Some Aspects of Algal Chemistry.

EIGHTH HUGO MÜLLER LECTURE DELIVERED ON OCTOBER 16TH, 1941.

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TWENTY years have passed since a Hugo Müller lecture has been directed to a botanical subject. On that occasion, the late Professor Benjamin Moore dealt with the fundamental problem of photosynthesis (J., 1921, **119**, 1555). It must be admitted that from the angle of organic chemistry our knowledge of this all-important chemical process has not advanced appreciably during the intervening years. In many other directions, however, the past twenty years have proved astonishingly fruitful in adding to our knowledge concerning the diverse materials elaborated by the living plant. Thus we have witnessed on the one hand the unravelling of the structures of the sugars, starches, and cellulose, those all-important primary products of photosynthesis. Further, the essential structure of chlorophyll has been elucidated and its total synthesis will doubtless be achieved within the next decade. Again, other pigments common to the Higher Plants, the anthocyanins and carotenoids have also been fully examined and their constitutions determined. A whole host of other biologically active substances of most varied character, hormones, vitamins, and enzymes, have been isolated and in many cases synthesised, and the detailed study of their reactions has led to a fuller understanding of biological processes.

It is curious that in this galaxy of discovery the study of the Algæ has, from the chemical aspect, largely remained in a side-channel, the more so as among members of this branch of the Thallophyta are to be found many of the simplest types of unicellular forms which one would reasonably expect to provide ideal material for the investigation of the still obscure life processes of the living cell, notably

TABLE I.

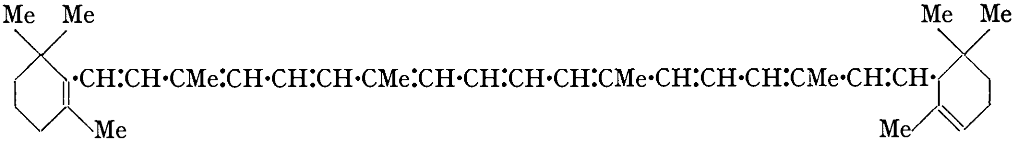
Algæ.

Class.	Pigments.	Food reserve.	Cell wall.	Structure.	Reproductive cells.	Habitat.	No. of Species.
Chlorophyceæ	Grass-green algæ. Plastids contain same pigments, in same proportion, as Higher Plants	Usually starch	Mainly cellulose	Unicellular or filamentous or multicellular	Motile cells with equal flagellæ. Sexual and asexual reproduction	Freshwater with marked terrestrial tendency. Few marine	5700
Xanthophyceæ	Yellow-green plastids. Similar pigments to Chlorophyceæ, but with excess of carotene	Usually oil or leucosin	Rich in pectin compounds and silica	Unicellular or simple filaments	Motile reproductive cells with flagellæ unequal in length	Mainly freshwater	150
Chrysophyceæ	Yellow-golden brown plastids	Fats and leucosin	Pectin, cellulose, sometimes silica	Unicellular or colonial	Motile cells with one or two cilia. Sexual reproduction not known	Mostly freshwater. Particularly plankton in lakes	210
Bacillariophyceæ (Diatoms)	Yellow or brown plastids	Fats and volution, never starch	Pectin substance, impregnated with silica	Unicellular	No ciliated cells	Freshwater and marine	5300
Cryptophyceæ	Brownish plastids	Starch or starch-like compound	Mostly naked protoplast	Unicellular	Motile cells. Two unequal flagellæ	Mostly freshwater. Few marine	30
Dinophyceæ	Golden to chocolate-brown plastids (few species without plastids)	Starch or oil	Elaborated cellulose plates	Mostly unicellular	Motile cells with cilia. Sex reproduction rare	Mostly marine	960
Chloromonadineæ	Bright green plastids with excess of xanthophyll	Oil		Unicellular	Motile cells with two almost equal cilia	Freshwater only	*ca.* 6
Euglenineæ	Grass-green or colourless plastids	Paramylon (related to starch) or fats	No cell wall, *i.e.*, naked protoplast	Unicellular	Motile cells with one, two or three flagellæ. Sexual reproduction very rare	Almost all freshwater	335
Phæophyceæ	Normal pigments masked by golden-brown fucoxanthin; proportion of chlorophyll *b* less than in Chlorophyceæ	Stored in dissolved state like sugar-storing Higher Plants	Inner of cellulose, outer of pectin compound	Always multicellular. Largest and most complex Algæ	Motile cells with two lateral cilia. Sexual reproduction normal	Almost exclusively marine and generally of colder waters	900
Rhodophyceæ	Pigments contain red, water-soluble phycoerythrin in addition to usual pigments. Sometimes blue phycocyanin as well, especially under intense illumination	Insoluble floridean starch. Some contain a galactoside of glycerol as well	Cellulose and pectin compounds	Mostly multicellular, somewhat complex plant body	No motile cells at all. Sexual reproduction of specialised kind	Mostly marine. A few species in freshwater	2500
Myxophyceæ	Unlike all other algæ, pigments *not* in plastids but diffuse through outer layers of protoplasm. Blue-green colour due to addition of blue phycocyanin, sometimes red phycœrythrin as well	Sugars and glycogen	Mainly cellulose. Pectin outer. Mucilage coat	Unicellular or filamentous	No motile reproductive cells. No sexual reproduction	Mainly freshwater	1400

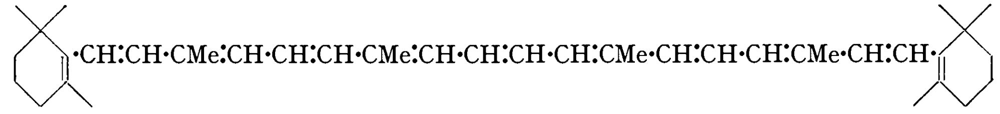
photosynthesis and reproduction. To some extent, this lack of interest may be due to the difficulty of obtaining the required material, but it seems to me that a more likely explanation is the lack of sufficiently close collaboration in this country between the various branches of Science. In directing attention to-day to the Algæ, I intend to confine my remarks to the lipoid constituents with special reference to the carotenoids and sterols. According to the eminent algologist Fritsch, the Algæ are most suitably divided into eleven distinct Classes (Fritsch, " Structure and Reproduction of the Algæ," Cambridge University Press, 1935). This classification is based very largely upon the colour of the pigments contained in the chromatophores, but other factors, such as reserve foods, form and propagative methods, are also taken into account. A summary of the main characteristics of each Class is reproduced in Table I, from which it will be noted that the colour factor is indeterminate, a fact borne out by a literature search, which reveals an almost complete lack of precise information concerning the individual pigments of the various Classes (compare also Boresh, " Handbuch der Pflanzenanalyse," Vienna, 1932). The first methodical attempt to clarify the position was made by Kylin (*Z. physiol. Chem.*, 1927, **166**, 39), who, using a capillary method of analysis, identified by means of absorption bands ten carotenoids in various types of Algæ. As, however, Kylin's work was purely of a qualitative nature, and as many of the pigments named by him have no parallel in modern carotenoid nomenclature, it seemed desirable to undertake a fresh and comprehensive study of the subject, examining representatives of each Class, including as far as possible Algæ of the various Orders.* Our aims were, first, to relate the algal pigments, so far as possible, to those of the Higher Plants, or failing such relationship, to elucidate their chemical constitution. We hoped further to establish by this chemical analysis the validity or otherwise of the accepted botanical relationship between colour and classification and finally we considered it likely that this study would throw light upon other biological and physiological factors. Although it has as yet been impossible to cover the whole field, we have been successful in examining Algæ selected from seven of the eleven Classes into which Fritsch has classified them. Moreover, during the course of our work details of investigations carried out in certain Continental laboratories have appeared with the result that our knowledge concerning the carotenoid pigments of the Algæ is now almost as extensive as that of the Phanerogams.

