pigments from sea-water samples was obtained, and these corresponded to the pigments expected on the basis of microscopic examination of algal types in the sample.

Mean $R_F$ values for the chloroplast pigments of marine algae are given in Table 2, and Table 3 shows quantitative data on the pigment composition of some algae. The extinction coefficients used for the carotenoids are those quoted by Goodwin (1955), but where these are unknown the extinction coefficient for carotene was used (Allen, Goodwin & Phagpolngarm, 1960). Extinction coefficients for chlorophylls *a* and *b* are given by Smith & Benitez (1955). No extinction values for pure chlorophyll *c* are available, and the provisional value of 22·0 given by Smith & Benitez (1955) was used. This extinction value was considered more accurate than the 'specific pigment units' used for the estimation of chlorophyll *c* by Richards & Thompson (1952).

A red cryptomonad, *Chroomonas erroticon nom. prov.*, which has recently been obtained in uni-algal culture, contained chlorophylls *a* and *c*, carotenes, three unidentified xanthophyll fractions, as well as a pink fluorescent water-soluble phycoerythrin fraction with an absorption maximum at 550 mμ, which is as yet unidentified.

### *Decomposition products*

Pheophytins were prepared as described by Smith & Benitez (1955) and $R_F$ values for pheophytins *a*, *b* and *c* are shown in Table 2. Pheophytin *a* was always present in extracts of old cultures and in plankton samples that contained much decomposing material. Pheophytin *b* was not observed in any extracts of the algae examined. In extracts

