THE COMPARATIVE BIOCHEMISTRY OF THE CAROTENOIDS

CAROTENOIDS

their comparative biochemistry

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1954
Chemical Publishing Co., Inc.
212 Fifth Avenue, New York, N.Y.

First American Edition
1954
CHEMICAL PUBLISHING CO., INC.
New York N.Y.

Printed in the United States of America

PREFACE

The carotenoids are not only amongst the most widespread of the naturally occurring groups of pigments, but probably also have the most varied functions; witness their known roles in photokinetic responses of plants, in phototropic responses of fish and as vitamin A precursors in mammals and birds. Pigments with such wide distribution and such diverse functions are obviously of great interest to biological scientists with very different specializations, especially as it is unlikely that the study of the functions of carotenoids is anywhere near complete.

The primary aim of the present work is to discuss the distribution, biogenesis and function of the carotenoids throughout the plant and animal kingdoms in such a way that, because of, rather than in spite of its biochemical bias, it will be of value to workers interested in all the biological aspects of these pigments. The biochemical approach is considered the most effective because, generally speaking, most progress in the study of carotenoids in living material has been achieved using biochemical techniques, be they applied by zoologists, botanists, entomologists, microbiologists or other specialists; what is even more important is that a consideration of the present position makes it certain that further fundamental progress will also only be made along biochemical lines.

Although many good accounts of the pure chemistry of the carotenoids are available, the most recent and comprehensive being Karrer and Jucker's *Carotinoide* (Birkhauser, Basel, 1948), (now available in an English translation by E. A. Braude and published by Elsevier) sufficient descriptive chemistry has been included to make this book adequately self-contained and to allow the discussion to be followed without undue difficulty. The most up-to-date spectrographic data have also been included, because spectrophotometric techniques are of great importance in identifying carotenoids in biological systems.

The first comprehensive survey of the biochemistry of carotenoids was made in 1922 by the late L. S. Palmer (*Carotinoids and Related Pigments*, Chemical Catalog Co., New York); this was followed in 1934 by Zechmeister's *Carotinoide* (Springer, Berlin) and Lederer's *Les Caroténoides des Plantes* (Hermann, Paris), and in 1935 by Lederer's *Les Caroténoides des Animaux* (Hermann, Paris). Since then a survey such as the present one has not appeared. In order to present a full picture, much of the pre-1934 work has been reconsidered and, as far

as is known, every important contribution which has appeared since that date has been discussed. Two peripheral aspects of the subject have, however, been omitted, namely (a) the qualitative and quantitative changes which the carotenoids of plant materials undergo in storage or during processing into food and (b) the carotene (pro-vitamin A) requirements of different animal species ; it was felt that the former, about which a great deal has been written, was too technological to be suitable for inclusion in the present volume, whilst the latter is more suitable for a monograph on vitamin A.

The very wide distribution of the carotenoids in Nature suggests that, in spite of the superficially diverse functions ascribed to them in different living tissues, there may be some factor or property through which all these functions will eventually be correlated ; any suggestion as to the nature of this common property can perhaps come most readily from a comparative approach. Apart from critically surveying the literature this book has been constructed so as to focus attention on comparative data and their possible implications. If the comparative aspects do not always appear to have been given sufficient explicit consideration it is because essential data are still lacking ; it may even be hoped that when research workers realise fully the lacunae, they will be stimulated to carry out investigations on comparative lines. If this does occur then the author will feel that the book has served one of its main purposes.

To many biochemists the word ' carotenoid ' stimulates the mental response ' vitamin A precursor ' and no more. There is a need, which it is hoped this book fulfils, to emphasize to all concerned, directly or indirectly, with carotenoid biochemistry that a much wider view must now be taken of these pigments and that in the course of elucidating their biogenesis, metabolism, and functions, very significant advances with wide implications for our understanding of living processes are to be expected.

My sincere thanks for considerable help during the writing of this book are due to many friends and colleagues ; it should be emphasized however, that none of them can be considered in any way responsible for any peculiarities which may exist in the book. Professor R. T. Williams (St. Mary's Hospital Medical School) read and criticized the original typescript ; Mr. D. A. Coult (Department of Botany, The University of Liverpool) read the section on plant carotenoids and corrected many errors of nomenclature ; Dr. J. Glover (Department of Biochemistry, the University of Liverpool) devoted considerable time to correcting both the galley and the page proofs, and made many valuable suggestions. Miss B. M. Morris and Miss M. W. Boggiano

between them produced an unblemished typescript from a far-from-perfect manuscript; the Staff of the Liverpool University Library (especially Miss E. Whelan) went to considerable trouble to trace and obtain obscure journals and monographs.

My greatest debt of gratitude is, however, due to Professor R. A. Morton, F.R.S. His encouragement stimulated me to begin this book and his continued unstinting help during the writing of it has been invaluable.

Conditions in the British publishing world are today extremely difficult and the long delays in publishing Scientific Books, especially monographs, tend to make them out of date before they appear. My Publishers have been most tolerant in dealing with my attempts to reduce this delay to a minimum. It is entirely due to their whole-hearted co-operation, that it has been possible eventually to include information available in this country up to the end of September 1951.

T. W. G.

CONTENTS

CHAPTER I

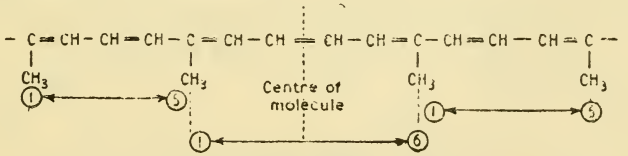
DEFINITIONS AND NOMENCLATURE

INTRODUCTION

The yellow alcohol-soluble pigments of autumn leaves were called xanthophylls by Berzelius[1] in 1837. Frémy[2] and Stokes[3] were the first to show that these pigments also occurred in green leaves, a possibility that had been first envisaged as early as 1827.[4] Later, following the classical work of Tswett[5] on the chromatographic separation of leaf xanthophylls which showed them to be a complex mixture of "polichromes", these pigments were divided into two classes: one very soluble in hydrocarbon solvents was called "carotenes" and the other, much less soluble in these solvents but very soluble in ethanol was called "xanthophylls"; the two classes were grouped together under the general term "carotenoids". The developments in recent years have made desirable the extension and standardization of these terms. Most of the ambiguities have recently been resolved in statements from the "Union internationale de Chimie"[6] and the Committee on Biological Nomenclature of The National Research Council of America.[7]

DEFINITION OF CAROTENOIDS

Karrer's definition of carotenoids, accepted by the "Union internationale de Chimie," states that "Carotenoids are yellow to red pigments of aliphatic or alicyclic structure, composed of isoprene units (usually eight) linked so that the two methyl groups nearest the centre of the molecule are in positions 1 : 6 whilst all other lateral methyl groups are in positions 1 : 5; the series of conjugated double bonds constitutes the chromophoric system of the carotenoids."



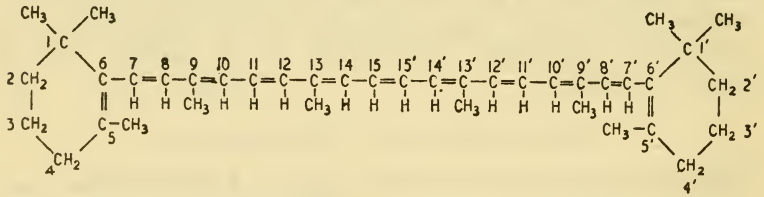
Arrangement of methyl groups around centre of a carotenoid molecule

This definition includes such naturally occurring compounds as vitamin A and azafrin only in so far as they are considered breakdown products (apocarotenoids) of carotenoids containing 40 carbon atoms. Although compounds such as bixin containing less than 40 carbon atoms (8 isoprene residues) are included in Karrer's definition, only those containing 8 isoprene residues are discussed in detail in this book; others are mentioned only in so far as they are concerned with the biochemistry of the C_{40} carotenoids.

NUMBERING AND NOMENCLATURE

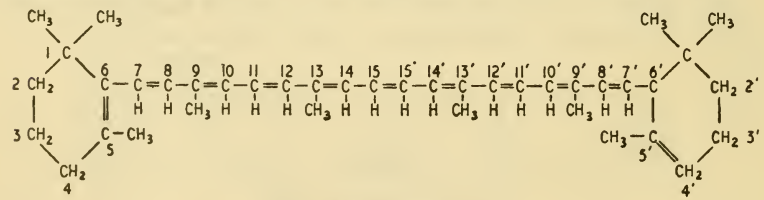
The conventions used in this book for describing carotenoids are, with one exception and some additions, those recommended by the "Union internationale de Chimie"[6] in a report by the "Commission de nomenclature de chimie organique et Commission de nomenclature de chimie biologique." A previous report by the American "Committee on Biochemical Nomenclature of the National Research Council"[7] differs slightly from the International Commission's report.

The carbon atoms of a carotenoid molecule are numbered according to the scheme proposed some years ago by Karrer, in which the carotenoid is divided into two parts, *viz.*

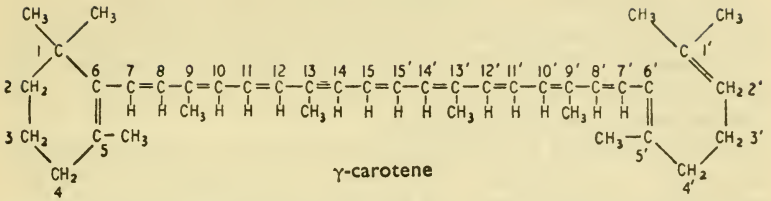


β-carotene

If the carotenoid under discussion is asymmetrical, the half containing the β-ionone residue is designated by plain numerals; *e.g.*



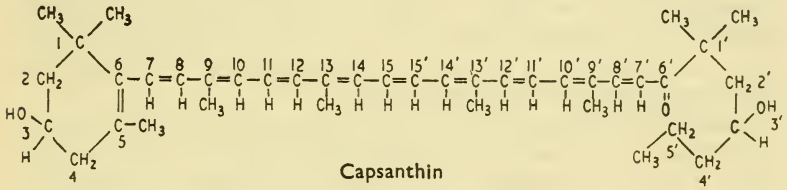
α-carotene



γ-carotene

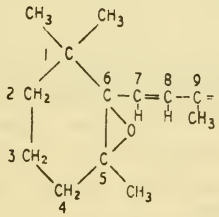
similarly an α-ionone-containing moiety would take preference over the open chain (ψ-ionone) moiety, although a naturally occurring compound containing such a combination has not yet been noted.

In the case of carotenoids in which the ionone ring is not opened at position 1 but at some other point, the Commission has made no recommendation, but Professor Karrer has informed the author that the numbering should remain the same. This point arises in the case of capsanthin.

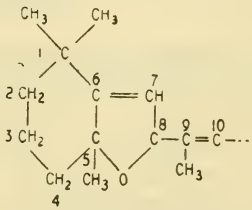


Capsanthin

The carotenoid hydrocarbons are designated by the term *carotenes*; carotenoids containing oxygen are to be regarded as derivatives of carotenes, and thus carotenoid alcohols, ketones, aldehydes and acids are characterized by the suffixes " ol," " one," " al," " oic," or by the prefixes " hydroxy," " keto," " aldo," " carboxy." The modification of the ending " xanthin " to " xanthol," " xanthone," etc., to indicate the function of the oxygen atom(s) is not permissible; *e.g.*, Cryptoxanthin or 3-hydroxy-β-carotene but *not* Cryptoxanthol. Karrer has recently observed the natural occurrence of carotenoid epoxides and oxides containing a furan ring (*see* p. 47); these are termed respectively 5 : 6-epoxycarotenoids, and 5 : 8-epoxycarotenoids, *e.g.*,



A 5 : 6-epoxycarotenoid



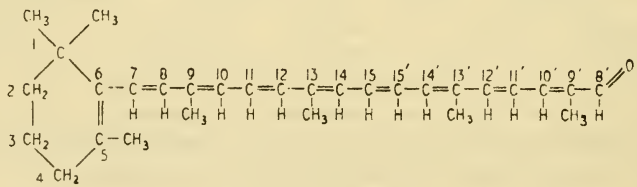
A 5 : 8-epoxycarotenoid

The Commission has not discussed carotenoids which, by virtue of their ability to undergo keto-enol tautomerism, exhibit acidic properties, *e.g.*, astaxanthin (*see* p. 169). In this book these will be termed *acid carotenoids*, and should not be confused with the carotenoid acids (carboxycarotenoids) just discussed.

In many countries the usual group name for the oxygen-containing carotenoids is *xanthophylls*. This name is not accepted by the International Commission because of Karrer's plea that a worker who first isolates a compound in a pure state has the privilege of naming that compound. The main oxygenated pigment of green leaves was first isolated pure by Karrer[8] and named *xanthophyll*. Later Kuhn and his collaborators[9] obtained the same pigment, called it *lutein*, and suggested *xanthophyll* as a group name for the hydroxy carotenoids.

One must respect Karrer's plea, but the influence of established custom, be it mistaken or not, cannot be completely ignored. It is hoped that the rule followed in this book will not be confusing; *lutein* (*xanthophyll*) will be used to designate Karrer's individual pigment wherever any possible ambiguity exists, otherwise *lutein* alone will be used. Xanthophylls will be retained as a group term; in this sense it will be generally used only in the plural.

Labile *cis-trans* isomers of the carotenoids are prefixed by "*neo-*" and suffixed by an identifying capital letter (*see* p. 9); *e.g.*, neo-β-carotene B, neo-β-carotene U. An aldehydic, ketonic, or carboxylic fragment obtained by degradation of a carotenoid takes the suffix, "al," "one," or "oic" only when the prefix "*apo*" is used. This prefix is followed by the numeral indicating the carbon atom of the aldehyde, ketone, or acid group, *e.g.*,



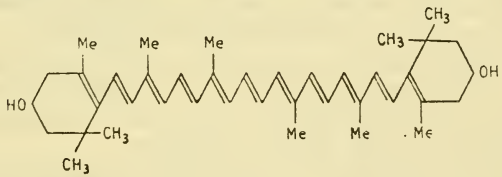
β-apo-8′-carotenal

It should be noted that this enumeration is different from that first proposed by Karrer,[10] by whom most of the work on apocarotenes has been reported. In the original scheme, the number following the prefix "apo" indicated the number of double bonds removed from the original carotenoid molecule; thus the above example would on the old system be termed β-apo-2-carotenal.

It is recommended that newly discovered carotenes of unknown structure be named by using the term " carotene " prefixed by Greek letters or terms appropriate to the source or properties of the carotenes, *e.g.*, " *ω-carotene*," or " *lycocarotene*." A mixture of α- and β-carotenes in which the β-isomer predominates (about 90-95 per cent. of the total) has been used by many workers without further purification ; in this work such a mixture will be termed " *carotene*."

New xanthophylls are to be named by using the term " xanthin " to which a prefix appropriate to its source is added. As structures of these pigments become known, they can be described in terms of the parent hydrocarbon, using the rules of nomenclature set out in this chapter ; *e.g.*, zeaxanthin (*not* zeaxanthol) becomes either 3 : 3′ -dihydroxy-β-carotene or β-carotene-3 : 3′ -diol. The author does not feel that the chemical names should oust well-established trivial names in most biochemical discussions ; in fact, it will be found that in the following pages chemical names of carotenoids of known structure are given together with the trivial names only when they are first mentioned ; thereafter the trivial names are used. Dyson[11] has applied his method of enumeration to the carotenoids.

Up to now the formulae of the carotenoids discussed have been given in full ; in the chapters which follow, unless there are good reasons not to do so, the formulae will be reproduced in a " shorthand " version, demonstrated in the following example :—



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PART I

CAROTENOIDS IN PLANTS

CHAPTER II

CAROTENOIDS IN LAND PLANTS

I. Phanerogams

Carotenoids are found in all green tissues of plants, and their occurrence and distribution in these tissues will be our first consideration.

LEAF CAROTENOIDS

CAROTENES

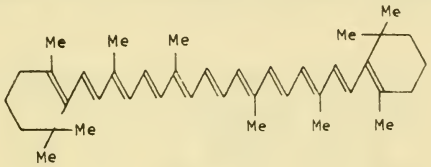
(i) β-*carotene.* The leaf carotenoids are associated with chlorophyll in the chloroplasts[1,2], probably as water-soluble protein complexes[3] but occasionally as lipid droplets.[4] The intense green of the chlorophyll components normally masks the orange-yellow of the carotenoids. It is only during early autumn, when the chlorophyll is destroyed and the leaves yellow, that the carotenoids become apparent to the naked eye. In the dying leaf the carotenoids themselves undergo changes which will be discussed later (*see* p. 19). The final bronze and red colours observed in falling leaves are not due to carotenoids but to water-soluble pigments not yet fully identified.