As already mentioned, we have included an examination of the sterols in our survey, and many interesting points have emerged from this study which, we believe, may not be without significance in relation to sexual reproduction.

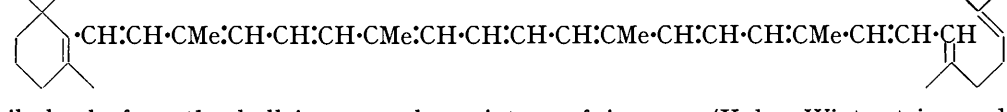
Before dealing with the individual lipoid pigments found in the Algæ, I would like to recall to you the structures of certain characteristic carotenoids of the Higher Plants. Here, first and foremost, stand carotene and " xanthophyll," which pigments invariably occur associated with chlorophylls *a* and *b* in all green leaves (Willstätter and Stoll, " Untersuchungen über die Assimilation der Kohlensaure," Berlin, 1913), and are therefore the most widely distributed, naturally occurring organic compounds. As is well known, at least three isomeric carotenes (α, β and γ) occur in Nature, the structures of which are shown in the formulæ (I), (II), and (III).



(I.)



(II.)



(III.)

Similarly, leaf xanthophyll is a complex mixture of isomers (Kuhn, Winterstein, and Lederer, *Z. physiol. Chem.*, 1931, **197**, 141), of which lutein (IV) is the main constituent, but according to Strain (" Leaf Xanthophylls," Carn. Inst. Wash., Pub. No. 490, 1938) the isomeric zeaxanthin (V) is also

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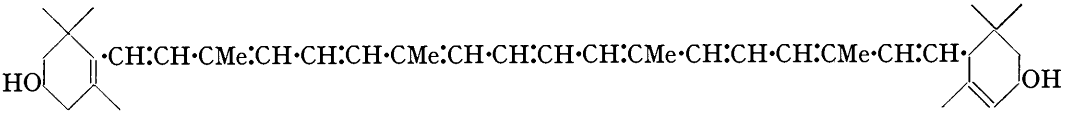
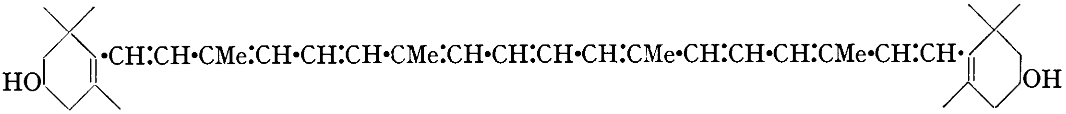
* I desire to take this opportunity of placing on record the indebtedness of my chemical collaborators and myself to Mr. P. W. Carter of the Botany Department, University College of Wales, Aberystwyth, for his enthusiastic co-operation, not only in the difficult task of collecting the various classes of pure Algæ, but more especially in connexion with the correlation of chemical factors with botanical classification.

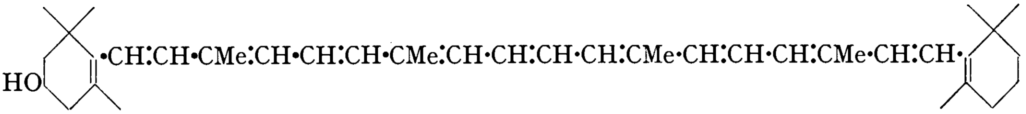
invariably present in small amount, as are also kryptoxanthin (VI) and the structurally indefinite pigments flavoxanthin, $C_{40}H_{56}O_3$, and violaxanthin, $C_{40}H_{56}O_4$. The only other carotenoids of the Higher Plants of which mention need be made are crocin and crocetin (VII), the former of which is the digentiobiose ester of the latter (Karrer and Kiki, *Helv. Chim. Acta*, 1929, **12**, 985). Crocetin itself is capable of existence in *cis*- and *trans*-forms and the corresponding dimethyl esters have been isolated and found to differ appreciably in their light absorption, the labile *cis*-isomer having its absorption maxima displaced towards the ultra-violet (Kuhn and Winterstein, *Ber.*, 1933, **66**, 209). I do not propose to discuss the methods of extraction and isolation of the individual algal pigments, as these have already been described in detail elsewhere (Carter, Heilbron, and Lythgoe, *Proc. Roy. Soc.*, 1939, *B*, **128**, 62; see also Kylin, *K. fysiogr. Sällsk. Lund. Forh.*, 1939, **9**, 1). Whenever possible we have attempted to isolate the pigments and characterise them by melting point, crystalline form, absorption maxima and specific colour reactions. Reliance solely upon spectroscopic or adsorption evidence can well be misleading, as the carotenoids readily undergo spontaneous isomerisation (Zechmeister and Tuzson, *Biochem. J.*, 1938, **32**, 1305; *Ber.*, 1939, **72**, 1340), giving labile isomerides which may spectroscopically simulate some other pigment. Thus β-carotene isomerises readily into ψ-α-carotene, which has absorption maxima identical with those of α-carotene (Carter and Gillam, *Biochem. J.*, 1939, **33**, 1320).



(IV.)



(V.)



(VI.)

$HO_2C{\cdot}CMe{:}CH{\cdot}CH{:}CH{\cdot}CMe{:}CH{\cdot}CH{:}CH{\cdot}CH{:}CMe{\cdot}CH{:}CH{\cdot}CH{:}CMe{\cdot}CO_2H$ (VII.)

Among the Algæ the Chlorophyceæ or Green Algæ comprise a very large number of diverse forms, many of which are freshwater, while others are terrestrial or marine. The first detailed examination of any member of this group was made by Willstätter and Page (*Annalen*, 1914, **404**, 237), who, working with *Ulva lactuca*, a marine green alga growing between tide marks, observed carotene and xanthophyll. We have now examined nine individuals of the Chlorophyceæ and, as shown in Table II, in every case these pigments have been observed. *Trentepohlia aurea*, a strictly terrestrial alga, requires special mention. It occurs attached to rocks, tree trunks, and generally in regions of rather high rainfall as an orange-yellow felt, the colour of which was attributed by Cohn to the presence of a special orange-red pigment which he named hæmatochrome (*Arch. microsk. Anat.*, 1867, **3**, 44). We have now found that the actual pigment is β-carotene, the content of which in this alga is unusually high, being of the order of 0·2% of the dry weight. As in dry leaves of land plants the percentage of carotene averages 0·05%, it will be seen that *Trentepohlia aurea* is one of the richest sources of carotene hitherto described (compare also Tischer, *Z. physiol. Chem.*, 1936, **243**, 103). On the other hand, xanthophyll is only present in minute amount, whereas in other Chlorophyceæ and also in the Higher Plants the xanthophyll–carotene ratio is of the order 2—5 : 1 (Willstätter and Page, *loc. cit.*; see also Seybold and Egle, *Jahrb. wiss. Bot.*, 1938, **86**, 50). Another interesting observation is that *Trentepohlia*, unlike other members of the Class, is deficient in sterol, and in this respect resembles members of the Myxophyceæ which will be referred to later.