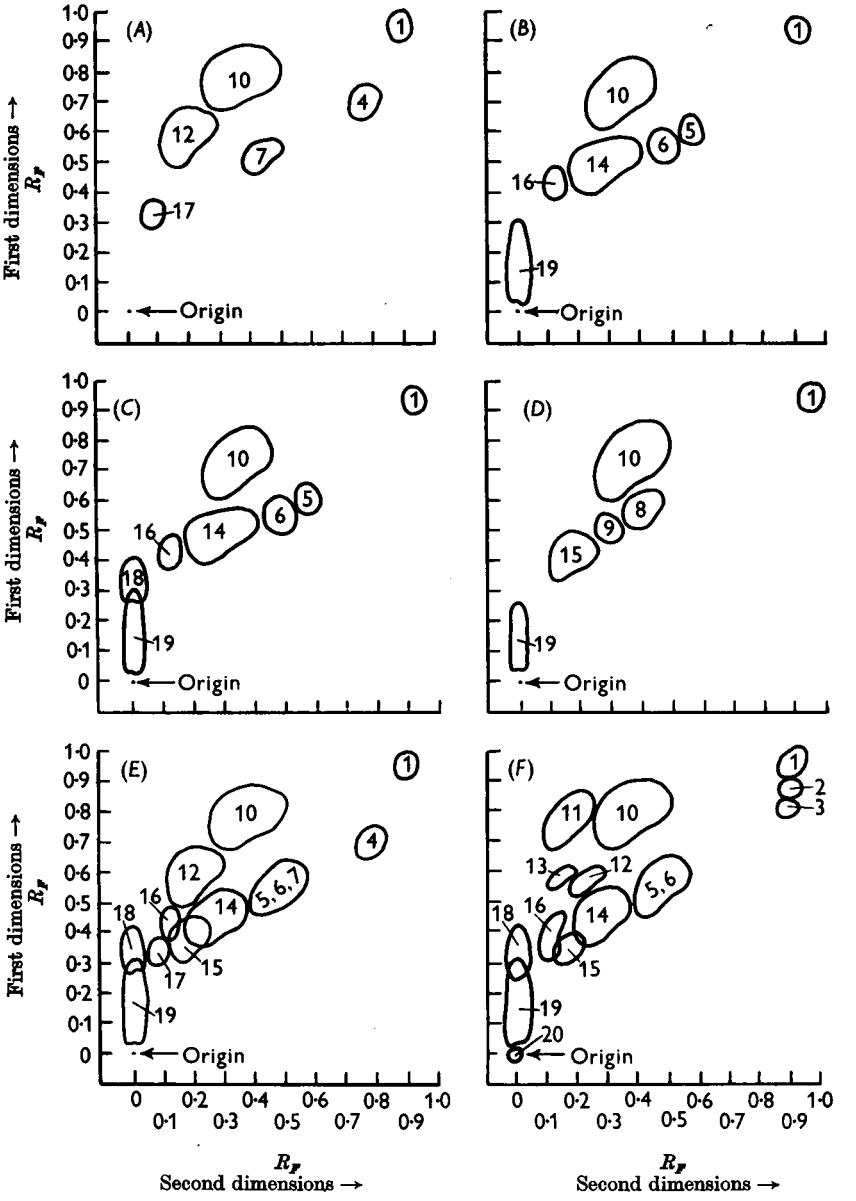


Fig. 1. Two-dimensional chromatograms of pigments in marine algae. (*A*) *Dunaliella tertiolecta*. (*B*) *Isochrysis galbana*. (*C*) *Skeletonema costatum*. (*D*) *Gymnodinium* sp. (*E*) Mixed extract of *Skeletonema costatum*, *Dunaliella tertiolecta* and *Gymnodinium* sp. (*F*) Sea-water extract containing mixed diatoms and dinoflagellates. Chromatographic solvent system: first dimension, 4% propan-1-ol in light petroleum; second dimension, 30% chloroform in light petroleum. 1, Carotenes (orange); 2, esterified astaxanthin (pink); 3, pheophytin *a* (grey-green); 4, lutein (yellow); 5, diatoxanthin (yellow); 6, diadinoxanthin (yellow); 7,violaxanthin (yellow); 8, dinoxanthin + diadinoxanthin (yellow); 9, neodinoxanthin + neodiadinoxanthin (yellow); 10, chlorophyll *a* (blue-green); 11, chlorophyll *a'* (blue-green); 12, chlorophyll *b* (olive green); 13, chlorophyll *b'*(olive green); 14, fucoxanthin (deep orange); 15, peridinin (reddish orange); 16, neofucoxanthin A and B (orange); 17, neoxanthin (yellow); 18, chlorophyllide *a* (blue-green); 19, chlorophyll *c* (light green); 20, pheophytin *c* (light greenish-brown).

derived from old cultures, or extracts that were aged for several days, the chlorophyll *a* and *b* spots sometimes separated into two zones during development in the chloroform–light petroleum solvent. These may be the isomerization products which Strain (1958) designated chlorophyll *a'* and chlorophyll *b'*.

### *Second chlorophyll* a *fraction*

Several of the marine algae (*Phaeodactylum tricornutum*, *Skeletonema costatum* and the flagellate *Sphaleromantis* sp.) and some of the sea-water samples that were collected under conditions of a diatom bloom showed a blue–green zone in addition to the usual chlorophyll *a* spot. This new blue–green zone ran just ahead of chlorophyll *c* after development with 4% propan-1-ol in light petroleum, but was never completely separated from it. This new chlorophyll did not move in chloroform–light petroleum. The absorption spectrum in acetone resembled closely that of chlorophyll *a*, with maxima at 663, 615, 580, 535, 429 and 417 mμ, but its chromatographic behaviour was quite different. The unknown pigment could be separated from chlorophyll *c* with ethyl ether as the chromatographic solvent. Under these conditions, the carotenes, xanthophylls and 'normal' chlorophyll *a* travelled together just behind the solvent front, followed by a discrete blue–green spot (the second

Table 2. *$R_F$ values of pigments from marine algae separated by two-dimensional chromatography*

These $R_F$ values are mean values for pigments separated from the ten marine algae studied. Because $R_F$ values in these systems may vary according to the quantity of pigments put on the paper, with the time of development and the organism used (Šesták, 1958), the values indicate the relative position of the pigments on the chromatogram, rather than absolute values.