As soon as the implications of the classical researches of Tswett[5] on the chromatographic separation of the lipid-soluble plant pigments were fully appreciated, developments in the isolation and chemical characterization of the carotenoids were extremely rapid. The outstanding work of Karrer and of Kuhn and their collaborators on the elucidation of the structure of the carotenoids has recently been fully and authoritatively discussed by Karrer and Jucker.[6] The most common carotene found in green leaves is β-*carotene*, in fact it is probably true to say that all leaves contain this pigment. The one report to the contrary which claimed that the green leaves of the Formosa tea plant contain exclusively α-carotene[7] has now been refuted.[8]

In the largest single investigation so far reported, Mackinney[9] identified β-carotene in 59 different species. The concentration of

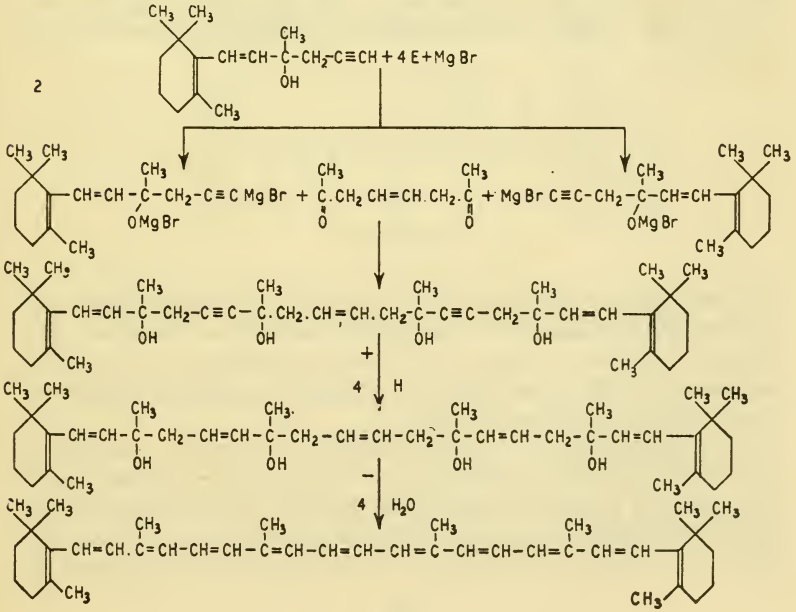
β-carotene varies from species to species but usually falls within the range 200-700 μg./g. dry weight. The carotene content of the leaves of a large number of plant species has been collected in an appendix (*see* p. 289). There is one report that β-carotene exists in timber (*Acacia acuminata*).[10]

The structure of β-carotene, which was finally elucidated by Karrer and his colleagues,[6] is :

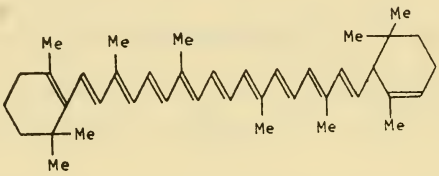


β-carotene

It is fitting to note here that Karrer[11-13] has crowned 20 years' work on carotenoid chemistry by achieving a total synthesis of β-carotene and lycopene (*see* p. 25), albeit in small yield. As this work was reported after the publication of Karrer and Jucker's monograph, the essential steps in the synthesis are included here, using β-carotene as an example.

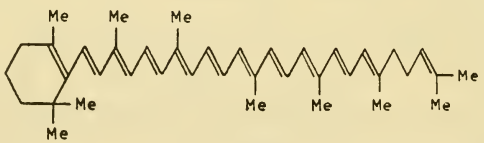


(ii) α-*carotene*. Although α-carotene is often associated with β-carotene in green leaves it is quite frequently absent, for neither spinach, nettle[14], artichoke, nor barley[15] leaves contain α-carotene. 68 per cent. of the species examined by MacKinney[9] contained α-carotene in their leaves in amounts varying from " a trace " to 35 per cent. of the total carotene fraction. Kuhn and Lederer's[14] earlier value for the α-carotene content of horse-chestnut leaves falls within this range; similar values are reported for the leaves of Japanese plants.[8]



α-carotene

(iii) γ-*carotene*. There is no convincing evidence that γ-*carotene* occurs to any appreciable extent in green leaves except in those of the Californian marsh dodder (*Cuscuta salina*)[16], in which it is the major carotenoid component; 20-25 mg. of crystalline γ-carotene was isolated from 1 kg. of these leaves compared with only 12·5 mg. of β-carotene. Kuhn and Brockmann[17] elucidated the structure of γ-carotene:



γ-carotene

(iv) *cis-isomers*. It is obvious that molecules with structures such as the carotenoids possess, have many spatial possibilities. Since the other carotenes reported to occur in leaves are stereoisomers of the parent β-carotene, it will be convenient at this point to discuss briefly the stereochemistry of the carotenoids.

Gillam and El Ridi[18] in 1935-6 were the first to draw attention to the lability of carotenoids in solution, and since then Zechmeister[19] and his co-workers have reinvestigated the problem at great length and in 1944 Zechmeister produced an authoritative review on the subject. The all-*trans* form of a carotenoid, the most stable form because of its

low energy content, can undergo *trans* → *cis* isomerization in solution, yielding a complex equilibrium mixture of isomers. These changes are accelerated by light, heat, and by the addition of iodine or acid. Any *cis*-isomer similarly treated will produce the same equilibrium mixture as the all-*trans* isomer. These isomers can be separated chromatographically and are named according to the positions they occupy relative to the parent all-*trans* substance on an adsorption column. Any *cis*-isomer is named by prefixing " neo- " to

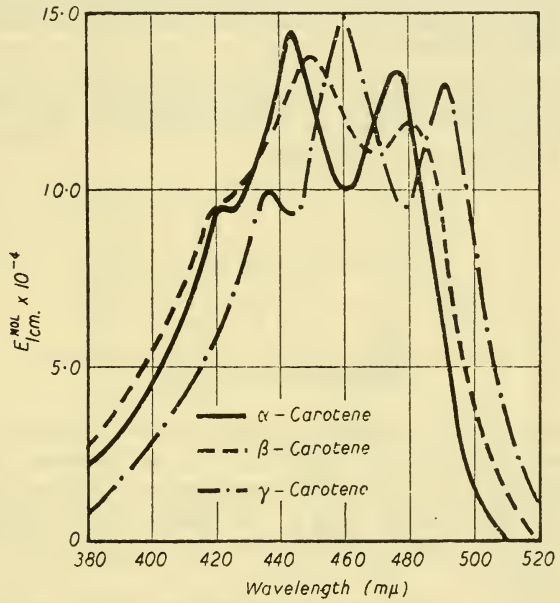


Fig. 1.—The absorption spectrum in hexane of α-carotene, β-carotene and γ-carotene. (*From Zechmeister, L. (1944)* Chem. Rev., **34**, 267.)

the name of the parent compound and then adding the letters " T, U, V," etc., or " A, B, C," etc., according to its relative position on the column, T, U, V, if adsorbed above, and A, B, C, if adsorbed below the all-*trans* form. A glance at the diagram (Fig. 2) will make this clear. It is interesting to note that Gillam and El Ridi's isomer of β-carotene, pseudo-α-carotene, is, according to Zechmeister's notation, neo-β-carotene B.

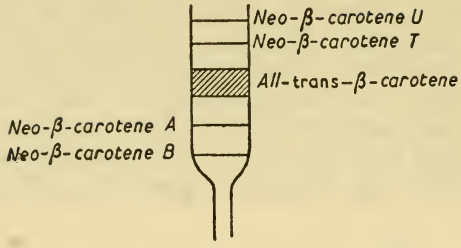
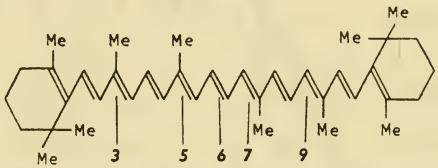


Fig. 2.—Illustrating the naming of stereoisomers of carotenoids according to the positions they occupy on an adsorption column relative to the parent all-*trans* compound.

Not all the double bonds in a carotenoid molecule are capable of *trans* → *cis* rotation because of steric hindrance; only 5 double bonds in β-carotene can rotate thus:



All-trans-β-carotene (rotation can only take place around double bonds *3*, *5*, *6*, *7*, *9*).

As a result of this restriction, the term "all-*cis*" applied to carotenoids is not the antithesis of "all-*trans*." In an "all-*trans*" molecule every double bond does have a *trans*-configuration, whereas in an "all-*cis*" molecule all possible *trans* → *cis* rotations have occurred, but there are always present a number of double bonds with the "*trans*-" configurations.

Occasionally the most abundant naturally occurring carotenoid has a *cis*-configuration. Zechmeister terms this a "pro-carotenoid," *e.g.*, pro-γ-carotene: the naturally occurring all-*trans* forms are given no special designation.

The demonstration of the ease with which carotenoids undergo stereoisomerization makes it very difficult now to assess some of the earlier work on the carotenoids present in plant tissues; extractions were often carried out with some vigour using hot solvents and with no precautions to exclude bright sunlight. Thus a number of claims to have isolated a series of new and unidentified carotenoids must be treated with caution until re-investigations under modern conditions rule out *cis* → *trans* isomerization.

Modern investigators have been stimulated by Zechmeister's work to search for *cis*-isomers in living tissues. Beadle and Zscheile[20] claim to have isolated a *neo*-β-carotene from spinach, asparagus, and broccoli leaves, but Griffith and Jeffrey[21] could find no trace of neo-β-carotenes in fresh extracts of tobacco leaves. A long and critical examination of a large number of fresh grasses has, however, convinced Kemmerer, Fudge and Fraps[22] that the carotene fraction of a grass contains, on the average, 77·7 per cent. of β-carotene, 12·9 per cent. of *neo*-β-carotene U, and 9·4 per cent. of *neo*-β-carotene B. Both these isomers have also recently been observed in the leaves of cauliflower, carrot, lettuce, spinach, fenugreek and rape[23]. Zechmeister,[19] from spectrophotometric considerations, is inclined to believe that *neo*-β-carotene U is *3*-mono-*cis*-β-carotene, and *neo*-β-carotene B is *3* : *6*-di-*cis*-β-carotene, where the italicized numerals indicate double bonds and not carbon atoms.

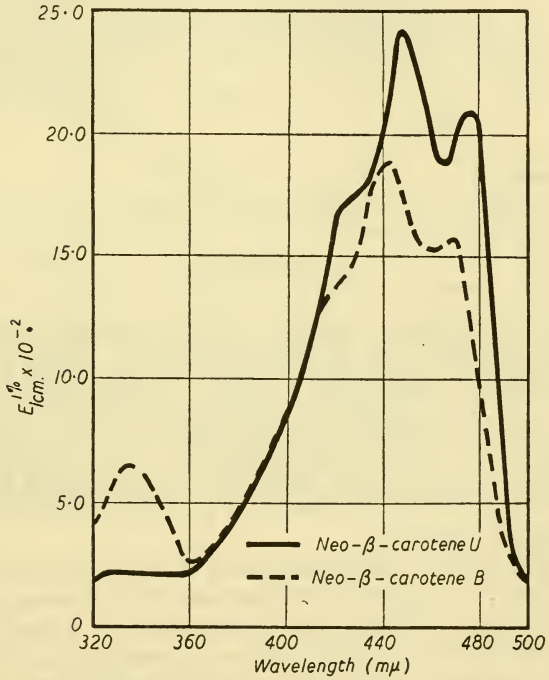
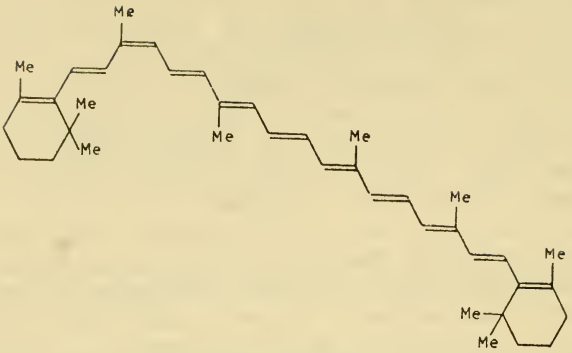
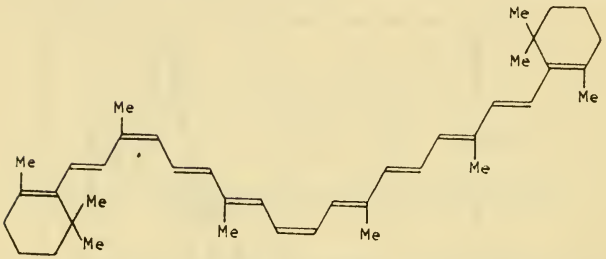


Fig. 3.—Absorption spectrum in iso-octane of Neo- β-carotene B, Neo-β-carotene U. (*From Bickoff, E. M., White, L. M., Bevenue, A. and Williams, K. T. (1948)* J. Ass. Off. Agric. Chem., p. *633.*)



Neo-β-carotene U



Neo-β-carotene B

(v) *Colourless polyenes.* Recently attention has been turned to the colourless fluorescing and non-fluorescing unsaponifiable compounds which accompany carotenoids in fruits. These substances, which are dealt with in detail later, are carotenoids in which varying numbers of double bonds have been saturated. They have not usually been considered to occur in green tissues but recently Porter and Burns[24] have reported the presence of tetrahydrophytoene (? eicosahydrolycopene) in leaves.

Some phylogenetic relationships based on the carotene distribution in leaves have been suggested by Mackinney[9]; the safest generalization which appears justified is that if one member of a family contains no α-carotene, then it is unlikely that other members will do so. The amount of α-carotene in any plant can be forecast with some degree of accuracy, but no explanation is yet available of the sporadic appearance of this carotenoid. Table 1 summarizes data on the carotenes of green leaves.

TABLE 1.—*Leaf Carotenes*

PIGMENT	m.p.	ABSORPTION SPECTRA MAXIMA (mμ.)			
		petroleum (b.p. 70-80)	hexane	CS_2	$CHCl_3$
α-Carotene (1)	187–188°	478, 477·5	475, 445	509, 477	485, 454
β-Carotene (2)	184°	483·5, 452, 426	477, 450, 425	520, 485, 450	497, 466
γ-Carotene (3)	178, 131·5	495, 462, 431		533·5, 496, 463	508, 475, 447
Neo-β-Carotene B (4)	—	475·5, 443·5	456 in Wesson oil (5) and 470 in *iso*-octane (6)		and 443
Neo-β-Carotene U (4)	122–123°	481, 450		512·5, 478·5	474,447 in *iso*-octane (6)

(There is still some discrepancy concerning the m.p. of γ-carotene (*see* Zechmeister and Schroeder (3) for a discussion.)

REFERENCES TO TABLE 1.

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2. KARRER, P. (1945), *Vierteljahrsch. Naturforsch. Ges. Zurich*, **90**, 1.
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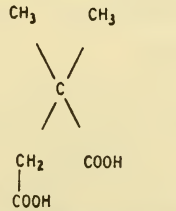
XANTHOPHYLLS

Much of our knowledge of leaf xanthophylls is due to a series of researches by Strain which he has reported in detail.[25] He has not only confirmed the earlier findings of Kuhn, Winterstein and Lederer[26] that the predominating carotenoid is *lutein* (xanthophyll), but has also isolated, by chromatography on magnesia, a number of minor components. It should be noted at this point that the position of the two hydroxyl groups in lutein are not completely fixed although they are generally accepted as being at 3- and 3′-.

Oxidation of lutein (xanthophyll) with $KMnO_4$ yields a mixture 1 : 1-dimethylsuccinic and dimethylmalonic acids but no geronic or 1 : 1-dimethylglutaric acids.[27] This indicates that, as there is one

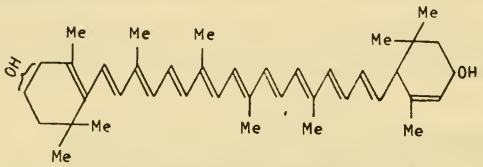


1 : 1-dimethylsuccinic acid

Dimethylmalonic acid

hydroxyl group in each ring,[28] they must occupy positions 3- or 4- and 3′- or 4′-.