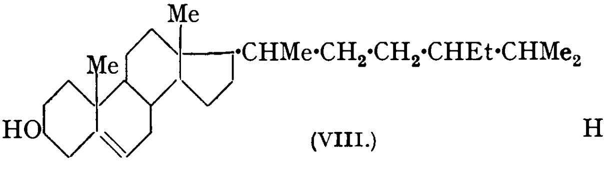
We have not in all cases attempted a complete separation of the carotene isomers, but in general β-carotene very largely predominates, sometimes indeed to the complete exclusion of the α-isomeride. Similarly, under " xanthopyll " we mean here the mixed hypophasic pigment which is predominantly lutein. In many cases, in addition to the typical lutein absorption maxima at 5060, 4730, and 4410 A., other maxima at 5000 and 4720 A. have been observed which may be due either to *neo*lutein (Zechmeister and Tuzson, *loc. cit.*), or possibly to some closely related xanthophyll of the type Strain (*loc. cit.*) has observed in the xanthophyll of the Higher Plants. Kylin (1939, *loc. cit.*) claims that he has obtained spectroscopic evidence of the presence of Strain's violaxanthin *b* in the green algæ examined by him. This pigment corresponds to the phylloxanthin of his earlier publications (*Z. physiol. Chem.*, 1927, **166**, 39)

TABLE II.

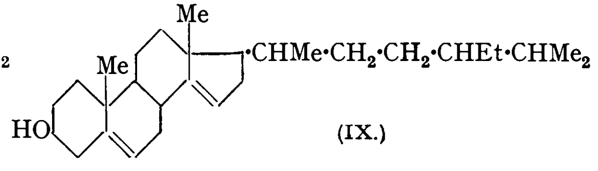
Genus and species.	Order.	(1).	(2).	(3).	(4).	(5).	(6).	(7).	(8).	(9).	(10).	(11).	(12).
Chlorophyceæ.													
Ulva lactuca	Ulotrichales	+	.	.	+	.	.	.	.	.	+	.	.
Enteromorpha compressa	,,	+	.	.	+	.	.	.	.	.	+	.	.
Cladophora Sauteri	Cladophorales	+	.	.	+	.	.	.	.	.	+	+	.
Trentepohlia aurea	Chætophorales	+	.	.	+	.	.	.	.	.	.	.	.
Pleurococcus Nægelii	,,	+	.	.	+	.	.	.	.	.	+	.	.
Œdogonium	Œdogoniales	+	.	.	+	.	.	.	.	.	+	.	.
Zygnema pectinatum	Conjugales	+	.	.	+	+	.	.	.	.	+	.	.
Vaucheria hamata	Siphonales	+	.	.	+	.	+	.	.	.	+	.	.
Nitella opaca	Charales	+	.	.	+	.	.	.	.	.	+	+	.
Xanthophyceæ.													
Botrydium granulatum	Heterosiphonales	+	.	.	.	.	.	+	.	+	+	.	.
Bacillariophyceæ.													
Nitzschia closterium	Pennales	+	.	.	+	+	.	.	.	.	.	+	+
Chrysophyceæ.													
Apistonema Carteri	Chrysomonadales	+	.	.	+	+	.	.	.	.	.	+	+
Thallochrysis litoralis	,,												
Glœochrysis maritima	,,												
Phæophyceæ.													
Pilayella littoralis	Ectocarpales	+	.	.	+	+	.	.	.	.	.	+	.
Ectocarpus tomentosus	,,	+	.	.	+	+	.	.	.	.	.	+	.
Sphacelaria cirrhosa	Sphacelariales	+	.	.	?	+	.	.	.	.	.	+	.
Stypocaulon scoparium	,,	+	.	.	?	+	.	.	.	.	.	+	.
Cladostephus spongiosus	,,	+	.	.	.	+	.	.	.	.	.	+	.
Dictyota dichotoma	Dictyotales	+	.	.	?	+	.	.	.	.	.	+	.
Chorda filum	Laminariales	+	.	.	.	+	.	.	.	.	.	+	.
Laminaria digitata	,,	+	.	.	.	+	.	.	.	.	.	+	.
Fucus ceranoides	Fucales	+	.	.	.	+	.	.	.	.	.	+	.
Fucus vesiculosus	,,	+	.	.	.	+	.	.	.	.	.	+	.
Ascophyllum nodosum	,,	+	.	.	.	+	.	.	.	.	.	+	.
Halidrys siliquosa	,,	+	.	.	.	+	.	.	.	.	.	+	.
Rhodophyceæ.													
Porphyra umbilicalis	Bangiales	+	.	.	+	.	.	.	.	+	.	+	.
Lemanea mamillosa	Nemalionales	+	.	.	+	.	.	.	.	+	+	.	.
Gelidium corneum	Gelidiales	+	.	.	+	.	.	.	.	.	+	.	.
Dilsea edulis	Cryptonemiales	+	.	.	+	.	.	.	.	.	Too little material available for identification		
Polyides rotundus	,,	+	.	.	+	.	.	.	.	.			
Corallina officinalis	,,	+	.	.	+	.	.	.	.	.	.	.	+
Polysiphonia fastigiata	Ceramiales	+	.	.	+	.	.	.	.	.	+	.	.
Polysiphonia nigrescens	,,	+	.	.	+	+	.	.	.	.	.	+	.
Ceramium rubrum	,,	+	.	.	+	.	.	.	.	.	+	.	.
Chondrus crispus	Gigartinales	+	.	.	+	.	.	.	.	.	+	.	.
Gigartina stellata	,,	+	.	.	+	.	.	.	.	.	+	.	.
Phyllophora membranifolia	,,	+	.	.	+	.	.	.	.	.	+	.	.
Ahnfeltia plicata	,,	+	.	.	+	.	.	.	.	.	.	.	+
Rhodymenia palmata	Rhodymeniales	+	.	.	+	.	.	.	.	.	.	+	.
Plocamium coccineum	,,	+	.	.	+	.	.	.	.	.	+	.	.
Myxophyceæ.													
Oscillatoria rubescens	Hormogonales	+	+	+	+	.	.	+	+	.	.	.	.
Rivularia atra	,,	+	+	+	+	.	.	.	.	.	.	.	.
Rivularia nitida	,,	+	+	+	+	.	.	.	.	.	.	.	.