| Pigments | $R_F$ First dimension | $R_F$ Second dimension |
|---|---|---|
| Carotenes | 0·96 | 0·96 |
| Chlorophyll *a* | 0·84 | 0·29 |
| Chlorophyllide *a* | 0·38 | 0 |
| Chlorophyll *b* | 0·65 | 0·10 |
| Chlorophyll *c* | 0·20 | 0 |
| Esterified astaxanthin | 0·87 | 0·88 |
| Lutein | 0·74 | 0·73 |
| Violaxanthin | 0·65 | 0·48 |
| Neoxanthin | 0·32 | 0·05 |
| Diatoxanthin | 0·57 | 0·60 |
| Diadinoxanthin | 0·54 | 0·44 |
| Dinoxanthin | 0·54 | 0·44 |
| Fucoxanthin | 0·49 | 0·28 |
| Neofucoxanthin A and B | 0·49 | 0·08 |
| Peridinin and neoperidinin | 0·51 | 0·23 |
| Pheophytin *a* | 0·87 | 0·96 |
| Pheophytin *b* | 0·70 | 0·89 |
| Pheophytin *c* | 0·00 | 0·00 |

Table 3. *Pigment composition of some marine algae*

Values given are mg. of pigment/g. (dry wt.) of cells; +, present; –, not detected.

| Pigments | Chlorophyceae | | | | Bacillariophyceae | | | Chrysophyceae | | Dinophyceae |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Dunaliella tertiolecta* | *Nanno-chloris atomus* | *Chlorella stigma-tophora* | *Tetra-selmis suecica* | *Phaeo-dactylum tricornutum* | *Skeletonema costatum* | *Nitzschia closterium* | *Isochrysis galbana* | *Sphalero-mantis* sp. | *Gymno-dinium* sp. |
| Chlorophyll *a* | 5·56 | 1·23 | + | + | 5·22 | 0·38 | 3·26 | 2·96 | 2·43 | 0·97 |
| Chlorophyll *b* | 2·63 | 0·61 | + | + | – | – | – | – | – | – |
| Chlorophyll *c* | – | – | – | – | 1·34* | 0·21* | 1·18* | 1·14* | 0·68* | 0·57* |
| Carotenes | 0·99 | 0·07 | + | + | 0·31 | 0·02 | 0·12 | 0·38 | 0·10 | 0·09 |
| Lutein | 1·43 | 0·23 | + | + | – | – | – | – | – | – |
| Violaxanthin | 0·61 | 0·05 | + | + | – | – | – | – | – | – |
| Neoxanthin | 0·29 | 0·11 | + | + | – | – | – | – | – | – |
| Diatoxanthin | – | – | – | – | – | 0·03† | 0·12† | 0·40† | 0·13† | – |
| Diadinoxanthin | – | – | – | – | 0·22† | 0·03† | 0·15† | 0·40† | – | 0·17† |
| Dinoxanthin | – | – | – | – | – | – | – | – | 0·10† | |
| Neodiadinoxanthin | – | – | – | – | – | – | – | – | – | 0·02† |
| Neodinoxanthin | – | – | – | – | – | – | – | – | – | |
| Fucoxanthin | – | – | – | – | 4·15 | 0·43 | 3·78 | 1·88 | 1·68 | – |
| Neofucoxanthin A and B | – | – | – | – | 1·20 | 0·15 | 1·51 | 0·53 | 0·64 | – |
| Peridinin and neoperidinin | – | – | – | – | – | – | – | – | – | 0·52† |
| Esterified astaxanthin | – | 0·08† | – | – | – | – | – | – | – | – |
| Total chlorophylls | 8·19 | 1·84 | | | 10·04 | 1·09 | 7·53 | 7·07 | 4·88 | 3·01 |
| Total carotenoids | 3·32 | 0·54 | | | 5·88 | 0·66 | 5·68 | 3·59 | 2·65 | 0·80 |