It seems certain that the position of the group attached to the α-ionone ring is 3′- because an hydroxyl group in position 4′- would have enolic properties; the attachment in the other ring is accepted as being as position 3- only by an "analogy of symmetry" in a molecule which is asymmetrical. In many eyes, however, the tentatively suggested position (3-) in the β-ionone ring has now become an accepted fact and it is therefore important to emphasize its equivocal position. Further, recent work by Goodwin and Taha[29] indicates that position 4 might well be the correct one.



Lutein (Xanthophyll)

TABLE 2.—*Leaf Xanthophylls*
(after Strain unless otherwise stated)[25]

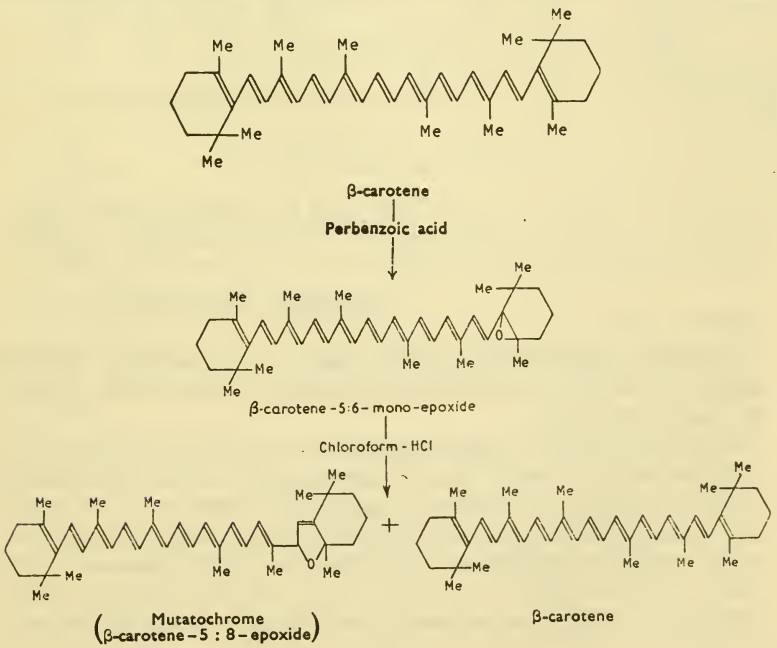
PIGMENT	RELATIVE ABUNDANCE	m.p.	ABSORPTION SPECTRA MAXIMA (mμ.)			
			CS_2	Petroleum	Chloroform	Benzene
Mixture I	13·25		separated	into 5 unidentified pigments		
Mixture II	5		separated	into 2 unidentified pigments		
Mixture III	1		several	inseparable	pigments present	
Neoxanthin	20	137-145°	493, 463	466, 437	476, 447	477, 447
Flavoxanthin c	4·5	169-172°	478, 450		459, 430	462, 431
Flavoxanthin b	6	167-171°	479, 449		459, 430	461, 432
Flavoxanthin*	4·75	184° (32)	478, 447·5	450, 421	459, 430	
Violaxanthin*	6·5	200° (32)	501, 470, 440	472, 443	482, 451·5, 424	484, 454
Violaxanthin b	6·5	187-191°	500, 469		481, 452	484, 454
Zeaxanthin	2	215·5° (4)	518, 483, 450	483, 451	494, 462, 429	
Isolutein	1·25	197-8°	503, 473			486, 457
Lutein epoxide†	—	192° (34)	501·5, 472	471, 442		482, 453
Lutein	62·5	193° (4)	508,475, 445	477·5, 447·5	487, 456, 428	
Cryptoxanthin	trace	169° (4)	518, 483, 453	485·5, 452, 420	497, 463, 433	

The reference numbers are to the end of Chapter 2.
* These data are for pigments isolated from flowers.
† Not observed by Strain.

Table 2 indicates the xanthophylls isolated by Strain and the relative amounts of each obtained; data reported subsequent to Strain's investigation are also included. Strain resolved mixtures I, II, and III (Table 2) into a number of components by further chromatography on $CaCO_3$ and powdered sucrose. None of these components was isolated or identified, although it seems unlikely that they were all oxidative artefacts.

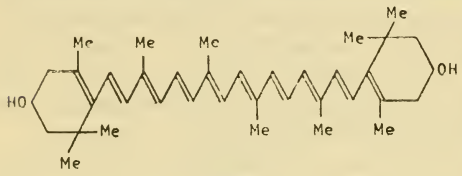
The flavoxanthins are designated *b* and *c* and violaxanthin, *b*, to distinguish them from the pigments isolated by Kuhn and his collaborators from flower petals (*see* p. 46); these pigments differ from Kuhn's pigments in chromatographic behaviour, m.p. and in optical rotations but are spectroscopically indistinguishable. Whether they are the separate pigments which Strain[25,30] considers them is still doubtful. Assuming for the moment that they are not identical they must be very closely related to the corresponding flower pigments.

It is only recently that the structures of violaxanthin and flavoxanthin have been determined, although their empirical formulae have been known since 1931-2.[31,32] Karrer's school working on the oxidation of carotenoids discovered that those members containing a β-ionone residue could be oxidized to 5 : 6-expoxides by treatment

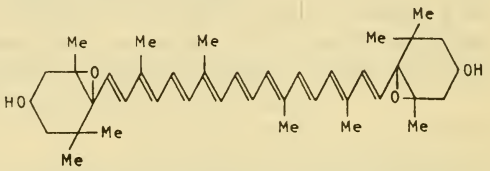


with either perphthalic or perbenzoic acid in chloroform solution. In the presence of traces of hydrogen chloride a chloroform solution of a 5 : 6-epoxide isomerizes producing a furanoid type of compound. (5 : 8-epoxide) and at the same time regenerating a small amount of the parent compound.[33]

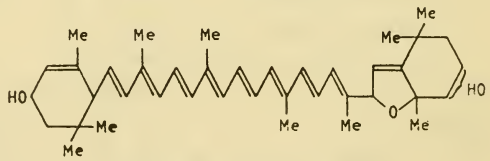
Violaxanthin and flavoxanthin have now been shown to be compounds of this type[34,35]; violaxanthin is the di-epoxide of zeaxanthin (*3 : 3′-dihydroxy-β-carotene, β-carotene 3 : 3′-diol) and flavoxanthin is the furanoid isomer of lutein (xanthophyll).[36]



Zeaxanthin



Violaxanthin (5:6, 5′:6′ -diepoxyzeaxanthin)



Flavoxanthin (5:8-epoxylutein)

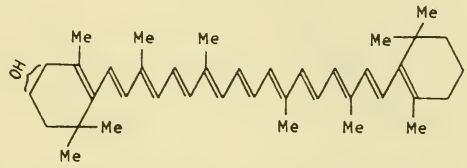
Zeaxanthin which Kuhn and Brockmann[37] had previously failed to observe in leaves was noted by Strain in traces (about 2 per cent. of the total xanthophylls).

Whereas β-carotene predominates over α-carotene in leaves, it is the dihydroxy-α-carotene (lutein (xanthophyll)) which predominates over the dihydroxy-β-carotene (zeaxanthin). There is yet no proof that xanthophylls are produced *via* the corresponding hydrocarbons (*see* p. 84) so no significance can, at the moment, be attached to this

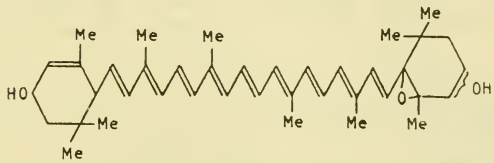
* The same doubt exists concerning the position of the OH groups in zeaxanthin as in the β-ionone ring of lutein (*see* p. 13).

reversal of relative abundance between the carotene and xanthophyll fractions.

Small amounts of *cryptoxanthin* (3- or 4-hydroxy-β-carotene)[38] and iso*lutein* also occur in leaves. *Iso*lutein, a carotenoid of unknown structure has, according to Strain, an empirical formula $C_{40}H_{56}O_4$ or $C_{42}H_{60}O_4$; it will be interesting to see if, in the light of recent experience, *iso*lutein turns out to be lutein -5 : 6-epoxide ($C_{40}H_{56}O_3$) which was observed in the leaves of *Lotus corniculatus* and *Arnica montana* by Karrer, Jucker and Krause-Voith[39] and which is now considered to be a normal component of green leaves.[40]



Cryptoxanthin (3 or 4-hydroxy-β-carotene)



Lutein (xanthophyll) 5:6-epoxide

Hydroxy carotenoids can occur naturally in the form of esters, as was first shown by Kuhn, Winterstein and Kaufmann.[41] In fresh green leaves, however, the amount of esterified xanthophylls present is a very small percentage of the total.[42]

Although the ratio of α : β-carotene can differ widely amongst different species (p. 8), Strain[25] has shown that the xanthophyll mixture remains quantitatively and qualitatively the same from species to species.

One of the most interesting observations made recently is that rhodoxanthin (*see* p. 32) occurs in the leaves of the gymnosperms *Ceratozamia mexicana* and *Haworthia coarctata* v. *krausii*.[185] This confirms and gives added interest to the early Tswett observations, and those of Monteverde and Lubimenko[42B], who stated that rhodoxanthin occurred in the winter foliage of *Thuja virginica*, *Taxus baccata*, *Cupressus naitnockii*, *Retinospora plumosa*, *Juniperus virginiana*, and *Gnetum* sp.

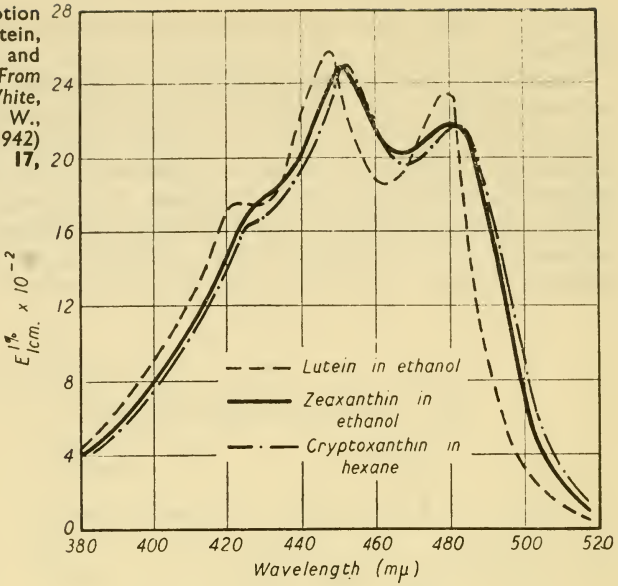


Fig. 4.—The absorption spectrum of Lutein, Zeaxanthin and Cryptoxanthin. (*From Zscheile, F. P., White, J. W., Beadle, B. W., and Roach, J. R.* (1942) Plant. Physiol., **17**, *331.*)

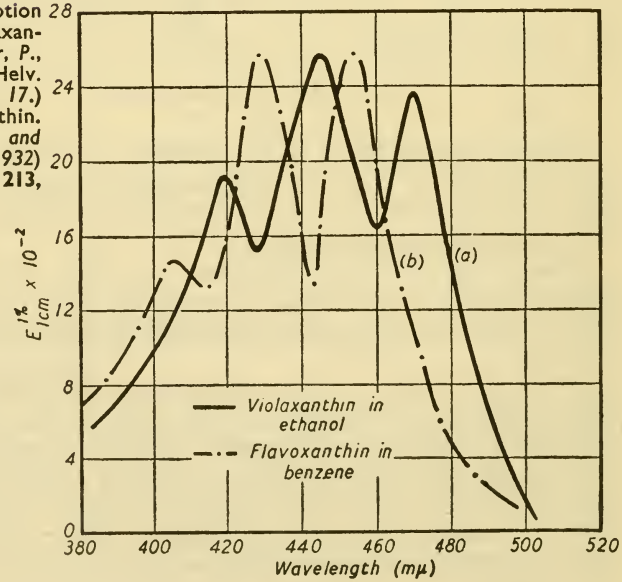


Fig. 5.—The absorption spectrum of Violaxanthin (*from Karrer, P., and Jucker, E.* Helv. Chim. Acta, **26**, *17.*) and Flavoxanthin. (*From Kuhn, R., and Brockman, H.* (1932) Hoppe-Seyl. Z., **213**, *192.*)

As rhodoxanthin is normally considered to be a carotenoid characteristic of fruit, and as it never occurs in the leaves of angiosperms (it does, however, exist in the water weed *Potamogeton* (*see* p. 57)) it may be the basis of an important differentiation between angiosperms and gymnosperms. This is an aspect of carotenoid biochemistry which demands much further study.

CAROTENOIDS IN THE DEVELOPING PLANT; FADING OF LEAVES

A considerable amount of work has been carried out on the quantitative aspects of carotenoid formation in the developing plant, not only because of its intrinsic importance, but because of the importance of green leaves as a source of vitamin A precursors in the nutrition of herbivorous animals. On a wet weight basis representative values for the carotene and lutein (xanthophyll) content of a forage grass are 45 and 60 μg./g.[43] Variations between the carotene content of the leaves of different species can, however, be considerable; for example, elm leaves contain twice as much as do willow leaves.[44] For further details the reader is referred to the appendix (p. 289) where all reported quantitative data on carotenoids in leaves are collected.

As a source of vitamin A precursors the leaf blade is extremely important, for not only is its carotene concentration 5-50 times greater than that of the mid-rib or the petiole, but also it contains over 90 per cent. of the total leaf carotene. Further, the leaves contain over 90 per cent. of the total plant carotene although they are less than 50 per cent. of the total weight.[45/47] The mid-ribs appear to contain slightly more carotene than do the petioles.[45]

Working with turnip tops, Bernstein, Hamner and Parks[48] showed that β-carotene is distributed fairly uniformly throughout the leaf blade. Their results also indicate that the carotene concentration in leaves picked at midday is less than in those picked either in the morning or at night (*see* p. 75). Similar diurnal variations are reported for lucerne.[49] Further data, taking into account variations from plant to plant, are necessary before one can unreservedly accept this phenomenon of diurnal variation. Markley,[50] using wheat plants, showed that inter-plant variations can be considerable.

Carotenoid formation begins immediately after germination takes place[51] and continues rapidly during the early period of active growth[52] (*see also* p. 66). It is at the period of maximal growth rate that carotene concentration is also maximal; this has been repeatedly demonstrated for a variety of species growing in a variety of climates in

the United States,[47,48,51,53-64] Russia,[65,66] Finland,[67] England,[68] South Africa,[69] Canada,[70] Australia,[71] Germany,[72,73] Japan,[74,75] Norway,[76] and India.[23] The point of maximal carotene concentration occurs relatively soon after germination, for example, 5 weeks after drilling in oat plants[77] and 8 days after the plants appear above ground in French beans.[78] The failure to detect an early maximum may account for the general decrease in carotene concentration during the period of active growth reported in one investigation in India[79] and one in Palestine.[80]

A later investigation in India,[23] however, showed that this maximum did appear in all but one of a number of vegetable species examined; the exception was lettuce, and no explanation of this anomalous behaviour has yet been offered.