Column (1) Carotene; (2) Myxoxanthin; (3) Other epiphasic pigments; (4) Xanthophyll; (5) Fucoxanthin; (6) Violaxanthin; (7) Flavoxanthin; (8) Myxoxanthophyll; (9) Other hypophasic pigments; (10) Sitosterol; (11) Fucosterol; (12) Other sterols.

which he distinguishes from ordinary xanthophyll by the blue-green colour given with 25% hydrochloric acid. He also claims to find traces of zeaxanthin (previously named by him phyllorhodin). We ourselves have identified violaxanthin only in *Vaucheria hamata*, from which a crystalline specimen was obtained. This member of the Siphonales is known to be abnormal, being one of the few Chlorophyceæ which regularly store their food reserve as oil instead of starch, and the arrangement of its cilia is also unusual. It is intriguing to speculate on the cause of these divergencies and one wonders how far they find their origin in the unusual pigmentation of the chromatophore—is the pigment the directive influence or do the abnormal factors mentioned above determine the nature of the pigment? An examination of *Zygnema pectinatum*, a yellowish-green alga found in ponds and streams, has revealed the presence of fucoxanthin, which is the characteristic carotenoid of the Phæophyceæ. According to the botanists no abnormality in the life cycle or habitat of this alga is known, and here again the occurrence of an unusual xanthophyll can only be a matter for conjecture; is the chemical analysis revealing the phylogenetic past or is it pointing to some impending change? Apart from these abnormalities, this recent work strongly supports the view held by most algologists, that the terrestrial plants originated among the more advanced members of the Chlorophyceæ. Our examination of the sterols present in members of this Class adds weight to the above view. It is known that the characteristic sterol of the Phanerogams is sitosterol (VIII) and we have observed its presence without exception in all the Chlorophyceæ examined by us, independent of habitat (Carter, Heilbron, and Lythgoe, *loc. cit.*). In the case of *Cladophora sauteri* and *Nitella opaca*, both members of somewhat specialised orders, the presence of fucosterol of probable structure (IX), the typical sterol of the Phæophyceæ, has also been observed (Heilbron, Parry, and Phipers, *Biochem. J.*, 1935, **29**, 1976).



(VIII.)



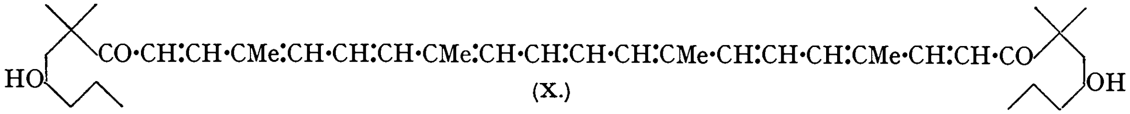
(IX.)

The Xanthophyceæ, named owing to their yellow-green chromatophores, form a small distinctive Class which at one time was included with the Chlorophyceæ. Unfortunately we have only been able to examine one member, the common mud-alga *Botrydium granulatum.* β-Carotene has been definitely identified and among the hypophasic lipochromes we have observed the presence of a pigment having well-defined absorption maxima at 4750 and 4470 A. and giving a strong blue colour with 25% hydrochloric acid. The absorption values are in exact agreement with those recorded by Kuhn and Brockmann (*Z. physiol. Chem.*, 1932, **213**, 192) for flavoxanthin. The only information in the literature concerning the xanthophyll of the Xanthophyceæ is the recognition that it gives a blue colour with concentrated hydrochloric acid. This could apply to either violaxanthin or flavoxanthin, but the spectrographic evidence excludes the former, and thus it may well be that flavoxanthin is characteristic of all the Xanthophyceæ.

Pascher has suggested (*Ber. deutsch. Bot. Ges.*, 1921, **39**, 236) a relationship, probably from a common ancestry, between the Xanthophyceæ, Bacillariophyceæ and Chrysophyceæ. His views are based on a number of similarities such as absence of starch, deposition of silica, and certain resemblances in the structure of the cellular envelopes. In order to ascertain whether this evidence of relationship extends to the carotenoids and sterols we have examined *Nitzschia closterium* as representing the Bacillariophyceæ (Diatoms) and also a mixture of three members of the Chrysophyceæ, *Apistonema Carteri Anand.*, *Thallochrysis litoralis Anand.*, and *Glœochrysis maritima Anand.*, kindly collected and classified for us by Professor Fritsch. From both Classes we have isolated identical carotenoids, *viz.*, carotene, lutein, and fucoxanthin; the analogy extends also to the sterols, where we find only fucosterol (see Table II). This analysis strongly supports Pascher's view that the Bacillariophyceæ and Chrysophyceæ have points of affinity and we suggest they are also possibly connected with the more primitive orders of the Phæophyceæ (Ectocarpales). On the other hand no affinity between these Classes and the Xanthophyceæ is observed, although admittedly the opportunity of drawing conclusions has been restricted by paucity of material.

Reference has already been made to the Phæophyceæ or Brown Algæ, a Class possessing botanical features of quite distinctive character. In appearance they vary in colour from olive-green to dark brownish-black and many varieties are among the largest of marine flora. The typical chromolipoid is fucoxanthin, which was first isolated and analysed by Willstätter and Page (*loc. cit.*) and given the formula $C_{40}H_{56}O_6$ or $C_{40}H_{54}O_6$. The pigment has absorption maxima at 5100, 4770, and 4450 A. and in this respect resembles α-carotene and lutein. It is further characterised by the deep blue colour it gives with hydrochloric acid and more especially by its extreme lability towards alcoholic alkali, which converts it into *iso*fucoxanthin (Heilbron and Phipers, *Biochem. J.*, 1935, **29**, 1369), which is more basic in character than the natural pigment as shown by the fact that it gives a blue colour with even dilute acetic acid. In *iso*fucoxanthin the absorption maxima are displaced towards the ultra-violet with the band heads at 4740 and 4480 A., similar to flavoxanthin. This susceptibility to alkali sharply differentiates fucoxanthin from violaxanthin and flavoxanthin. The structure of fucoxanthin is still obscure, but its ease of conversion into *iso*fucoxanthin recalls the similar conversion of astaxanthin into astacene (*q.v.*). Of its six oxygen atoms, four are present as hydroxyl groups (Heilbron and Phipers, *loc. cit.*) and catalytic hydrogenation (Karrer, Helfenstein, Wehrli, Pieper, and Morf, *Helv. Chim. Acta*, 1931, **14**, 614) discloses the presence of ten double bonds, all of which, judging by the absorption data, must be conjugated. All attempts to demonstrate the presence of carbonyl groups have proved abortive, but this does not necessarily exclude their presence, as capsorubin (X) also fails to form any ketonic derivatives (Zechmeister and

v. Cholnoky, *Annalen*, 1935, **516**, 30). Apart from fucoxanthin, Willstätter and Page (*loc. cit.*) record the presence of both carotene and xanthophyll in the Phæophyceæ, although the latter pigment was only assessed colorimetrically and never actually isolated.



(X.)