* Values for *E* not available; provisional value of 22·0 (Smith & Benitez, 1955) was used. † Values for *E* not available; $E^{1\%}_{1\,cm.}$ (2500) for carotene was used.

chlorophyll *a* derivative). Chlorophyll *c* moved a little from the origin. The basicity test of Willstätter & Stoll (1913) showed this material to be a chlorophyllide derivative of chlorophyll *a*, and it was subsequently found that the conversion of chlorophyll *a* into the new form resulted from the presence of a highly active chlorophyllase in some of the algae. The occurrence and properties of this enzyme in marine algae will be discussed elsewhere.

## DISCUSSION

Some workers have tacitly assumed that two-dimensional chromatography is too slow for the resolution of labile plant pigments (Šesták, 1958), but the method which is described here is rapid and simple, and no apparent decomposition of pigments occurs if the precautions of quick extraction and absence of light are observed.

Complete separation of all the minor xanthophyll components was not achieved by the method, although for any one organism the xanthophyll resolution was considered satisfactory. In mixtures of organisms the xanthophyll spots showed a certain degree of overlapping, particularly peridinin and fucoxanthin. These two pigments could be resolved by a third development in the direction of the second dimension with higher proportions of chloroform to light petroleum (up to 60 %, v/v).

Astaxanthin, which is usually thought to be an animal carotenoid, but which has been described in the green alga *Haematococcus pluvialis* (Kuhn, Stene & Sorensen, 1939; Goodwin & Jamikorn, 1954), was found in an esterified form in the green $\mu$-flagellate *Nannochloris atomus*, which was isolated from the coastal waters off Sydney. The concentration of this pigment increased appreciably with the age of the culture.

The xanthophylls in the two members of the Chrysophyceae which were examined are of some interest. Both organisms contained fucoxanthin and isomers as the major carotenoids and diatoxanthin as a minor pigment. *Isochrysis galbana* showed in addition the presence of small quantities of diadinoxanthin, whereas *Sphaleromantis* contained a yellow pigment similar to dinoxanthin, with absorption maxima at 477 and 444 m$\mu$ in ethanol. Allen *et al.* (1960) identified fucoxanthin and diatoxanthin in members of the Chrysophyceae, and Dales (1960) found fucoxanthin and diadinoxanthin in cultures of *Isochrysis galbana*. The latter author claimed that chlorophyll *c* was not present in any of eight members of the Chrysophyceae which he examined, whereas chlorophyll *c* was found in all cultures of *Isochrysis galbana* and *Sphaleromantis* studied in the present work. The failure to find chlorophyll *c* apparently resulted from the use of light petroleum, in which chlorophyll *c* is insoluble, instead of diethyl ether in the extraction procedure.

The quantitative measurements showed that the concentration of chlorophylls was approximately 1½–3 times the concentration of the carotenoids in the organisms studied. The concentration of the accessory chlorophylls *b* and *c* was of the same order of magnitude as that of chlorophyll *a*, although the chlorophyll *c* values are tentative figures only, owing to uncertainty about the true extinction values for chlorophyll *c*. In the diatoms and golden-brown flagellates fucoxanthin and isomers constituted 80–90 % of the total carotenoids, and peridinin accounted for 70 % of the total carotenoids in the dinoflagellate cultures. Each of the minor components in these organisms (carotene, diatoxanthin, diadinoxanthin etc.) represented about 5 % of the total carotenoids. Lutein, in the green flagellates, accounted for 40 % of the total carotenoids, with carotene, violaxanthin and neoxanthin present in quantities ranging from 10 to 20 % of the total.