It is well established that a period of drought reduces somewhat the carotene concentration of plants,[67,68] but the claim that plants which have wilted slightly, with consequent loss of carotene,[62] subsequently recover their carotene content with the recovery of turgidity when placed in water[81] still requires confirmation. A period of drought often coincides with the maturation of plants but there appears no doubt that the fall in carotene concentration at maturation is a specific result of this condition and is not due to a possible concomitant period of drought. This variation in carotene concentration during maturation is exhibited even on the same plant. In turnip tops[56,82] and tobacco leaves,[83,84] for example, the lowest (oldest) leaves have a carotene concentration somewhat lower than that of the highest leaves and even more striking differences were noted in leaves of different ages taken from maize plants.[47] Typical values which have been obtained for the youngest and oldest leaves were 800 and 547 μg./g. (dry wt.) respectively. Similar results were obtained by Nagel[73] for the leaves of the tobacco plant; he also found that even in single leaves the youngest parts (tips) contained a higher concentration than did the rest of the leaves. *Betula verrucosa* behaves in the same way.[336]

The higher concentrations of carotene in the upper stem regions compared with the lower regions are, in all probability, also due to the age factor.[46]

It seems possible that legume leaves do not show this drop so markedly[79] and Snyder and Moore[53] claim that the carotene content of soya bean leaves grown in America not only increases right up to maturity but also continues to do so until three weeks after the first flowers appear. In Australia, however, although legumes maintain their maximum concentration longer, their concentration at full maturation is as low as that of any other crop.[71] On the other hand

there appears to be no doubt that the effect of drought is much less marked with legumes than with other plants.[54]

Although at maturity the carotene concentration can be as low as one half the maximal value,[53] this does not mean that the total amount of carotene in the plant is reduced. There is no completely satisfactory evidence on this point, but when a survey of the available data is made,

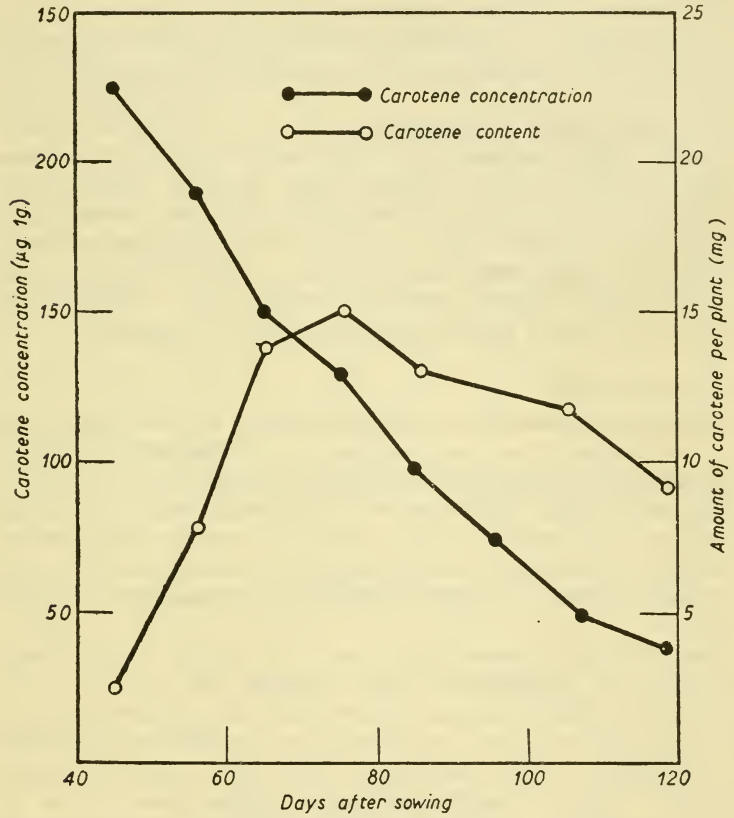


Fig. 6.—Showing the variation in the carotene concentration and carotene content in the developing maize (*after Porter, J. W., Strong, F. M., Brink, R. A., and Neal, N. P. (1946)* J. Agric. Res., **72**, *169*).

it emerges that during the later stages of maturation, the carotene *content* of a plant remains rather more constant than does the concentration. (*See* Fig. 6.)

From the point of view of total yield, it is probably best to harvest

maize at the " medium dent " stage of maturity, although the carotene concentration is highest at the pollen-shedding stage ;[47,53] as soon as flowering begins there is a rapid drop in carotene concentration.[67,68] In pasture grasses which produce a second cutting the carotene concentration generally recovers during the early autumn ;[56,62] exceptions to this are " big blue stem " (*Andropogon furcatus*) and buffalo grass (*Buchloe dactyloides*), autumn samples of which were almost devoid of carotene.[56] An observation which may become important in practical animal nutrition is that late cuttings may actually have a higher carotene concentration than have the first cuttings,[47,57] although the evidence is not unequivocal.[85,86] It is further claimed that frequent clippings increase the yield per acre.[63] However, in some vegetables which can be " wintered," *e.g.*, sprouting broccoli, kale, collards, there is no doubt that a marked drop in carotene concentration occurs during the winter ; for example, the concentration in collards fell from 7·9 mg. per cent. (wet wt.) in August to 2·8-4·6 mg. per cent. in mid-winter.[60,87] Chard does not show this drop.[87] Winter wheat, on the other hand, is a good source of carotene for cattle.[88]

Fading of Leaves

Although the carotene concentration of leaves varies during growth there are apparently no great variations in the relative distribution of carotenoids. The ratio of total carotene concentration to total xanthophyll concentration, which can vary considerably with species (the normal ratio lies between 1 : 1 and 1 : 8 but can rise to 1 : 15 in alpine plants)[74,89,90] does not seem to alter much during growth ; when leaves die, however, extensive qualitative and quantitative changes occur.

The yellowing of leaves in the autumn is due to the preferential destruction of chlorophylls which unmasks the colour of the carotenoids, but these themselves are undergoing changes. This was first appreciated by Tswett, who found that the yellow leaf carotenoids were epiphasic in a light petroleum—90 per cent. aqueous methanol partition.* This is a characteristic property of carotenes, but as the pigments behaved on chromatography similarly to xanthophylls he called them " autumn carotenes."

Much later von Euler, Demole, Weinhagen and Karrer[91] showed that these " autumn carotenes " were without appreciable vitamin A activity and agreed with the suggestion of Kuhn and Brockmann[37]

* When a solution of carotenoids in light petroleum is shaken with methanol containing 10 per cent. (v/v) of water, the carotenes and their mono-hydroxy and mono-keto-derivatives remain in the petrol (the epiphase), whilst the di- and poly-hydroxy-carotenoids are transferred to the methanol (the hypophase).

that they were xanthophyll esters which are known to exist in small amounts in green leaves.[31] Quantitative considerations indicated that if this were true some carotenes must have been converted into xanthophylls; this is unlikely from other considerations (*see* p. 84) and in 1934 Karrer and Walker[92] showed that "autumn carotenes" are not xanthophyll esters but unidentified oxidation products of both carotenes and xanthophylls. Even as late as 1937 these oxidation products were still being mistaken for carotenes.[93] During autumn necrosis, then, both types of pigment are oxidized, but the carotenes rather more quickly.[73]

Strain[25] states that the predominating pigment in yellow leaves is *zeaxanthin* which persists even after the leaves have fallen. This is attributed to the relatively greater stability of zeaxanthin, for there appears to be no question of its formation from other carotenoids.

It is interesting to find that normally yellow leaves appear to have a carotenoid system similar to that of necrosed leaves, for it is reported that leaves of the *aurea* variety of the elder, *Sambucus nigra*, contain excess "xanthophylls." The mixture of carotenoids in the young yellow leaves of *Euonymus japonica* is typical of that of autumn leaves[26] and Egle[94] has demonstrated that carotenoids of the autumn leaves of tropical evergreens are very similar to those found in the necrosed leaves of deciduous plants.

It will be obvious from what has been stated previously that the carotenoid picture in green leaves is reasonably simple. Very few green leaves differ from this general pattern and, when they do, it is only in minor details, *e.g.*, in the proportion of α-carotene present. This lack of species specificity disappears when the carotenoids of other plant tissues are considered. It is from the point of view of the production of characteristic carotenoids that fruit, blossoms, etc., are important, for, with perhaps one or two exceptions (*see* p. 269), they are relatively poor sources of the nutritionally important carotenoids (*see* p. 24).

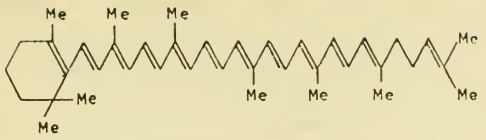
FRUIT CAROTENOIDS

(i) *β-carotene.* Generally, the preponderant leaf carotenoids, β-carotene and lutein (xanthophyll), are found in fruit, but they are often only minor constituents; for example, Le Rosen and Zechmeister,[95] found that the red flesh of the fruit of *Celastrus scandens* contained β-carotene to the extent of only 3 per cent. of the total carotenoid pigment and the presence of lutein (xanthophyll) was not recorded. The fruit of *Cotoneaster occidentalis* contain no β-carotene but here, as in the case

of *Pyracantha coccinea*, only small amounts of carotenoids are present.[96] However, β-carotene (together with small amounts of its *cis*-isomers) is the only carotene present in Badami mango fruit,[97] and in a number of other varieties of mango this and lutein (xanthophyll) are the predominant pigments.[98-102] According to the variety, β-carotene represents between 36 and 53 per cent. of the total carotenoids present.[103] In *Pyracantha angustifolia* β-carotene is a major constituent of the pigment fraction which only contains very small amounts of xanthophylls;[96] a similar situation is reported in buriti palms,[104] elderberries,[105] dates,[106] melons,[107,107A] beach plums,[108] and cannonball fruit.[109] According to Webster, Black and Cross[110] Concord are the best variety of grapes from the carotenoid point of view. The avocado pear appears to have a carotenoid distribution similar to green leaves, for it contains 2·5 μg./g. of total carotenoids, of which 0·6 μg. is carotene. 10 per cent. of this carotene is the α-isomer.[111] Papayas,[112] guavas[112] and the berries of *Rubus chamaemorus*[113] also contain β-carotene. It is interesting to compare the β-carotene content of fruit with the leaves of the plant producing the fruit; rose hips (*Rosa cinnamomea* and *R. rugosa*) grown in Russia contain 5 mg. per cent. (wet wt.) compared with a value of 40 mg. per cent. found in the corresponding leaves.[114] Apart from these comparatively few examples β-carotene is not normally the major carotene in fruit, for either α-carotene, γ-carotene, or lycopene is the most common fruit carotene. Further details concerning the carotene content of fruit are given in the appendix (p. 294).

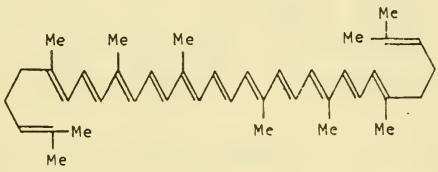
(ii) *α-carotene*, although not a distinctive fruit carotenoid, generally occurs in greater amounts, both absolutely and in relation to β-carotene, than it does in leaves. It constitutes up to 40 per cent. of the total pigment in red palm oil,[115] 25 per cent in banana flesh,[116] 25 per cent. in the chestnut,[115] and 15 per cent. in the rowan berry (*Sorbus aucuparia*).[117] Red palm oils are probably the richest sources of α-carotene known, but other palm oils, *e.g.*, *Attalea gomphococca* Mart. contain much less; apparently carotenes only are present in the latter oils.[118]

(iii) *γ-carotene* was first isolated in 1932 from the fruit of the lily of the valley (*Convallaria majalis*) by Winterstein and Ehrenberg;[119] the former investigator also demonstrated its presence in the fruit of *Gonocaryum pyriforme*.[120] The Kuhn[121,122] and Karrer[123] schools soon afterwards elucidated its structure; it is a monocyclic carotenoid containing a β-ionone and a ψ-ionone residue.



γ-carotene

(iv) *Lycopene*, an acyclic carotene[124,125] devoid of vitamin A activity, has been known in crystalline form since Millardet isolated it in 1876 and named it solanorubin. It is the main carotenoid pigment in the fruit of the red tomato (*Lycopersicum* spp.),[126] but is subordinate to β-carotene in the green fruited species (*L. peruvanium* and *L. hirsutum*)[127] and probably does not exist in the golden varieties.[128] It occurs in a number of other fruits such as rose hips (*Rosa canina*),[129] water melons (*Cucumis citrullis*),[130,131] apricots (*Prunus armeniaca*),[132,133] the palm (*Seafortia elegans*),[134] pink grape fruit,[135] and cow-berries.[136] Zechmeister and Cholnoky[137] in 1943 gave a comprehensive list of berries which contain this almost universal fruit carotenoid; recently Zechmeister has made the list complete up to 1947.[138] Peaches occupy an interesting position because European varieties contain lycopene[135] as well as α- and β-carotenes, whereas American varieties on the other hand do not contain any lycopene.[139]

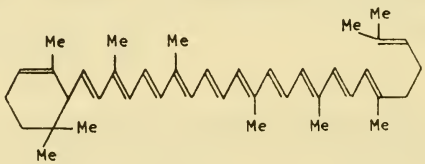


Lycopene

The preponderance of lycopene over β-carotene in some fruits is considerable. In commercial varieties of *L. esculentum* the lycopene content of the fruit is on the average 16 times greater than that of β-carotene; the values varied between 11 and 32 μg./g. (wet wt.) of lycopene and 2·1 and 8·0 μg./g. of β-carotene;[127] these values can be altered considerably by suitable breeding[140] (*see* p. 67). Similarly Jacoby and Wokes[140] found about seven times as much lycopene as carotene in rose hips and woody nightshade berries; in the case of rose hips the values were 101-834 μg./g. and 74-187 μg./g. (wet wt.) respectively. The carotene/lycopene ratio is about 1 : 12 in water

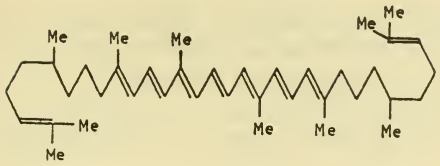
melons,[141] and 1 : 8 in European peaches.[135] On the other hand, Hunter and his colleagues[142,143] found only small amounts of lycopene in red palm oil. According to Brockman[132] the apricot contains only 2-3 mg. per cent. (wet wt.) of lycopene compared with 50-80 mg. per cent. of β-carotene. It is interesting to note at this point that whilst apricots contain only carotenes,[132] xanthophylls predominate in peaches.[136]

(v) δ-*carotene.* Winterstein[144] gave the name δ-carotene to a fraction obtained from the fruit hulls of *Gonocaryum pyriforme.* It also occurs in traces in ordinary tomatoes[145] (*Lycopersicum esculentum*), but crosses of *L. esculentum* and *L. pimpinellifolium* can contain up to 40 μg. of δ-carotene/g. fruit.[146] From such crosses Porter and Murphey[147] have recently been able to isolate δ-carotene in crystalline form (Table 4). It contains eleven double bonds ten of which are conjugated, one closed ring (which cannot have the β-ionone configuration because the pigment has no Vitamin A activity) and one open ring. Winterstein had previously suggested the following structure for δ-carotene. This cannot be completely reconciled with the findings of Porter and Murphey[147], although it may well be near the truth.



(?)δ-carotene

(vi) ζ-*carotene.* A carotenoid, first isolated from carrots by Strain in 1939[148] (*see* p. 54) and which, although not yet prepared in a completely pure state, has been well characterized chromatographically,[149,150] spectroscopically[151] and chemically,[152] is ζ-carotene. The suggestion[150] that because of the similarity in the position of their visible absorption bands ζ-carotene and aurochrome (Karrer and Jucker[150,153]) are probably identical, has been disproved by carrying out mixed chromatograms with the two pigments. Aurochrome is much more tightly absorbed than is ζ-carotene,[152] which has now been shown to be in all probability 5 : 6, 7 : 8, 5′ : 6′, 7′ : 8-octahydrolycopene[152] and to have no vitamin A activity.[154]



ζ-carotene

It has not been reported in the fruit of normal market tomatoes but exists in the selections made by Porter and Lincoln,[146] especially crosses of *L. hirsutum* with the common commercial species *L. esculentum*; concentrations of up to 80μg./g. fruit have been achieved in this

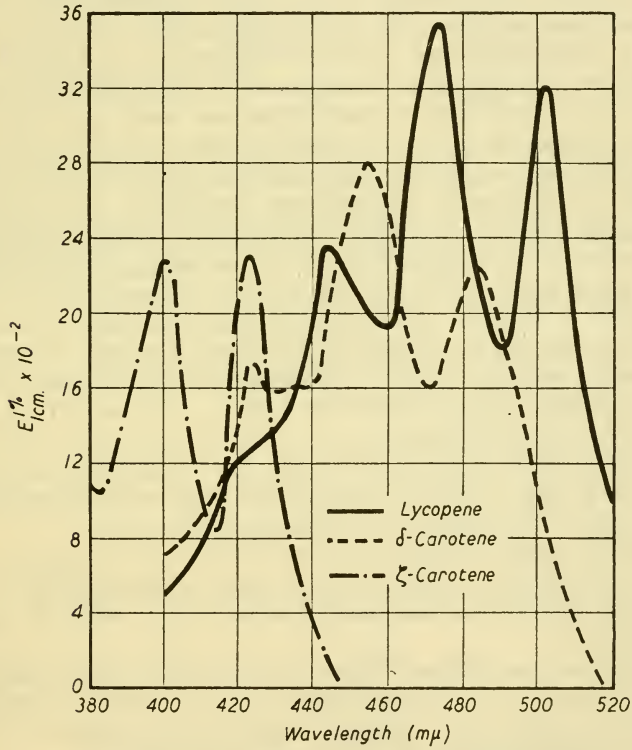


Fig. 7.—The absorption spectrum (in hexane) of Lycopene, δ-carotene and ζ-carotene. (*From Porter, J. W., and Zscheile, F. P. (1946)* Arch. Biochem., **10**, *537.*)

way. κ-Carotene, first observed in yellow maize[155] (*see* p. 43) is, in all probability, ζ-carotene as is the " carotene I " reported from the same source by Baumgarten, Bauernfeind and Boruff.[156] This being so, we can assume that ζ-carotene occurs in some Indian varieties of orange (*Citrus aurantium*) (reported as κ-carotene).[157]

(vii) *Other carotenes* (*including colourless polyenes*). Porter and Lincoln[146] have examined in great detail the carotenoid composition of many tomato crosses involving *L. esculentum*, *L. hirsutum* and *L. pimpinellifolium*; they have found what appear to be a series of derivatives of lycopene produced either by the step-wise addition of four hydrogen atoms to the parent carotenoid or (as they consider more probable) the step-wise removal of four hydrogen atoms from a saturated precursor (*see* p. 68 for a full discussion). The first in this series is *tetrahydrolycopene* which is considered to be identical with *neurosporene* first reported in *Neurospora crassa* and lately in other fungi (*see* p. 103); then follows *octahydrolycopene* which is probably ζ-*carotene*, already discussed. This completes the coloured members of the series for the next compound is *phytofluene* which is colourless but exhibits a characteristic bluish-green fluorescence in ultra-violet light. Zechmeister[158-160] and his colleagues first obtained phytofluene from tomatoes and other plant sources not containing chlorophyll (*e.g.*, flower petals and fungi) and its quantitative occurrence in some fruit is recorded in Table 3. A little later Porter and Zscheile[145] independently also reported the presence of this colourless polyene in tomatoes. Zechmeister and Sandoval,[159,160] although not able to isolate phytofluene in crystalline form, showed that it is a C_{40} compound ($C_{40}H_{84\pm2}$) with the carotenoid skeleton.