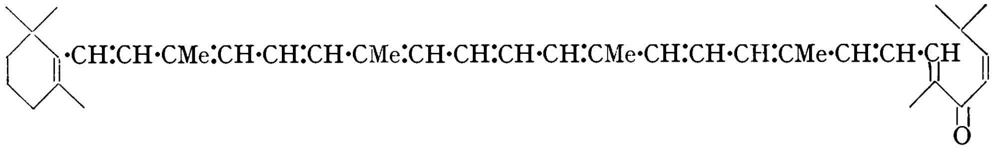
In our investigations of the Phæophyceæ we have extended the survey to include members from various Orders of these brown algæ and find that, despite the great diversity in the life cycles of the individual species, all reveal the most marked uniformity of pigmentation with fucoxanthin largely replacing lutein as the main hypophasic pigment. Indeed, as shown in Table II, we were only able to demonstrate the presence of the latter spectroscopically in the lower members of the Class and not in the more massive plants. A curious phenomenon has been noted in the case of dry *Fucus vesiculosus*; here fucoxanthin is absent but zeaxanthin makes its appearance and Heilbron and Phipers (*loc. cit.*) suggest that this pigment is a *post mortem* product of the fucoxanthin. This view is not shared by Kylin (1939, *loc. cit.*), who claims to find by spectroscopic and chromatographic methods xanthophyll (lutein ?), zeaxanthin and violaxanthin *b* in small amounts in fresh *Ectocarpus siliculosus* and *Fucus vesiculosus*. He suggests that as the alga dries, the fucoxanthin is rapidly destroyed (see Willstätter and Page, *loc. cit.*) and that the zeaxanthin already present, being more resistant to oxidation than the other xanthophylls, is naturally observed with ease in the dry material. We know of no experiments which support the latter contention, but the whole question will be re-investigated so soon as opportunity permits. Another striking feature of the Phæophyceæ is the universal occurrence of fucosterol (IX) and total absence of sitosterol (VIII). This again sharply differentiates the Phæophyceæ both from other Classes of Algæ and from the Higher Plants. At the same time a connexion can be traced in so far as both fucosterol and sitosterol contain the same length of branched side-chain, fucosterol being a diethenoid member of the general phytosterol C_{29}-group (Heilbron, Phipers, and Wright, J., 1934, 1572 ; Coffey, Heilbron, and Spring, J., 1936, 738).

It is interesting to note that whereas in the Chlorophyceæ the chlorophyll components *a* and *b* are the same as in the Higher Plants, in the Phæophyceæ, Rhodophyceæ, Diatoms and Cyanophyceæ, chlorophyll *b* is absent (Seybold and Egle, *loc. cit.*) and therefore cannot be an integral factor in the photosynthetic process, unless one is to postulate a completely different mechanism for the Higher Plants and Green Algæ from that obtaining in other Classes.

The next Class to come under review is the Rhodophyceæ, whose members are mainly marine. The colour of these Red Seaweeds is not due solely to carotenoids but also to the presence of water-soluble red phycœrythrin and sometimes as well blue phycocyanin, both of which are pyrrole derivatives of the urobilin type. In general agreement with Kylin (1927, *loc. cit.*) we find carotene and lutein throughout all members of the seven Orders examined (Carter, Heilbron, and Lythgoe, *loc. cit.*). An unexpected abnormality has been observed in the case of *Polysiphonia nigrescens*, an alga of striking red-black colour. This definitely contains both fucoxanthin and fucosterol, whereas we have commonly found the sterol to be sitosterol in the other Rhodophyceæ which we have examined. This abnormality of pigment and sterol is curious, as *Polysiphonia nigrescens* is morphologically a perfectly normal diplobiontic member of the Ceramiales Order.

Another interesting group comprises the Myxophyceæ, also known as Cyanophyceæ or Blue-Green Algæ, a very ancient Class whose members inhabit both fresh and salt water and are among the simplest of plants, being devoid of sexuality, propagation being mainly by plain fission. Unlike all other Algæ, its members do not possess a proper chromatophore, the photosynthetic pigments being diffused through the outer layers of protoplasm. Myxophyceæ contain a specific carotenoid for which the name phycoxanthin was originally suggested by Kraus and Millardet in 1866. A more detailed examination of an alga of this Class (*Calothrix scopulorum*) was made by Kylin (1927, *loc. cit.*), who, using his capillary method, qualitatively noted carotene together with three other pigments to which he gave the names myxorhodin-α and -β and calorhodin. In 1935 Heilbron, Lythgoe, and Phipers (*Nature*, 1935, **136**, 989) isolated from *Rivularia nitida*, together with carotene and lutein, another carotenoid in crystalline form for which the name myxoxanthin was suggested. This alga is a terrestrial species, but the same pigment was obtained from a related marine species *Rivularia atra* and later a convenient source was found in the freshwater species *Oscillatoria rubrescens*, growing as a scum on one of the Hampton reservoirs of the Metropolitan Water Board. From this source, from which a substantial weight of alga was accumulated, a second new pigment, myxoxanthophyll, was also isolated, together with much carotene and lutein.

By employing micro-methods throughout, Heilbron and Lythgoe (J., 1936, 1376) were able to prove that myxoxanthin was a typical carotenoid monoketone having the formula $C_{40}H_{54}O$ and characterised by a single absorption band at 4880 A. It contains twelve ethenoid linkages and the carbonyl group must be conjugated with the chromophoric system, since the optical maximum of the oxime is displaced 100 A. nearer the violet end of the spectrum (compare Kuhn and Brockmann, *Ber.*, 1933, **66**, 828). Biological tests point to the presence of an unsubstituted β-ionone ring (vitamin A activity) and since the absorption spectrum of myxoxanthol, the aluminium *iso*propoxide reduction product, is identical with that of both γ-carotene and rubixanthin (eleven conjugated ethenoid linkages), the carbonyl group must be at the termination of such a conjugated system. From the fact that myxoxanthin, like astacene (*q. v.*), possesses only a single absorption band we conclude that its structure is (XI) rather than (XII), which would be expected to exhibit the normal triplet spectrum. In the case of the hypophasic myxoxanthophyll the quantity of material available has unfortunately precluded a detailed examination, but analysis has given the formula $C_{40}H_{56}O_7$ (± 2H) and, from the tenacity with which it is retained by alumina in a column, it obviously contains a multiplicity of hydroxyl groups. Tischer (*Z. physiol. Chem.*, 1938, **251**, 109) has independently examined the carotenoids of another blue alga *Aphanizomenon flos-aquæ*, from which he isolated four pigments, principally an epiphasic lipochrome named aphanin and a hypophasic member, aphanizophyll. Of these, aphanin is seemingly isomeric with myxoxanthin, being according to Tischer (*Z. physiol. Chem.*, 1939, **260**, 257) derived from β-carotene and not from γ-carotene as is the case with myxoxanthin. Similarly aphanizophyll is considered by Tischer to be different from myxoxanthophyll, but further evidence is required before a definite decision can be reached. Whether future work establishes complete identity or not, it is abundantly clear that this Class synthesises a type of pigment which differentiates it quite sharply from other Algæ. Another striking feature is the complete absence of sterols (Carter, Heilbron, and Lythgoe, *loc. cit.*). Bearing in mind that in this Class there is a total lack of sexuality, the question as to whether any correlation exists between sterol content and sexuality naturally frames itself, and although no definite answer can at this stage be supplied it opens up an intriguing avenue for further investigation. If any connexion between sexual reproduction and the presence of sterols be ultimately established, the question of *Trentepohlia aurea*, of which mention has already been made, obviously needs reviewing on the botanical side. Whereas so far as is known this alga is sexually normal, it nevertheless is now growing in a habitat unusual for a green alga and moreover the genus is normally tropical. It may be that the disappearance of sterol under both the temperate and the terrestrial conditions forecasts an impending loss of sexuality. Some evidence in favour of this suggestion may be found in the case of *Pleurococcus Nægelii*. In this alga the only reproduction known is vegetative and although sterol has not altogether disappeared the quantity found was conspicuously small. Cognate to this question of sterol content and sexuality it is significant that the Bacteria which are also lacking in sexuality are likewise devoid of sterols.