The four green flagellates (Chlorophyceae) studied contained chlorophylls *a* and *b* and carotenes and gave three prominent xanthophyll zones corresponding to lutein, violaxanthin and neoxanthin. The three diatoms (Bacillariophyceae) and the two golden-brown flagellates (Chrysophyceae) showed a similarity in their pigment composition with chlorophylls *a* and *c*, carotenes and fucoxanthin and isomers as the major xanthophylls. The dinoflagellate (Dinophyceae) was distinguished by the presence of peridinin in place of fucoxanthin as the major xanthophyll, in addition to chlorophylls *a* and *c*, carotenes and the minor xanthophylls shown in Fig. 1. It is therefore apparent from the present work that chlorophylls *a*, *b* and *c*, the carotenes and the xanthophylls lutein, fucoxanthin and peridinin are quantitatively the most important photosynthetic pigments found in algae which comprise the phytoplankton organisms studied.

## SUMMARY

1. Two-dimensional paper chromatography with propan-1-ol–light petroleum and chloroform–light petroleum mixtures separated the chloroplast pigments of marine algae belonging to the classes Chlorophyceae, Bacillariophyceae, Dinophyceae and Chrysophyceae.

2. The pigments separated included chlorophylls *a*, *b* and *c*, their pheophytins, the carotenes, the xanthophylls lutein, violaxanthin, neoxanthin, fucoxanthin and isomers, peridinin, esterified astaxanthin and a number of minor xanthophyll components.

3. The concentration of the accessory chlorophylls *b* and *c* was of the same order of magnitude as that of chlorophyll *a*. Fucoxanthin and peri-

dinin, and their isomers, constituted 80–90 % of the total carotenoids in the organisms which contained these pigments.

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# Intrinsic Factor: Active and Inhibitory Components from the Mitochondria of Human Gastric Mucosal Cells

BY W. H. TAYLOR, BARBARA J. MALLETT AND K. B. TAYLOR
*Department of Chemical Pathology, Ashton Street, Liverpool 3, and Nuffield Department of Medicine, Radcliffe Infirmary, Oxford*

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Cell particles, separated by high-speed ultracentrifuging from preparations of human and rat gastric mucosa, have been shown to contain as much as half of the intrinsic-factor activity of the whole preparation (Taylor, 1955). The activity of the human particles may be removed by disruption with water, in which the active material subsequently dissolves, but in the rat the activity remains bound to the disrupted particles (Taylor, Mallett, Witts & Taylor, 1958*b*). The activity from the human particles may be further concentrated into a 'substance E' (O'Brien, Taylor, Turnbull & Witts, 1955), which on paper electrophoresis, electrophoresis in the Tiselius apparatus and Svedberg ultracentrifuging cannot be resolved into more than one component. Substance E promotes the absorption of $^{60}$Co-labelled vitamin $B_{12}$ in patients with pernicious anaemia in doses of about 10 mg., but this represents a low order of activity and it has been provisionally suggested that substance E may be a precursor of intrinsic factor (O'Brien *et al.* 1955). Further investigations into the activity of substance E and related studies are now reported.

## METHODS

Substance E was prepared as described by O'Brien *et al.* (1955) from human gastric mucosa.

Paper electrophoresis was carried out at room temperature for 16 hr. in a horizontal bath with a current of 1 mA/4 cm. width of paper strip and diethylbarbituric acid–sodium diethylbarbiturate buffer (pH 8·6, *I* 0·06). The paper strips were stained with 1 % bromophenol blue in ethanol saturated with mercuric chloride.

Ultracentrifugal studies were carried out in the Oxford Svedberg ultracentrifuge under the guidance of Professor A. G. Ogston, F.R.S.

Intrinsic-factor activity was assayed in patients with pernicious anaemia in remission by giving 0·5 $\mu$g. of $^{60}$Co-labelled vitamin $B_{12}$ in aqueous solution by mouth, alone and then with preparations of intrinsic factor, and comparing the amounts of radioactivity appearing in the stools. Measurements of activity were made in a ring of Geiger counters, similar to that described by Bradley (1957). In order to compare quantitatively results obtained with different fractions and patients, the increase of absorption