It contains seven unsaturated linkages, five of which are conjugated. They did not feel justified in further defining its structure but Porter and Lincoln[146] consider it to be dodecahydrolycopene.

Phytofluene does not occur in green plant leaves or in the leaves of the tree *Cinnamonum camphorum* at any stage of development.

Recently phytofluene has been reported in the wood of *Acacia acuminata*.[162A]

Zechmeister and Pinkard[161] have lately found in ripe tomatoes a compound very similar spectroscopically to phytofluene but differing in chromatographic and partition behaviour; it appears to be a hydroxy phytofluene and has been named *phytofluenol*.

The colourless unsaponifiable substance which precedes phytofluene on an adsorption column[145] is now considered to be hexadecahydrolycopene[146] and has been named *phytoene*; tomato selections have

TABLE 3.—*The occurrence of phytofluene in some fruit* (*from Zechmeister and Sandoval* Arch. Biochem. (1945), 8, 425)

Species	Amount present mg./kg. fresh material
Zantedeschia aethiopica	1·1
Cucumis citrillus	2·2
Cucumis melo	0·6
Cucurbita maxima	+
Zea mays	0·6
Eugenia uniflora	0·7
Butia eriospatha	0·3
Pyracantha angustifolia	14·7-27·7
Pyracantha yunanensis	0·4
Rosa canina	1·8
Prunus domestica	1·0
Prunus persica	0·8
Citrus aurantium—juice	0·3
—outer rind	1·5
—inner rind	2·3
Capsicum annum—skin	4·6
Lycopersicum esculentum—ripe	6·0-10·6
—unripe	2·0

been obtained containing up to 43 μg. of this substance per g. of fruit. The last material in this series reported by Porter and Lincoln[146] is *tetrahydrophytoene* (*i.e., eicosahydrolycopene*). Little information about this highly saturated carotenoid is available at present but it apparently absorbs light at 220 mμ.

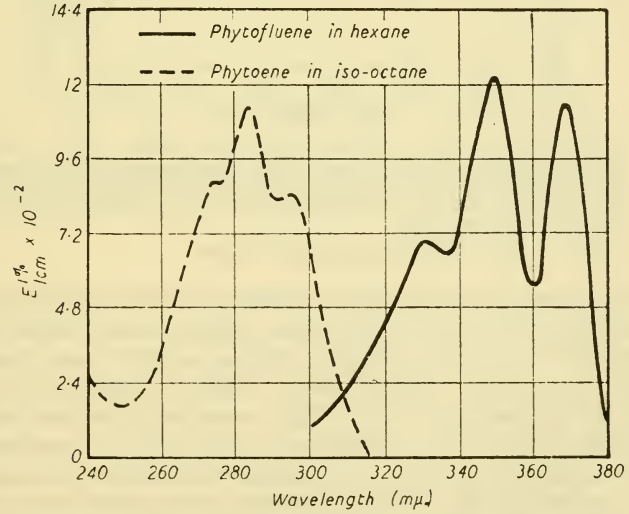


Fig. 8.—The absorption spectrum of Phytofluene and Phytoene. (*From Porter, J. W., and Zscheile, F. P.* (*1946*) Arch. Biochem., **10**, *537*.)

There still remains to be identified the pigment designated " Unidentified II " by Porter and Zscheile[145] and which disappeared in advanced generations of the selections containing it; it appears to contain 8 conjugated double bonds[145] and its spectrum is recorded in Table 4.

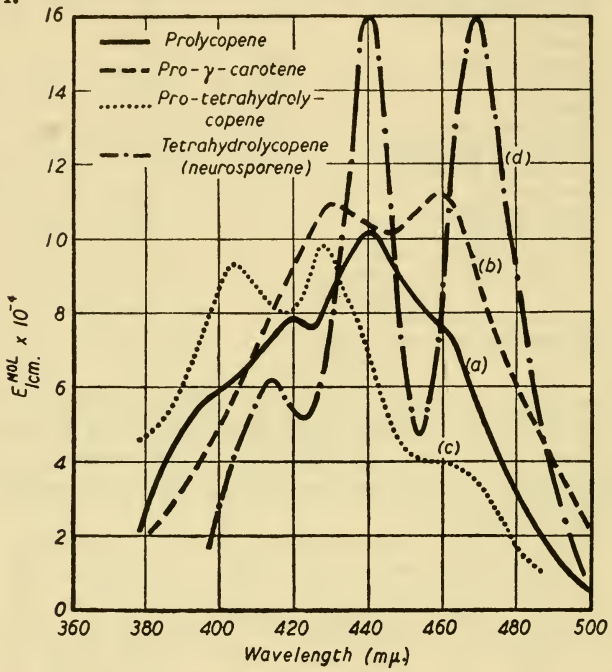


Fig. 9.—The absorption spectrum in hexane of (*a*) Prolycopene, (*b*) Pro-γ-carotene, (c) Pro-tetrahydrolycopene and (*d*) Tetrahydrolycopene (Neurosporene). (*From Zechmeister, L. (1944)* Chem. Rev., **34**, *267 (a & b), Porter, J. W., and Zscheile, F.P. (1946)* Arch. Biochem., **10**, *537. (c), and Haxo, F. (1949)* Arch. Biochem., **20**, *400. (d).*)
Note.—The E values for (c) are only relative.

(viii) *cis-isomers* of the carotenes have been observed in the fruit of tomatoes,[145,146,152] the palms *Butia capitata* and *B. eriospatha*,[163] mangoes,[101,157] peaches, papayas, guavas, apricots, water melons, bananas, pineapples, grapes, musk melons, oranges[154,164] and *Pyracantha angustifolia*.[163,165] The most important are *neo*-β-, β-carotenes B and U[157], prolycopene,[146,154,166] pro-γ-carotene[163] and protetrahydrolycopene.[146] The β-carotene isomers have already been discussed (p. 8). Prolycopene and pro-γ-carotene are stable pigments which contain, respectively, 5 or 7 and 4 or 5 of their double

bonds with the *cis*-configuration. *Protetrahydrolycopene* (previously named all-*cis*-lycopene,[167] poly-*cis*-ψ-carotene[168] and unidentified I[145]) is also a poly-*cis*- compound.

In addition to prolycopene Zechmeister and Pinckard[165] isolated 6 further lycopene isomers from *P. angustifolia* berries and named them poly *cis*-lycopenes I-VI, according to their adsorptive power, the first member being the most strongly adsorbed. The absorption spectra of these isomers are recorded in Fig. 10.

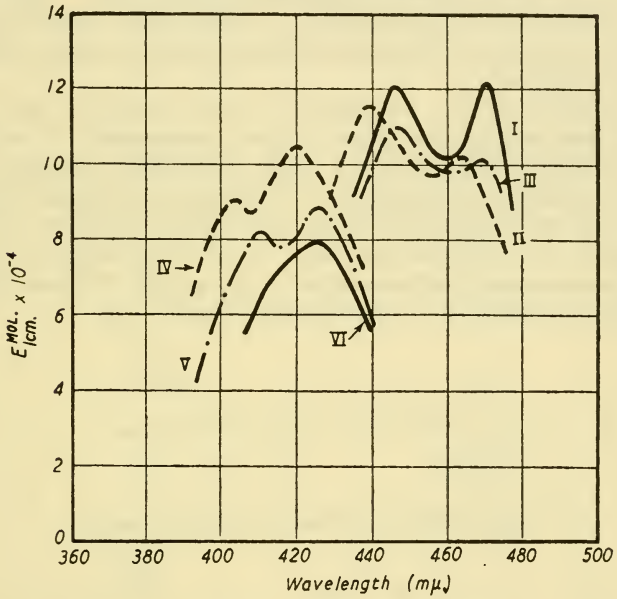


Fig. 10.—The absorption spectrum in hexane of Prolycopenes I—VI. (*From Zechmeister, L., and Pinckard, J. H. (1947)* J. Amer. Chem. Soc., **69**, *1930.*)

II. Xanthophylls

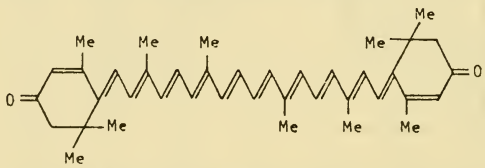
Turning to fruit xanthophylls, they differ from leaf xanthophylls in three main features, (*a*) they are generally esterified, (*b*) there is a different quantitative distribution, and (*c*) many fruit contain xanthophylls peculiar to themselves.

Cryptoxanthin,[37] although only present in leaves in traces, constitutes one-third of the total carotenoids of the berries of *Physalis alkekengi* L. (*P. franchetti* Hort.) from which it was first isolated; Karrer and Schlientz[169] consider that the pigment isolated from *Carica papaya* and *Citrus poonensis* and termed *caricaxanthin*[170] is

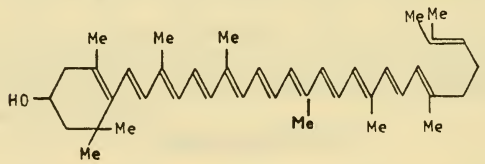
cryptoxanthin palmitate. There is more cryptoxanthin than β-carotene in the rind of Seville oranges[171,172] and it constitutes about 30 per cent. of the total carotenoid of the pulp of Indian oranges, but is slightly less abundant (25 per cent.) in the rind.[157]

Physalien,[173] the pigment responsible for the deep red colour of mature berries of *P. alkekengi* is zeaxanthin dipalmitate[40]; there is no zeaxanthin in the unripe sepals.[174] Zeaxanthin is apparently the principal carotenoid in fruit of the palm *Cycas revoluta*[175] and it also occurs in kaki fruit (*Diospyros kaki*).[176] *Violaxanthin* and *flavoxanthin* have been reported in *Cotoneaster occidentalis* and *Pryacantha coccinea*[95] berries, respectively.

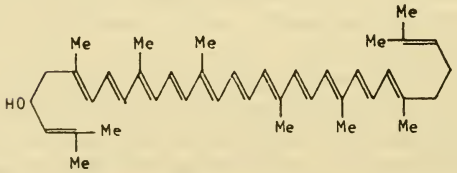
Xanthophylls having considerable species specificity have been isolated from fruits of yew (*Taxus baccata*), *rhodoxanthin*,[177] rose hips, (*Rosa rubiginosa*) *rubixanthin*,[178] woody nightshade (*Solanum dulcamara*) *lycoxanthin* and *lycophyll*;[179] red peppers (*Capsicum annuum*), capsorubin and capsanthin;[180] false bittersweet (*Celastrus scandens*), celaxanthin,[181] oranges *citroxanthin*;[182] and from spindle tree berries (*Euonymus europaeus*) *antheraxanthin*[183] (*see* p. 50). Citroxanthin has recently been identified as *mutatochrome*[184] (*see* p. 15).



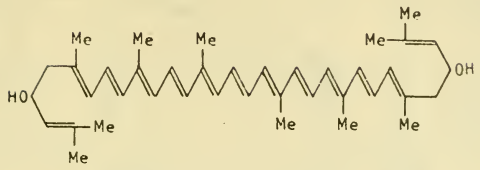
Rhodoxanthin (3 : 3′-diketodehydro-β-carotene)



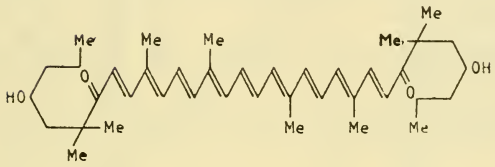
Rubixanthin ($C_{40}H_{56}O$) (3-hydroxy-γ-carotene)



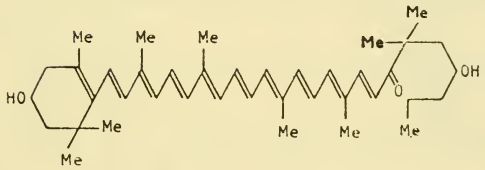
Lycoxanthin ($C_{40}H_{56}O$, 3-hydroxylycopene)



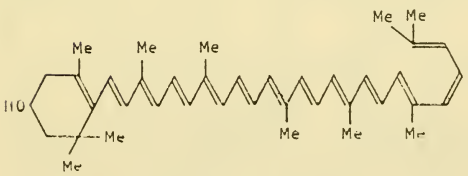
Lycophyll ($C_{40}H_{56}O_2$ 3:3- dihydroxylycopene)



Capsorubin ($C_{40}H_{60}O_4$)



Capsanthin ($C_{40}H_{58}O_3$)



Celaxanthin ($C_{40}H_{56}O$)
(3-hydroxy-3′-dehydro-γ-carotene)

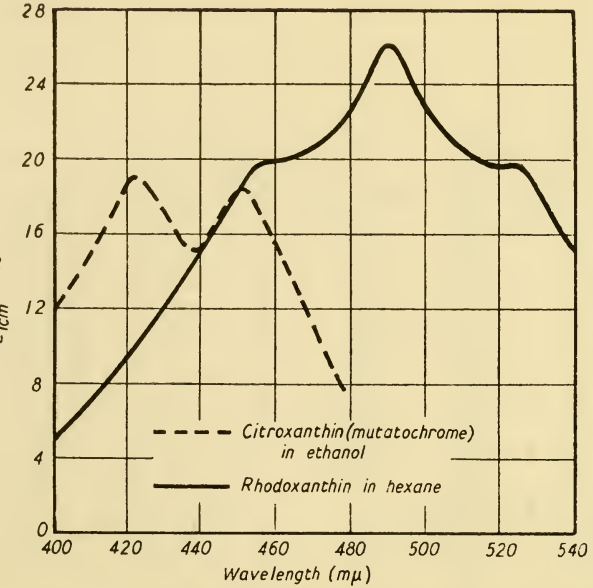


Fig. 11.—The absorption spectrum of Citroxanthin (Mutatochrome). (*From Karrer, P., and Jucker, E. (1947)* Helv. Chim. Acta, **30,** *536.*) and Rhodoxanthin in hexane (*from Kuhn, R., and Brockmann, H. (1933)* Ber. deutsch. chem. Ges., **66,** *828*).