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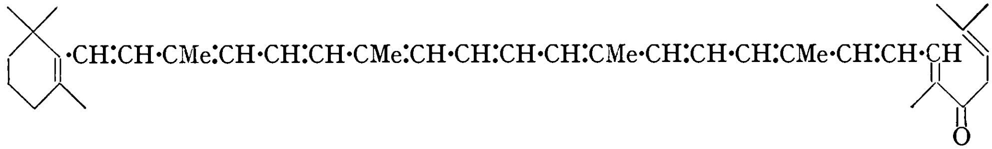
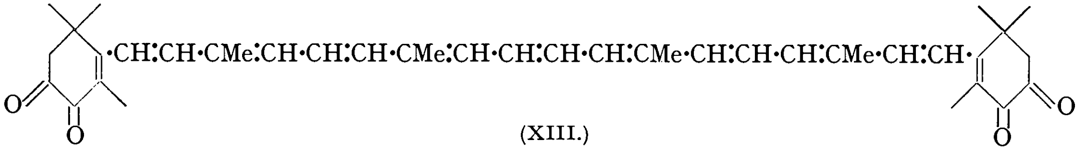
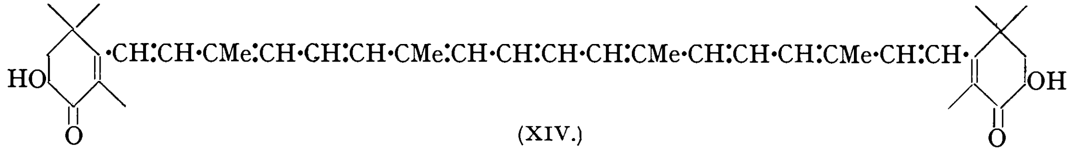


(XII.)

Finally I would like to draw attention to the Euglenineæ, members of which are nearly all fresh-water and are sharply defined naked Flagellates of obscure origin which show a definite trend in the direction of animal organisation (Fritsch, *loc. cit.*). The chromatophores, when present, are bright green and not uncommonly members occur in such numbers as to give a characteristic coloration to the water. The Euglenineæ also possess saprophytic tendencies and it is somewhat doubtful whether the colourless forms should not be referred to the animal kingdom, as they have many characteristics of typical Protozoa. In addition to green Euglenineæ, members are known which are deep red (*e.g.*, *E. sanguinea*), the colour being due to the presence of the red pigment known to botanists as hæmatochrome (Cohn, *loc. cit.*). This pigment is also found in *Hæmatococcus pluvialis*, a member of the Volvocales belonging to the Green Algæ. Tischer (*Z. physiol. Chem.*, 1936, **239**, 257) has recently examined *Euglena heliorubescens*, which is rich in this red pigment, which he calls euglenarhodon. In addition chlorophylls *a* and *b*, β-carotene, lutein, and zeaxanthin are present. According to Tischer, euglenarhodon is a tetraketone of formula $C_{40}H_{48}O_4$, isomeric with astacene, the typical Crustacean pigment first isolated in pure state by Kuhn and Lederer (*Ber.*, 1933, **66**, 488) and shown to have the structure (XIII). The same red pigment was also isolated by Tischer from the green alga *Hæmatococcus pluvialis* (*Z. physiol. Chem.*, 1937, **250**, 147; 1938, **252**, 225), but Kuhn, Stene, and Sörensen (*Ber.*, 1939, **72**, 1688) have independently examined it



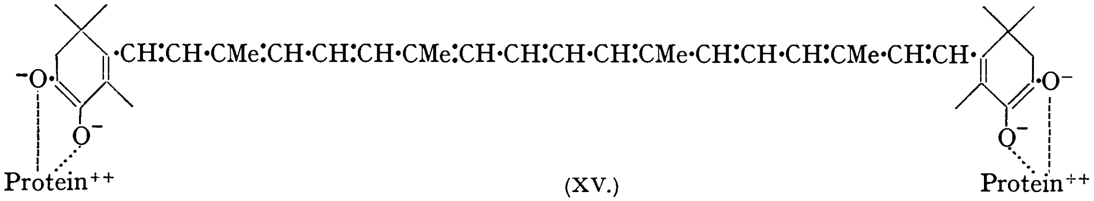
(XIII.)



(XIV.)

and proved that euglenarhodon and astacene are identical. Actually the natural pigment present in *Hæmatococcus pluvialis* is not astacene but an ester of a xanthophyll to which the name astaxanthin (XIV) is given, astacene being an artefact produced by aerial oxidation during hydrolysis.

Astaxanthin is also the natural pigment of the lobster, occurring in the form of an ester in the red epidermis, and in the green eggs it is present as the double enolate of β-carotene combined with protein (XV) (Kuhn and Sörensen, *Ber.*, 1938, **71**, 1879). The presence of astaxanthin in the Euglenineæ is of



(XV.)

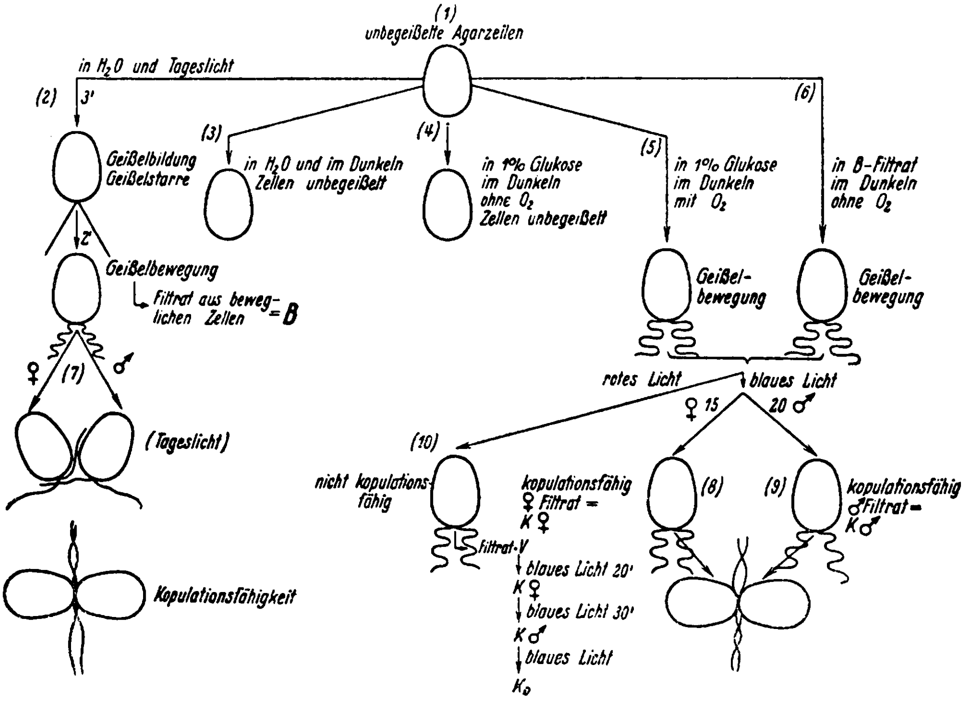
much interest in view of the position occupied by these Flagellates in bridging the gap between the vegetable and the animal kingdom. Its occurrence in *Hæmatococcus pluvialis* is more difficult to comprehend; it may be noted, however, that the red pigment is apparently not present in the normal green motile Sphærella but only makes its appearance at the onset of the resting phase or with variation in constitution of its habitat. Apart from its occurrence in the Crustaceæ astaxanthin has also been found in many invertebrates and also in some of the lower vertebrates (Jones, *Ann. Reports*, 1940, **37**, 309). It has also been isolated from the retina of the hen (Wald and Zussmann, *J. Biol. Chem.*, 1938, **122**, 449), where its rôle is probably bound up with the process of colour vision, just as vitamin A is connected with this phenomenon in human vision. In this connexion the occurrence of eye-spots (stigmata) in nearly all motile cells of the Algæ merits attention. These appear as a reddish or brownish-red dot in the superficial layer of the cytoplasm and have for long been associated by botanists with hæmatochrome. In view of our more precise knowledge there can be little doubt that the pigment is astaxanthin.