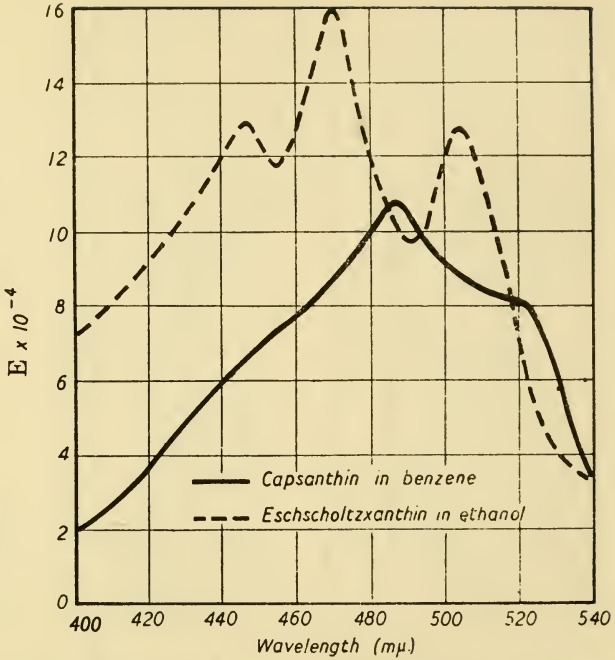


Fig. 12.—The absorption spectrum of Capsanthin (*from Zechmeister, L. (1944)* Chem. Rev., **34,** *267.*) and Eschscholtzxanthin (*from Strain, H. H. (1938)* J. biol. Chem., **123,** *425*).

TABLE 4.—*Fruit Carotenoids**

PIGMENT	m.p.	ABSORPTION SPECTRUM MAXIMA (mμ.)				
		CS_2	$CHCl_3$	Light petroleum (b.p. 60–80°)	Hexane	Benzene
Pro-γ-Carotene[163] ..	118–119°	493·5, 460·5	473, 444	464, 435		477, 447·5
δ-Carotene[120,147]	—	526, 490, 457	503, 470, 440	488, 456, 430‡	490, 458, 428	
ζ-Carotene[151]	—				425, 400, 378	
Lycopene[125]	175°	547, 507	520, 485, 456	506, 474		
Prolycopene[166]	111°	500·5, 469·5	484, 453·5	470, 443·5		485, 455·5
Poly-*cis*-lycopene I[165] ..	93–95°				444–445	
Poly-*cis*-lycopene II[165] ..	85–87°				441–442	
Poly-*cis*-lycopene III[165] ..	105–106°				443–446	
Poly-*cis*-lycopene IV[165] ..	—				426	
Poly-*cis*-lycopene V[165] ..	—				431–432	
Poly-*cis*-lycopene VI[165] ..	—				433	
Tetrahydrolycopene (? neurosporene)[146] ..	124°				433, 410†	
Protetrahydrolycopene[145]	—				430, 407†	
Phytofluene[160]					332, 348, 367–8	338, 355, 374
Phytoene[145]	—				275, 285, 296†‡	
Tetrahydrophytoene[146] ..					? 220	
Unknown II[143]					453, 428†	
Rhodoxanthin[177]	219°	564, 525, 491	546, 510, 482	521, 487, 454	524, 489, 458	542, 503·5, 474
Rubixanthin[178]	160°	533, 494, 461	509, 474, 439	495·5, 463, 432	494, 462, 432	
Lycoxanthin[179]	168°	547, 507, 473		503, 472, 443		521, 487, 456
Lycophyll[179]	179°	546, 506, 472		504, 473, 444		505, 474, 444
Capsorubin[180]	198–201°	543, 503·5, 470		507, 474, 444	502, 470, 471	524, 489, 455
Capsanthin[180]	175–176°	542, 503		504, 474·5		520, 486
Celaxanthin[181]	209–210°	562, 521, 487		520, 486·5, 456		
Citroxanthin[182, 183] (mutatochrome) ..	167°	489·5, 459	469, 435	456, 427		

* Carotenoids recorded in previous tables are not included here; references at end of Chapter II.

† Read from the published curves.

TABLE 5.—*Carotenoid Distribution in Fruit and Seeds*

	α-Carotene	β-Carotene	γ-Carotene	Lycopene	δ-Carotene	ζ-Carotene	Lutein	Rhodoxanthin	Cryptoxanthin	Zeaxanthin	Lycoxanthin	Violaxanthin	Rubixanthin	Taraxanthin	Mutatochrome	Capsanthin	Celaxanthin	Antheraxanthin	Reference
Actinophloeus angustifolia				+															2
A. macarthurii				+															2
Aglaonema nitidum, *A. oblongifolium*, *A. simplex*				+															2
Ananas sativus		+					+												14
Arbutus unedo	+	+		+			+		+	+		+							49
Archontophoenix alexandrae				+															2
Areca alicae				+															2
Arum italicum				+															2
A. maculatum				+															12
A. orientale		+		+			+												12
Asparagus officinalis									+										15
Attalea gomphococca		+																	6
Bryonia dioica				+															2, 15
*Butia capitata**																			13
Calyptrocalyx spicatus				+															2
Capsicum annoum															+	+			18, 47, 76
C. frutescens		+														+			50, 57
C. japonicum		+																	70
Carica papaya									+			+							46, 47
Celastrus scandens		+															+		66
Citrus aurantium	+			+			+		+	+		+			+				32, 35, 77
C. grandis	+			+					+			+							36
C. madurensis				+			+		+	?		+							38
C. poonensis		+					+		+			?							39
Citrullus vulgaris	+	+	+	+															59, 60
Convallaria majalis	+	+	+	+			+												15, 16
Cotoneaster occidentalis							+					+							21
Cucumis citrullus				+															67, 68
Cucurbita maxima	+	+					+					+							61
Cuscuta subinclusa													+						72, 73
C. salina													+						
Cycas revoluta										+									69
Diospyros costata	+	+		+					+										52
D. kaki				+					+										53
Elaeis guineensis		+		+															7, 8
Elaeis melanococca		+		+															7
Erythroxylon coca				+															2
E. novogranatense				+															30, 31
Euonymus europaeus†				+						+							+		42, 75, 78
E. japonicus				+															13
Gonocaryum obovatum	+	+	+	+															15, 16
G. pyriforme	+	+	+	+	?														
Gossypium spp.		+					+												43, 44
Hippophae rhamnoides										+									12
Iris pseudacorus												+							74
Lathyrus sativus		+																	83
Luffa spp.		+					+												62
Lycium barbaratum										+									15
L. hamimifolium										+									54
Lycopersicum esculentum †		+	+	+		+													55, 56, 71
L. hirsutum †		+	+	+		+													26, 71,
L. peruvianum †		+	+	+		+													79, 80
Mangifera indica	+	+					+												40, 41
Momordica balsamina				+			+												63, 64
M. charantia		+		+															64
Musa paradisiaca		+					+												19
Nenga polycephalus				+															2
Pandanus polycephalus				+															2
Passiflora coerulea				+															45
Physalis alkekengi ‡									+	+									57
Prunus armeniaca		+	+	+					+	+									21
P. persica			+				+		+	+									22

* pro-γ-carotene
† also colourless polyenes and prolycopene.
‡ = *P. franchetti*.

TABLE 5.—*Carotenoid Distribution in Fruit and Seeds—continued*

	α-Carotene	β-Carotene	γ-Carotene	Lycopene	δ-Carotene	ε-Carotene	Lutein	Rhodoxanthin	Cryptoxanthin	Zeaxanthin	Lycophyll	Lycoxanthin	Violaxanthin	Rubixanthin	Taraxanthin	Mutatochrome	Capsanthin	Celaxanthin	Antheraxanthin	Reference
Ptychandra elegans				+																2
P. glauca				+																2
Pyracantha angustifolia * ..		+																		20, 81, 82
Pyrus aucuparia		+																		71
Rosa canina		+	+	+			+			+				+	+					24, 24, 25
R. damascena } ..		+	+	+			+			+				+	+					25
R. rubiginosa }																				
R. rugosa	+	+	+	+										+						26
Rubus chamaemorus	+	+	?	+						+				+						27
Sabal serrulatum		+																		9
Seaforita elegans				+																68
Solanum balbisii				+																2
S. decasepalum				+																10
S. dulcamara											+	+								18
S. esculentum				+							+									13
S. hendersonii										+										15
S. lycopersicum		+		+			+													58
Sorbus aucuparia	+	+																		28, 29
Synaspadix petrichiana ..				+																2
Tabernae-montana pentasticta				+																2
Tamus communis				+							+	+								17, 18
Taxus baccata								+												1
Trichosanthes spp.				+																65
Triticum vulgare		+					+													3
Vaccinium vitis-idoea ..		?		+			+			+										48
Vigna sinensis		+					+													30
Zea mays	+		+			+	+		+	+										4. 5, 54

* also pro-γ-carotene and prolycopenes.

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The most important feature which emerges from this list of xanthophylls in fruit is the first appearance of two new types of carotenoids, (*a*) those containing a carbonyl group : rhodoxanthin, capsorubin, and capsanthin and (*b*) those in which an oxygen atom is incorporated into a furan ring (a 5 : 8-epoxide), mutatochrome. With regard to rhodoxanthin, it should be pointed out that an unconfirmed report exists indicating that it occurs in the leaves of *Haworthia coarctata* v. *Krausii* and *Ceratozamia mexicana.*[185] (*see also* p. 17).

Chemical and spectrographic data on fruit carotenoids are recorded in Table 4 and their qualitative distribution in different species in Table 5.

Ripening of Fruit

There is complete agreement amongst numerous workers that the carotenoid content of fruit increases considerably with maturation; for example, the carotene content of red peppers is more than thirty times greater than that of the green peppers;[186,187] similar results have been obtained with orange rind,[188,190] orange pulp,[189] orange juice,[112] pumpkins (*squashes*),[191] rose hips (*R. spaldingii*,[192] *R. cinnamomea* and *R. rugosa*),[111] tomatoes,[145] mangoes[193-196] and a number of citrus fruits.[197]

There does, however, exist one early report that banana skins maintain a constant carotenoid content during ripening.[193]

Rind or peel of fruit very often is the region of the highest concentration of carotenoids;[188,197-201] for example, apple peel has a concentration five times greater than that of the flesh,[202] whilst 50-75 per cent. of the total carotenoids of oranges exists in the rind.[147]

The fact that accumulation of carotenoids in maturing fruit is accompanied by a commensurate disappearance of chlorophylls has given rise to considerable speculation on the possibility of the conversion of chlorophylls into carotenoids. This will be dealt with later (*see* p. 66) for at the moment it is only important to consider the qualitative changes occurring in the carotenoid distribution during maturation. The overall picture indicates an increase in total carotenoids and an increase in the carotene : xanthophyll ratio (*i.e.*, the preferential formation of carotenes, especially noticeable in mangoes)[103] and an increase in the amount of xanthophylls present as esters.

Kuhn and Brockmann[176] showed that the carotenoid distribution in the green sepals of *P. alkekengi* is very similar to that in green leaves (xanthophylls : carotenes, 3 : 1). On ripening, artificially in the presence of O_2, lutein (xanthophyll) disappears, carotene increases and physalien appears.

There is relatively a much higher concentration of carotenes than of xanthophylls in mature orange peel.[139] The lycopene : carotene ratio changes in ripening rose hips.[203] However, in the case of *Pyracantha angustifolia* berries, ripening produced a considerable increase in all pigments except β-carotene which remains at almost the same level;[97] in ripening palms the α : β-carotene ratio remains constant.[143]

Factors influencing Carotenoid Production in Ripening Fruit

Naturally most of the work which has been reported has been carried out on the economically important tomato. Fruit formed under growing conditions unfavourable to fruit production contain more lycopene than do well-developed fruit. This variation is not a function of the size of the fruit nor is it caused by the formation of other carotenoids at the expense of lycopene.[204] In general, fruit gathered unripe continue to produce carotenoids, but vine ripened tomatoes always contain more than do fruit ripened in storage.[205,206] Stored tomatoes can synthesize up to 1200 μg. of carotenoids per day.[207]

(i) *Oxygen*

Oxygen is needed for the ripening process, for artificial ripening in an atmosphere of ethylene or carbon dioxide prevents the expected increase in the carotenoid content of tomatoes, paprika,[207A] *Physalis alkekengi*[173] and *Tamus communis*.[207A] Similarly oranges fail to synthesize carotenoids in an atmosphere of ethylene and in the case of limes, lemons and grapes ripened in ethylene, there is in fact a slight loss during maturation.[208]

(ii) *Light*

The well-known domestic habit of ripening tomatoes in dark cupboards and drawers indicates that light is not essential for carotenoid synthesis during maturation. Information concerning the precise rôle of light is however scanty and occasionally contradictory. The observation that with commercial strains of tomatoes, fruit matured on the vine in the dark (by bagging the fruit) contain less carotenoids than do those grown normally in the light,[209,210] indicates that a photochemical factor is concerned in carotenogenesis. However, an increased synthesis apparently occurs in the dark in the case of albino (Clark's albino) and golden (Ruby Gold) tomatoes and Elberta peaches, Humbolt nectarines and Royal apricots. This may be a true varietal and species difference but other uncontrolled factors such as temperature may have been operating. A separation of photo- and thermochemical effects is difficult to achieve, and, as will be seen later, the thermal factor is of major importance in carotenoid synthesis. A further factor which has not yet been adequately explored is the probability that there is an optimum light intensity for synthesis, and that the optimum value may vary for each carotenoid component; for example, on ripening tomatoes in "normal" sunlight lycopene proceeds at a greater rate than does β-carotene synthesis,[122] but in "strong" light the former is slowed down and the latter speeded

up.[211] As lycopene is by far the major pigment of commercial tomatoes, the net effect of "strong" light might well be to reduce the total amount of carotenoids synthesized. Further, there is some evidence which suggests that shaded tomatoes, although physiologically unripe, contain more carotenoids than do fruit fully exposed to the sun.[212] Here the important word is *fully* (probably connoting a high temperature), for the carotenoid content of oranges of the same age from the same tree varies according to the aspect of the fruit. Those facing the sun containing more than those facing away from the sun; this variation can even be observed on the opposite sides of the same specimen.[188]

That ultra-violet light may also play a part in carotene formation is suggested by the reports that tomato fruit produced in greenhouses and thus less exposed to ultra-violet radiations than out-of-door plants, contain less carotenoids than do those produced in the open.[205,209,213-215] As is the case with visible light, ultra-violet light inhibits carotenogenesis in the golden varieties.[216] Direct ultra-violet irradiation of excised green commercial tomatoes, however, retards the disappearance of chlorophyll and the appearance of carotene,[208] whilst in excised mangoes ultra-violet light actually increases the amount of carotenoids formed.[103]

(iii) *Temperature.* As early as 1913, Duggar[217] noted that carotenoid formation did not occur when unripe tomatoes were stored at 30°C. This has recently been confirmed by Went, Le Rosen, and Zechmeister,[204] McCollum,[218] Ellis and Hamner[205] and Sadana and Ahmad.[206] Ellis and Hamner found that green tomatoes held at 70°–80° F. failed to redden but became yellowish pink. Went *et al.* and Sadana and Ahmad found that unripe tomatoes stored above 30° failed to produce lycopene although formation of other carotenoids was unimpaired (*cf.* Smith's "light" experiments, p. 40). The mechanism of lycopene production was not affected, however, because on lowering the temperature the pigment soon appeared. A reasonably sharp optimum temperature (19°C.) was observed for lycopene formation. McCollum confirmed that high temperatures do not favour lycopene formation, and Sadana and Ahmad noted that carotene production was greater at 34°C. than at 38°C.

The carotenoid content of mangoes picked unripe increases on ripening in store,[102,103] as one would expect. The rate of formation of these carotenoids is accelerated at high storage temperatures but it is important to note the final amount synthesized is not affected by temperature variations.[103]

(iv) *Other Factors.* There is only one report, which is, however, very fully documented, that demonstrates that variation in mineral nutrients of the soil has no effect on the carotenoid content of tomatoes.[205] Similarly, treatment with β-naphthoxyacetic acid produces no alteration in carotene content.[219]

SEED CAROTENOIDS

In this section a wide interpretation of the term " seed " is accepted, for a discussion of various grass and cereal " seeds," which strictly are fruit, will be included.

Apart from the yellow maize which will be dealt with separately, grass and cereal seeds contain only small amounts of carotenoids in which xanthophylls predominate ; no specific carotenoids have been detected ; the pigments occur in both the flour and the bran.[220]

Cereals such as wheat and rye contain between 130 and 150 μg. per cent. (wet wt.) of carotenoids of which about 10 per cent. is carotene.[221-224] The greatest carotene accumulation occurs at the " milky " ripeness stage.[221] Underwood and Curnow,[225] report values of 800, 900 and 1,000 μg. per cent. (dry wt.) for wheat, oats and barley carotenoids respectively, but these differences are not considered significant. Milling and processing destroy a considerable portion of the carotenoids, *e.g.*, whole wheat flour contains about 10μg. per cent. of carotene and 150–200 μg. per cent.[226,227] (dry weight) of xanthophylls ; similar values are reported for rye, barley, and blue vetch flour.[228] The very low value for carotene in wheat flour has been confirmed by Goodwin and Morton,[229] and Malmberg and von Euler[230] actually found no carotene in Swedish flour. Zechmeister and Cholnoky[226] have listed early reports on the carotene content of flour and discuss reasons why, in these investigations, the carotene present was over-estimated.

Brockmann and Völker's[231] values for the carotene and lutein (xanthophyll) of a number of grass seeds used in avian nutrition (*see* Appendix I) are of the same order as those found for wheat, but it should be noted that, contrary to Brockmann and Völker's observations, Kritzler[232] reports β-*carotene* in canary grass and, rather oddly, only α-*carotene* in millet. A further interesting point is that arils of the seeds of the passion flower (*Passiflora coerulea*) contain only lycopene.[233] Carotenoids have also been reported in seeds of *Acer*, *Ginkgo*, and *Citrus nobilis*[234] and in coffee beans.[235]

The xanthophyllic fraction from seeds is heterogeneous and Strain[25] believes that its composition is very similar to that from green leaves ;

Seybold and Egle have confirmed this in both spores and seeds.[224] Lutein (xanthophyll) is the principal component of wheat[226,236,237] and *flavoxanthin* and *zeaxanthin* have been reported in canary seed and millet;[232] cryptoxanthin, however, appears to be absent from most seeds but may be present in peppers.[237,238]

Pumpkin seeds contain both carotenes and xanthophylls[238] but annatto seeds (*Bixa orellana*) contain neither carotene, lycopene nor cryptoxanthin;[239] as might be expected (*see* p. 32), red pepper seeds contain *capsorubin* and *capsanthin*.[238] Lycopene has been isolated from chaura (*Maytenus disticha*) seeds.[240]

Maize has long been known to contain β-carotene, cryptoxanthin and zeaxanthin,[241] and it was from this source that zeaxanthin was first isolated. Earlier workers reported a preponderance of cryptoxanthin, but it seems from recent work that maize contains about equal amounts of β-carotene and cryptoxanthin although the exact relative amounts do vary somewhat with varieties.[149,242,243] The total carotenoid content of maize also varies with variety, limiting values reported are 0·1–4·8 μg./g. (fresh wt.).[149,241-246] Other pigments reported in maize are α-carotene, ζ-carotene, (reported as χ-carotene and "unknown carotene I,"[149] although it should be noted that χ-carotene is reported to have vitamin A activity whilst ζ-carotene is inactive; *see* p. 27). γ-carotene,[109,246] lutein (xanthophyll) and probably *cis*-isomers of β-carotene.[247] Sorghum has about one-half the carotene content of maize.[248]

Considering bean and pea seeds, it is found that green (ready for picking) soya beans (*Soja glycine*),[248] cow peas (*Vigna sinensis*),[248] Lima beans (*Phaseolus lunatus*),[249] Thomas Laxton peas (*Pisum sativum*),[250,251] and French (snap) beans (*Phaseolus vulgaris*)[252,253] contain between 2 and 7 μg./g. (wet wt.) of carotene. Carotene has also been reported in cotton seeds.[254] The belief that the carotene content of peas depends considerably on the variety is apparently firmly based[253,255,256] (*see* Table 6) although it has been disputed.[247] Table 6 shows the varietal differences encountered during one investigation.

In developing pea seeds the accumulation of carotenoids parallels the synthesis of starch until just before maturation when the pigments begin to disappear.[258,259] At maturation the values drop precipitately; for example, typical values for peas and beans are 0·2–0·5 μg./g.;[245,255,259] some samples actually show no vitamin A activity when tested biologically.[260] One cannot, however, ignore reports that the carotene content of peas does not vary greatly during maturation.[257,261]

In the case of French beans, the variation in the carotene concentration of the beans during maturation does not parallel that of the pods. At early maturity the beans reach a value much higher than that of the pods, this then drops considerably at full maturity; the values for the pods vary little until they become markedly overmature. In spite of this, at all stages of development a pod contains larger amounts of carotene than do the beans which it contains.[252] The carotene distribution in beans and peas is very similar to that of leaves; for example,

TABLE 6.—*Carotene Content of some varieties or strains of Peas.**

	Variety or Strain	Amount (mg./100g. of wet wt.)
EARLY	Alah	·560
	Glacier	·406
	Hundredfold	·471
	Progress	·474
	Progress × Grand Stride	·451–·488
	Little Marvel × World Record	·454–·492
	Little Marvel × (Thomas Laxton × Phenomenon)	·383–·468
	Progress × World Record	·45
	Phenomenon × World Record	·444–·454
LATE	Creole	·408
	Miracle	·714
	Morse market	·462
	Perfection	·390
	Perfectah	·406
	Walah	·338
	Wando	·530
	Willet's wonder	·522

* From Heinze, P. H., Hayden, F. R., and Wade, B. L. (1947), *Plant Physiol.*, **22**, 548.

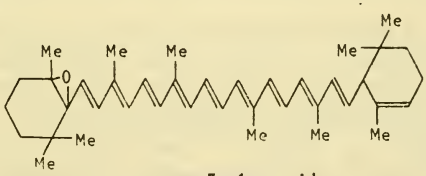
soya beans[248] and Lima beans[249] contain 80–90 per cent. of the β-isomer. Although few data are available concerning xanthophylls in beans and peas, it is likely that they are very similar to leaf xanthophylls. Nakamura and Sakan[262] report *taraxanthin*, a *fucoxanthin*-like pigment (*see* p. 132), and an *eloxanthin*-like pigment (*see* p. 57) as well as lutein (xanthophyll) in soya bean oil. Confirmation of these somewhat unexpected findings is required before they can be unreservedly accepted. Unpredictable oxidative changes may have taken place during the preparation of the oil. The carotene content of a number of seeds is recorded in Appendix I (*see* p. 294).

FLOWER PETAL CAROTENOIDS

The distribution of carotenoids in flower petals varies considerably; whilst some petals have a distribution somewhat similar to green leaves, others contain little or no carotenoids and others contain xanthophylls which, if not specific to a plant, are specific to petals. These specific carotenoids are, however, mostly epoxides.

Unique in carotenoid distribution are the flowers of the silky oak (*Grevillea robusta*). The xanthophylls, which are a complicated mixture, amount to only one-fifth of the total carotenoids (*cf.* leaves); the remaining four-fifths are entirely β-carotene. These flowers are recommended as a source of β-carotene, for 270 mg. of the crystalline pigment were obtained from 1 kg. of flowers.[263]

The sky blue flowers of the Brazilian tree *Jacaranda ovalifolia* also contain β-carotene almost exclusively but in comparatively small amounts (about 0·06 mg. from 1 kg. of flowers).[254] Other flowers, *e.g.*, *Tagetes patula*,[264] *Crepis aurea*,[265] *Cytisus* (*Sarothamnus*) *scoparius*,[266] *Lotus corniculatus*,[265] *Iris pseudacorus*[267] contain both α- and β-*carotene*, but *Caltha palustris*[268] and *Maratia praecox*[269] contain mainly α-carotene, associated with traces of β-carotene. γ-carotene has been reported in *C. scoparius*[266] and lycopene in *T. patula*[264] and *Gazania rigens.*[270, 271] α-Carotene-5 : 6-epoxide is found in *Tragopogon pratensis.*[272] Phytofluene has been found in a number of species (*see* Table 7) but, in particular, in *Jacaranda ovalifolia* it is much more abundant than β-carotene.[256] This is the first reported case in which phytofluene predominates over the coloured carotenoids.



α-carotene-5 : 6-epoxide

Of the xanthophylls found both in flowers and in leaves, lutein (xanthophyll) is probably the major example and the most widely distributed; Kuhn and Winterstein[273] give a list of flowers in which it has been detected and Karrer has found it in almost all the petals which he has been lately investigating; it occurs in both free and ester forms. Kuhn, Winterstein and Lederer[274] have isolated from *Helenium autumnale* lutein dipalmitate (m.p. 92°) which they have named *helenien.*

Esters from other sources, *e.g.*, *Tagetes aurea* have different melting points and although the component fatty acids have not been identified, this is good evidence for assuming that lutein is capable of esterifying with acids other than palmitic.

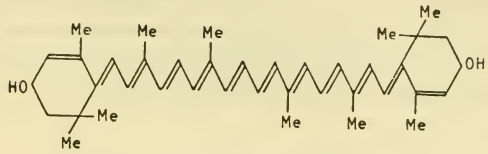
TABLE 7.—*The occurrence of phytofluene in some flowers (from Zechmeister and Sandoval* Arch. Biochem. (1945), **8**, 425)

Species	Amount present mg./kg. fresh material
Bignonia spp.	+
Tecomaria capensis	+
Canna spp.	0·2
Gazania rigens	32·5
Zinnia elegans	3·6
Gelsemium sempervirens	+
Eschscholtzia californica	5·0
Spartium junceum	0·1
Mimulus longiflorus	27·8
Photinia spp.	0·5
Fremontia californica	present

Violaxanthin, a minor component of leaf xanthophylls, is the major xanthophyll occurring in marigold flowers (*Calendula officinalis*)[275] and was first isolated from the yellow pansy (*Viola tricolor*);[276] it occurs in small amounts in *Tragopogon pratensis*[274] and *Forsythia intermedia*, Zabel.[277] Other leaf xanthophylls which have been isolated from flowers are flavoxanthin (*Ranunculus acris*,[273,278] *Taraxacum officinale*,[279] *C. scoparius*[267] and *Tragopogon pratensis*);[268] lutein (xanthophyll) -5 : 6-epoxide in *T. patula*,[264] *L. corniculatus*,[265] *C. scoparius*,[267] *Arnica montana*,[265] *Tragopogon pratensis*,[272] *R. acris* and *Laburnum anagyroides*;[270] zeaxanthin in *A. montana*[265] and cryptoxanthin in *Gazania rigens*.[270] The fruit carotenoid rubixanthin occurs in *T. patula*.[264]

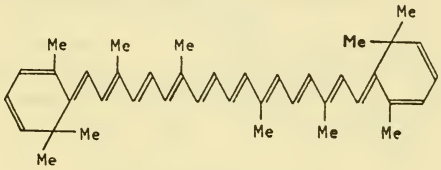
Eight carotenoids specific to flower blooms have so far been isolated (Table 9). *Eschscholtzxanthin* ($C_{40}H_{54\pm2}O_2$) first isolated from *Esch-*

scholtzia californica by Strain [280] has recently been investigated by Karrer and Leumann.[280A] They showed that it is probably 3 : 3′-dihydroxydehydro-β-carotene :



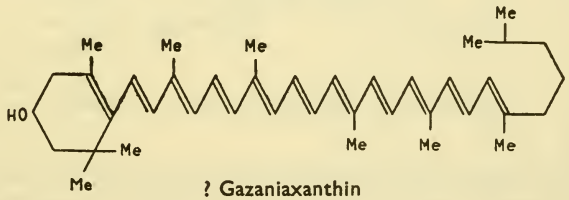
Eschscholtzxanthin

When treated with chloroform containing traces of hydrogen chloride it loses two molecules of water to form *anhydroeschscholtzxanthin* (absorption maxima 578,539,503 mμ in CS_2 : 531 and 500 mμ in light petroleum.)



Anhydroeschscholtzanthin

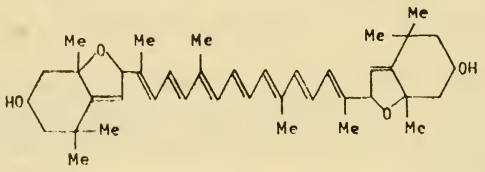
Petaloxanthin ($C_{40}H_{56\text{ or }58}O_3$) from *Cucurbita pepo*[282] *taraxanthin* ($C_{40}H_{56}O_4$) from *Taraxacum officinale*, *Impatiens noli me tangere*,[282] *Tussilago farfara*,[178] *Tragopogon pratensis*, and *R. acris*,[272] are both of unknown structure. *Gazaniaxanthin* isolated from *G. rigens* is probably a dihydrorubixanthin.[271] Four new carotenoids isolated



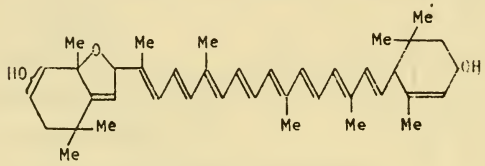
? Gazaniaxanthin

by Karrer and his associates are epoxides, three of them 5 : 8-epoxides : *auroxanthin*, 5 : 8, 5′ : 8′-*diepoxyzeaxanthin* ($C_{40}H_{56}O_4$), from *Viola tricolor* ;[279] *chrysanthemaxanthin*, 5 : 8-epoxy-3 : 3′-dihydroxy-α-carotene ($C_{40}H_{56}O_3$) from *Chrysanthemum* spp.,[267] *Tragopogon pratensis*, *R. acris*[272] and *Cytisus scoparius* ;[267] *rubichrome*, 5 : 8-epoxy-3-hydroxy-γ-carotene ($C_{40}H_{56}O_2$) from *Tagetes patula*[265] and

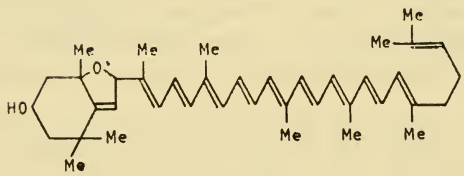
trollixanthin, a hydroxy lutein (xanthophyll)-5 : 6-epoxide ($C_{40}H_{56}O_4$), from *Trollius europaeus*,[283] *Caltha palustris*, *Laburnum anagyroides* and *Kerria japonica*.[264] The position of the third hydroxyl group in trollixanthin is still undecided. Chrysanthemaxanthin and flavoxanthin are probably *cis-trans* isomers, differing only in the spatial disposition of two oxygen atoms at positions 3 and 5 in the molecule.



Auroxanthin (5.8, 5'-8'-diepoxyzeaxanthin)



Chrysanthemaxanthin, Flavoxanthin
5 : 8-epoxylutein (xanthophyll)



Rubichrome (5 : 8-epoxy-3-hydroxy-γ-carotene)

Little is known of the metabolism of carotenoids during the development of flowers; but Karrer, Jucker, Rutschmann and Steinlin[272] have found that in spring, petals of *Viola tricolor* contain only small amounts of violaxanthin and auroxanthin, but that these increase considerably by autumn, and it has recently been reported that the carotene content of the petals of a number of plants increased from budding until a maximum was reached at flowering.[284] Table 8 records some characteristics of typical petal carotenoids and Table 9 the qualitative distribution of carotenoids in petals.

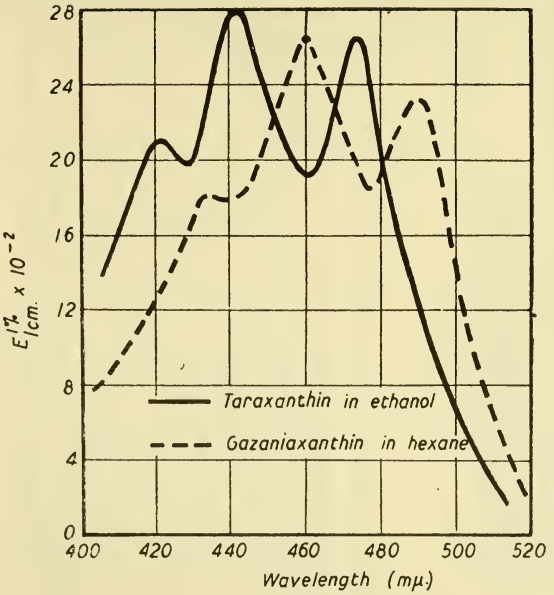


Fig. 13.—The absorption spectrum of Taraxanthin (*from Kuhn, R. et al. (1934)* Z. angew. Chem., **47**, *664*, and Gazaniaxanthin (*from Zechmeister, L. (1944)* Chem. Rev., **34**, *267*).