Reverting again to the lipoids of the Euglenineæ, Tischer (*Z. physiol. Chem.*, 1939, **259**, 163) states that he has isolated a small amount of ergosterol from *Euglena heliorubescens.* The evidence upon which this claim is based is somewhat slender but, if substantiated, further characterises this Class and links it with the Fungi.

In the foregoing, consideration has been centred upon pigmentation mainly in relation to the botanical classification of the Algæ with only incidental reference to any specific function of the respective pigments. I now wish to consider this functional aspect in some detail with special reference to the problem of sexual reproduction, the most fundamental of all physiological processes of the living organism.

One aspect of this problem has been examined during the past decade in great detail by the botanist F. Moewus, first at the Kaiser Wilhelm-Institut for Biology at Berlin and later in association with R. Kuhn at Heidelberg. It is beyond the scope of this lecture to do more than indicate in a general manner the extraordinarily important results which have been obtained by their joint efforts. For the purpose of their researches use was made in the first instance of the unicellular green alga *Chlamydomonas eugametos f. simplex* (Moewus, *Arch. Protistenkunde*, 1933, **80**, 469), in which male and female gametes are morphologically identical (isogamous). The formation of their cilia, development of motility, and finally their ability to copulate is brought about under the influence of light. These three photochemical processes are illustrated in the diagram, which is reproduced from a paper published by Moewus (*Jahrb. wiss. Botanik*, 1938, **86**, 753).

The gametes are grown on an agar medium and under these conditions are devoid of cilia. If, however, they are suspended in distilled water and illuminated by light from a 150 watt lamp, cilia develop in the course of about three minutes and after irradiation for a further two minutes begin to oscillate and the cell becomes motile. The same effect can be brought about by keeping the algal suspension in the dark and in the presence of oxygen with the addition of 1% of glucose. Cane sugar is without action, but other disaccharides, of which gentiobiose is the most effective, can replace glucose. The formation of the cilia and the development of gamete motility can also be brought about by the addition of a cell-free filtrate of gametes which has already become motile in light to a suspension of a dark culture even in absence of oxygen. It follows from this that a definite chemical substance soluble in water and capable of producing motility has been produced by the action of light. This substance has been identified with almost complete certainty as the saffron pigment crocin, which has been shown to be effective in producing motility in the amazing dilution of 1 : 250,000,000,000,000 (Kuhn, Moewus, and Jerchel, *Ber.*, 1938, **71**, 1541). The authors calculate that these figures imply that the activation of four sexual cells of *Chlamydomonas eugametos* is brought about by five molecules of crocin.



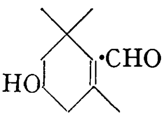
Even though the gametes have thus become motile, they are still not able to copulate and only acquire this property on further prolonged illumination. Whereas oscillation of the flagellæ is brought about in all parts of the visible spectrum, this second photochemical process is effected only by irradiation with blue or violet light, the active wave-lengths being the two mercury lines 4358 and 4961 A. Here again definite chemical substances are formed and discharged into the surrounding solution. This is shown by the fact that if a gamete suspension be suitably irradiated and filtered, copulation will ensue on addition of the filtrate to the appropriate unreactive " dark " gametes. The period of illumination is specific, being shorter for the female gametes than for the male, and over-irradiation gives only inactive filtrates. It is not essential for the formation of the copulatory materials to irradiate the sexual cells themselves with light of short wave-lengths. One can first illuminate a male or female cell suspension activated in the dark with glucose and oxygen with red light (6463 A.) for one hour and then filter and expose the inactive " red " filtrate (V) to the further action of short-wave light; the copulatory substances are then again formed. The time required for the assumption of female properties by the filtrate is from

20—30 minutes (K♀), after which it is no longer able to confer on female " dark " cells ability to copulate. After 60 minutes, however, the filtrate becomes able to render male " dark " cells active (K♂) and after 90 minutes' irradiation the filtrate (K_0) becomes permanently inactive.

Now all these phenomena can be exactly reproduced if in place of the filtrate (V) a light-sensitive pigment, *cis*-crocetin dimethyl ester (Kuhn and Winterstein, *Ber.*, 1933, **66**, 209), is employed. Under the influence of light this is converted gradually into the *trans*-form and it has been found that for this variety of *Chlamydomonas* the material conferring copulatory activity on the female " dark " gametes is a mixture of 3 parts of *cis*- and 1 part of *trans*-crocetin dimethyl ester and for the male " dark " gametes 3 parts of the *trans*-isomer and 1 part of the *cis*-ester. The V-material is thus the labile *cis*-crocetin dimethyl ester, and the K_0 material is the *trans*-isomer. It is only for this particular race of *Chlamydomonas* that the above ratios are effective; other species require different proportions of the two isomeric esters.

Although definite proof is lacking, it seems very probable that the dimethyl esters are actually the natural active materials, since it has been ascertained that a concentrated K_0 solution has exactly the same absorption bands as a solution of *trans*-crocetin dimethyl ester. The biological limit of activity of both esters is found at a dilution of 1 : 33,000,000,000; great as this is, it is not comparable with the effect of crocin in bringing about cell-motility.

In a still more recent memoir by Kuhn, Moewus, and Wendt (*Ber.*, 1939, **72**, 1702) considerable light is thrown upon the question of control of sex. For this purpose the hermaphroditic green alga *Chlamydomonas eugametos f. synoica* was selected. If this is treated by a filtrate of ♂-gametes of the diœcious strain, all the cells become male, whereas a filtrate from ♀-gametes will render all the cells female. It is thus clear that here again the gametes secrete substances controlling sex. These sexual materials serving to determine sex among hermaphroditic organisms, and thus bestowing *primary* sexual characteristics, are called termones. Although the termones in the active filtrates have not so far been isolated, it has been ascertained that the male termone (androtermone) in contradistinction to the female termone (gynotermone) is volatile in steam and soluble in ether. By heating the solution containing the gynotermone with dilute sulphuric acid or with baryta water, its female characteristics are destroyed and simultaneously the solution acquires the male termone properties. This points to the relationship of gynotermone to androtermone being of the type glycoside to aglucone and the fact that, unlike glycosides in general, the female termone was hydrolysed by alkali led to the conclusion that it might be picrocrocin, which reacts similarly. This was experimentally verified by bringing *synoica*-cells into contact with a solution containing 0·02 μ of picrocrocin in 1 c.c. of water; after 10 minutes all the cells had become female towards ♂-gametes of the same race. If the picrocrocin solution is just heated with acid, safranal is formed and under its influence all the cells become male. Although the authors consider that safranal is identical with the natural androtermone, the corresponding gynotermone is shown to be 1000 times more active than picrocrocin and this they attribute to the presence of a sugar different from glucose, and possibly gentiobiose. More recently Kuhn and Löw * (*Ber.*, 1941, **74**, 219) report that 4-hydroxy*cyclo*citral (XVI) is also an androtermone like safranal. Perhaps the most outstanding feature of this brilliant series of investigations is the remarkable insight it provides of the versatile manner in which Nature utilises one substance only, the carotenoid crocin (or the as yet undetected *proto*-crocin) to carry out all the fundamental physiological sexual functions of these green Algæ. Kuhn and Moewus * (*Ber.*, 1940, **73**, 547) have adduced physiological, genetic, and chemical evidence of the general correctness of their views. They have shown, for example, that when crocin is secreted, picrocrocin is also secreted and vice versa; when non-motile mutants were obtained, they were unable to propagate sexually and lacked the ability to secrete crocin and picrocrocin or safranal. Again only in those physical circumstances (temperature range, incidence of light, etc.) when the cells are able to couple do they secrete their coupling gamones (*cis-trans* crocetin ester mixtures). It is suggested that the *cis* : *trans* ratios have a similar significance to the numerical relations between individual amino-acids in proteins. It seems certain that the cells directly produce the proper mixture and that this mixture does not arise by photochemical rearrangement of *cis*-ester (Kuhn and Moewus,* *Ber.*, 1940, **73**, 559). Finally, in this connection Kuhn (*Angew. Chem.*, 1940, **53**, 1) announces the existence of an enzyme in male gametes capable of effecting the fission of picrocrocin. This glycoside-splitting ferment is in all probability localised in the cell nucleus and this he considers may be a fundamental constituent of the chromosomes and indeed may be actually identical with the gene M (controlling masculinity).



(XVI.)

In *Chlamydomonas eugametos* we have been dealing with a species of Algæ where sexual reproduction

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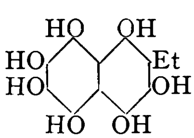
* This paper has unfortunately only been seen in the form of an abstract.

is isogamous, and I now wish to direct attention to an order of Algæ which reproduces oogamously and thus marks the highest stage in sexual reproduction. The members of the family Fucaceæ (Class phæophyceæ) reproduce in this manner by means of minute motile biciliate sperms and larger non-motile eggs.

The diœcious members of the Fucales such as *Fucus serratus* are especially convenient to study, as large quantities of both male and female gametes can be obtained in a pure state and free from any possible contamination by gametes of the other sex. In the Fucaceæ the antheridia and oogonia are produced within flask-shaped sunken cavities, the conceptacles. These occur at the periphery of somewhat swollen and mucilaginous receptacles. The gradual extrusion of groups of mature sperms and eggs through the openings (ostioles) of the conceptacles normally occurs between the tides and is probably brought about in part by the expansion of mucilage together with contraction on desiccation. In Nature the gametes are removed from the surface of the conceptacles by the incoming tide and fertilisation thus takes place outside the parent plant. The numerous minute biciliated sperms contain a small yellowish chromatoplast and the mature antheridia therefore appear orange-yellow, which colour they impart to the entire receptacle. This affords a means of distinguishing the male plant in the diœcious species of the Fuci at the time of gamete discharge. It seemed therefore of importance to determine experimentally the nature of the orange-yellow pigments characteristic of the sperm, as it appeared to us that such an investigation must inevitably throw light on this fundamental question of sexual reproduction. At the time this work was started by us (Carter, Cross, Heilbron, and Jones, unpublished work) we were not aware of the investigations of Kuhn and Moewus, and in any case the problem is different, as here only the male sperms are motile. Our experiments have been carried out with *Fucus serratus, Ascophyllum nodusum* and *Fucus vesiculosus* and have revealed that the pigment present in the male gametes consists almost entirely of β-carotene. In direct contrast to the results obtained with the male exudate the examination of the pigments of the corresponding female exudate has revealed that chlorophyll and fucoxanthin are responsible for the colour of the female ova. Unfortunately the advent of the war has so far prevented us from examining the respective gamete filtrates, but this we intend to do so soon as opportunity offers. In the meantime it would appear possible from the fact that β-carotene is to be observed mainly in the ♂-gametes that this carotenoid is bound up with their motility. It may be that under the influence of light and in presence of oxygen the hydrocarbon becomes partly oxidised to crocetin, which then acts as with *Chlamydomonas.* In support of this view Moewus (*Jahrb. wiss. Botanik,* 1938, **86**, 753) states that crude carotene extracted from both *Daucus carota* and green leaves, when exposed to light and air, gives an aqueous extract which acts in the same way as the female extract, but after longer standing in light the aqueous extract assumes the properties of the male copulatory substances. That carotene is utilised as a basis of physiologically active substances is familiar to all through its oxidative conversion into vitamin A in the animal body. The association of carotene with sexual processes is further illustrated in another direction by recent work carried out by Emerson and Fox (*Proc. Roy. Soc.*, 1940, *B*, **128**, 275) on the aquatic fungus *Allomyces.* These authors have observed that the orange colour of the motile male gametes in the sexual phase is due to γ-carotene present in high concentration.

Another problem to be elucidated in connexion with the sexual process in these Fucaceæ is the mechanism whereby the motile ♂-gametes become attracted to the egg. Kuhn and Wallenfels (*Ber.*, 1939, **72**, 1407) have shown that in the case of the sea-urchin, the ova secrete a definite compound, echinochrome A, into the sea-water which immediately attracts the sperms. The pigment is a quinone derived from leuco-echinochrome A (XVII) and although the mechanism of excitation is still unknown, it is suggested that, as echinochrome is a pigment of known redox potential, it may function as a typical co-ferment. It may be that fucoxanthin plays a similar rôle in these algal ova or that something akin to echinochrome is secreted. The latter possibility is supported by the fact that we have obtained cell-free extracts of ova of *Fucus serratus* which attract the sperms. This is the problem upon which in normal times we should have hoped to be working, but this, with other fascinating questions of like nature and interest, has had to be set aside for the time being in favour of investigations more relevant to the great struggle on behalf of the defence of civilisation.


(XVII.)