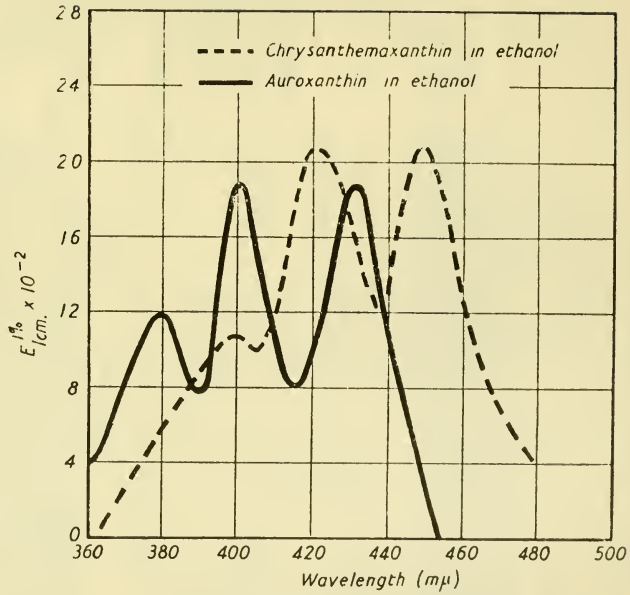


Fig. 14.—The absorption spectrum of Chrysanthemaxanthin (*from Karrer, P., and Jucker, E. (1943)* Helv. Chim. Acta, **26**, *626*), and Auroxanthin (*from Karrer, P., and Rutschmann, J. (1942)* Helv. Chim. Acta, **25**, *1624*).

TABLE 8.—*Characteristic Flower Carotenoids.**

PIGMENT	m.p.	ABSORPTION SPECTRA MAXIMA (mμ.)		
		CS_2	EtOH	$CHCl_3$
Taraxanthin[1, 2]	184–185°	441, 469, 501	417, 443, 472	
Petaloxanthin[3]	211–212°	481, 574·5	451·5, 482	460·5, 492
Escholtzxanthin[4, 13]	185–186°	475, 503, 536 474, 501, 542	446, 472, 502 448, 476, 505	456, 484, 513 456, 488, 520
Auroxanthin[5]	191–2°	423, 454	402, 428	
Chrysanthemaxanthin[6]	176–177°	451, 480·5	421, 448	430, 459
Rubichrome[7]	199°	472, 501		
Gazaniaxanthin[8]	133–4° 136–7°	461, 494·5, 531	462, 494·5	
Antheraxanthin[9]	211°	478, 510		460·5, 490·5
cis-Antheraxanthin[10]	110°	476, 506	445, 472	
α-Carotene-5 : 6-epoxide[11, 12]	175°	471, 503		454, 483
Flavochrome[12]	189°	451, 482	—	433, 461

* Those also occurring in leaves and fruit are not recorded here.

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CAROTENOIDS OF REPRODUCTIVE TISSUES

Characteristic carotenoids, *antheraxanthin*[285, 286] ($C_{40}H_{56}O_3$) 5 : 6-epoxyzeaxanthin) and a *cis*-isomer[287] have been isolated from the anthers of *Lilium tigrinum*; somewhat unexpectedly *capsanthin* was also isolated.[285, 286] Any suggestion that antheraxanthin may be a carotenoid specifically associated with reproduction in plants is apparently ruled out, for β-carotene, α-carotene and lutein (xanthophyll)-epoxide are the only carotenoids in the anthers of *Clivia miniata*.[184] Further, antheraxanthin probably exists in the fruit of *Euonymus europaeus*. The very close resemblance between the properties of antheraxanthin and petaloxanthin suggests that they may well be the same pigment (*see* Karrer and Jucker[4] for a comparison of

TABLE 9.—*The Distribution of Carotenoids in Flower Petals*

NAME	α-Carotene	β-Carotene	γ-Carotene	Lycopene	α-Carotene epoxide	Pro-γ-Carotene	Prolycopene	Lutein	Rhodoxanthin	Antheraxanthin	Violaxanthin	Zeaxanthin	Cryptoxanthin	Lutein-5 : 6-epoxide	Trollixanthin	Flavoxanthin	Chrysanthemaxanthin	Flavochrome	Taraxanthin	Auroxanthin	Petaloxanthin	Rubixanthin	Gazaniaxanthin	Rubichrome	REFERENCE
Acacia decurrens. v. *mollis*		+						+																	17, 18
A. discolor		+						+																	17
A. linifolia		+						+																	
A. longifolia																									
Arnica montana								+				+													5, 20
Aloe vera									+																1
Calendula officinalis		+	+	+				+			+														28
Caltha palustris	+	+		+				+																	9, 10
Cheiranthus sennoneri								+																	5
Chrysanthemum spp.		+						+						+			+								29, 30
Crepis aurea	+	+						+			+														20
Cucurbita pepo		+						+													+				56
Crocus sativus	+	+	+	+								+													7
Cytisus laburnum											+														9
Cytisus (Sarothamnus) scoparius	+	+						+						+		+	+								21, 22
Dimorphotheca aurantiaca				+																					9
Doronicum paradlanches								+																	5
*Eschscholtzia californica**								+			+	+													39, 40
Gazania rigens																									
(*a*) Portuguese		+	+					+														+	+		31
(*b*) Californian		+	+	+				+					+										+		32
Genista tridentata	+	+						+																	19
Grevillea robusta		+						+					+												8
Helenium autumnale								+																	5, 18
Helianthus annuus		+						+					+						+						18, 33
Heliopsis scabrae major								+																	5
Impatiens noli me tangere																			+						23
Iris pseudacorus		+						+			+			+											6
Kerria japonica		+						+						+											14, 15
Laburnum anagyroides		+						+			+														9, 14
Leontodon autumnnalis								+											+						23
Lilium candidum										?	+														2
Lotus corniculatus	+	+						+			+			+											20
Mimulus longiflorus		+	+	+	+	+	+						+												26, 42
Narcissus pseudo-narcissus								+																	5
Potentilla erecta		+						+				?				?									16
Pyracantha coccinea		+			+			+					+												27
Ranuculus acer	+	+				+		+			+			+		+	+	+	+						11, 12
Ranuculus arvensis		+						+																	9
Ranuculus steveni								+																	13
Rudbeckia neumanii								+																	18
Senecio doronicum								+				+													9
Silphium perfoliatum								+																	7
Sinapsis officinalis		+									+														9
Tagetes aurea								+																	7
Tagetes erecta								+																	18
Tagetes grandiflora			+	+				+			+														18
Tagetes nana								+																	18
Tagetes patula	+	+						+						+							+			+	18, 34
Taraxacum officinale †								+			+					+			+						35,36,37,41
Tragopogon pratensis	+	+			+			+			+			+		+									9, 12
Trollius europaeus		+						+						+	+										9, 14
Tropaeolum majus								+																	5
Tulip (yellow)											+								+						3, 4
Tussilago farfara											+								+						38
Ulex europaeus	+	+									+								+						19
Ulex gallii	+	+									+					+			+	+					19
Viola tricolor ‡		+						+			+	+				+			+						24, 25, 41
Viola (violet blue spp.)				+																					5

* Plus eschscholtzxanthin. † also tareoxanthin ‡ plus violeoxanthin.

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their properties). Lycopene probably occurs in the anthers of *Dahlia* species[288] and carotene and lutein in the anthers of tulips and narcissi.[289]

Carotenoids were first noted in pollen as early as 1892 when Bertrand and Poirault[290] detected them in the pollen of *Verbascum thapsiforme*. The subject was not reinvestigated until some fifteen years ago when Strain,[15] using physico-chemical methods, could not demonstrate the presence of these pigments in *Pinus radiata* and *P. ponderosa*. Later, Sekine and Li,[291] using the biological assay technique, could find no vitamin A-active carotenoids in the closely related *P. densiflora* and *P. thunbergi*. The yellow pigment obtained from the pollen of the broad-leaved reedmace (*Typha latifolia*) by Tischer[292] and named sporopollenien is not a carotenoid.

There are, however, reports that pollens do contain vitamin A-active materials[293] and Von Euler, Ahlström, Högberg and Pettersson[294] have detected carotenoids chemically in pollen of the white willow (*Salix alba*), black willow (*S. nigricans*), *Lilium candidum* and *Taraxacum officinale*. They confirmed their absence from *T. latifolia* and also were unable to detect them in the pollen of *Populus nigra*, *Pinns montana*, *Betula pubescens* and *Campanula persicifolia*. Mixed pollen gathered by bees from flowers of dandelion, plum, apple, clover, golden rod and aster, contained traces of carotene (0·2–0·7 μg./g.) but considerable amounts of xanthophylls (100–400 μg./g.).[295] When the

Table 10

The composition and approximate amount of Carotenoids in pollen and anthers of various species (from Karrer, P., Eugster, C. H., and Faust, M. (1950), *Helv. Chim. Acta*, **33**, 300).

Species	Amount of carotenoids present	Nature of pigments
Pollen		
Zea mays L. (var. Rheintaler)	small	Lutein esters, carotene (trace).
Helianthus annuus L.	large	lutein ester, carotene (trace), epoxides (trace).
Aster (unnamed single blooms)	significant	lutein esters, carotene (trace).
Helianthus tuberosus	significant	lutein esters, carotene (trace).
Alnus glutinosa (common alder)	almost none	—
Pinus mugo (*montana*)	almost none	—
Narcissus exsertus	small	?
Tilia platyphyllos (lime)	almost none	—
Chrysanthemum leucanthemum	large	—
C. coronarium	large	—
Acacia dealbata v. *Le gaulois**	—	α and β-carotene, α-carotene epoxide, lutein epoxide, traces of flavoxanthin and lutein
Anthers		
Colchicum autumnale	significant	lutein esters, lutein epoxide, carotene (trace).
Ranunculus campestris	significant	—
Antirrhinum majus maximum	present	—
Lilium umbellatum, *L. regale*, *L. willmottiae unicolor*, *L. maximee*	significant	*cis*-antheraxanthin, carotene (trace).

* Data reported by Tappi, G. (1949-50), *Atti accad. Sci. Torino*, **84**, 97.

pollen has fermented, the " bee-bread " so produced has a vitamin A-activity of 6 i.u./g.[296] Very recently, Karrer, Eugster and Faust[297] have surveyed the distribution of carotenoids in the pollen and anthers of a number of species; their observations are summarized in Table 10. It appears that esterified lutein (xanthophyll) is always the principal carotenoid component of pollen with β-carotene always present in traces. Except in the case of *Helianthus tuberosus*, epoxides were absent. In anthers, on the other hand, especially in *Lilium* spp. detectable amounts of epoxides (in particular, *cis*-antheraxanthin) were always encountered. A xanthophyll, perhaps esterified with different acids, occurred in a sample of deep orange beeswax, the principle pollen contaminant of which was probably from *Delonix regia*.[298]

ROOT CAROTENOIDS

(i) *Carotenes*

The most important roots from the carotenoid view-point are the carrot and the sweet-potato. The common potato contains only traces of carotenoids,[150] which have also been reported in the roots of beet,[299] *Brassica campestris*,[300,301] and *B. rapa*.[302] The carrot, as its name suggests, was the first recognized source of carotene which was obtained crystalline by Wackenroder in 1831.[303] Since then reports of investigations on carotene in carrots have been legion and the reader is referred to Zechmeister's treatise for a discussion of the early work.[303] In 1931 carrot " carotene " was resolved into 2 isomers, α- and β-*carotene*[14,304] and since then Mackinney, Aronoff and Bornstein[305] found α-carotene to constitute between 5 and 10 per cent. of the total carotene fraction of carrots, whilst Kemmerer and Fraps[306] found between 19·5 and 42·2 per cent. and Fujita and Ajisaka[8] 36–45 per cent.; Harper and Zscheile[307] reported even higher values giving an average figure of 46 per cent. Sadana and Ahmad[308] obtained very similar results in India, but correlated the α-carotene content with the colour of the carrot. Red varieties contained between 10 and 16 per cent. of α-carotene and orange varieties between 34 and 51 per cent. If the higher values just quoted are correct, carrots generally contain more α-carotene than do green leaves, for the highest value found in plants by Mackinney was 35 per cent.[16] Minor constituents of the carotene fraction of carrots are, *γ-carotene* (0·1 per cent. of total carotenoids),[123,309] *δ-carotene*, *lycopene*[305] (1/10 the concentration of *δ-carotene*),[307] and *ζ-carotene*,[150,309] Of these δ-carotene, ζ-carotene and lycopene do not occur in the carrot leaf.

Investigations on the carotene content of carrots have been far

too numerous to outline here, but generally carrots have been found to contain between 60 and 120 μg./g. (wet wt) of carotene, although some selected strains contain as much as 370 μg./g. (fresh wt.).[310] It now seems certain that carotene is present in carrots in a water soluble form attached to a protein.[311,312]

Puerto Rican sweet potatoes (*Ipomea batatas edulis*) can contain between 40 and 80 μg./g. (wet wt.) of β-carotene which is the predominant carotenoid present[313-316] for only traces of xanthophylls have been found.[317] The carotene concentration is about the same in the Red Velvet variety as in the common strains, but is much less (one third) in the Nancy Hall variety.[315] The common potato, on the other hand, contains very little carotene (about 0·1 to 0·2 μg./g.) the content varying only a little from " white " to yellow fleshed varieties;[150,318-321] *β-carotene* and probably *α-carotene* are present. We see then from the point of view of providing a source of vitamin A active carotenoids, potatoes are of negligible value, whilst sweet potatoes and carrots are potentially extremely good sources. Potatoes are useful in experimental diets which will just prevent vitamin A deficiency symptoms in rats.[322] Lycopene has recently been observed as the major component of the complex carotenoid mixture occurring in some swedes[302] whilst in others prolycopene appears to predominate.[322A]

(ii) *Xanthophylls*

The xanthophyll fraction of commercial carrots is quite small, being only 5–10 per cent. of the total carotenoids present;[323] however, the percentage varies from xylem to phloem, being higher in the former, where in the case of Danver's Half Long and Yellow Belgian, it reaches 30–50 per cent. of the total carotenoids.[307] In wild carrots at least 95 per cent. of the total pigments are xanthophylls, mostly monohydroxy derivatives,[227] and a similar high value (75–93 per cent.) is found for the xanthophyll content of yellow carrots.[306]

In potatoes the xanthophyll fraction constitutes about 90–95 per cent. of the pigment; this is in contrast to the commercial carrot and the sweet potato where it is the minor fraction. The concentration of xanthophylls in potatoes varies, according to variety, between 0·2 and 2·6 μg./g. (fresh wt.), the yellow fleshed varieties (*e.g.*, Katahdin) naturally containing most.[150,318,320,322]

The xanthophylls detected in potatoes are *β-xanthophyll** (sic), *tara-* or *violaxanthin*,[319] *lutein*, *auroxanthin* and *flavoxanthin*.[150] A

* β-xanthophyll was the name given by Tswett to a strongly adsorbed pigment occuring in green leaves.
As this has now been shown to be a mixture of pigments, its use has been abandoned.

number of other unidentified carotenoids noted by Brunstetter and Wiseman[150] may well be artefacts, for they were dealing with potatoes processed in a number of different ways (dehydrated, SO_2 treated, etc.).

(iii) *Distribution*

Commercial importance has no doubt stimulated the numerous quantitative investigations on the distribution of carotene in carrots. The phloem (cortex) has a 30 per cent. higher concentration than has the xylem* (core)[307,324,325] and contains 80 per cent. of the total root carotenoids.[325] The earlier work of Emsweller, Burrell and Borthwick[324] which indicated that the carotene concentration decreased from top to tip has been repeatedly confirmed.* Harper and Zscheile,[307] for example, found that although the centre and tip may have almost the same concentration, their concentration at the top is 50 per cent. greater than that at the centre or tip; further, Werner[325] reported that samples taken 1 in. from the root have concentrations of only 50 per cent. of those taken 1 in. from the stem. The concentration also increases in moving from the inner to the outer layers of the cortex.[307] As in the case of leaves the concentration of the carotene in the root increases with growth and becomes maximal about 100 days after sowing.[325-327] According to Smith and Otis[327] the difference between the carotene concentration of the cortex and the core, when measured on a dry weight basis, decreases during maturation; the quotient cortex/core for young and mature carrots being 1·5 and 1·2 respectively. These investigators also claim that in contrast to the well established synthesis of carotene during maturation, the xanthophyll content decreases; more supporting evidence is required before this can be accepted. The possibility of a relationship between carotene content and size and shape of the carrot has attracted some attention. Otis and Smith[327] and Schuphan[328] noted that the longer the carrot [? irrespective of state of maturation] the higher the carotene concentration. Pepkowitz, Larsen, Gardner and Owens[329] claim an inverse relationship between size and concentration in different varieties, and state that small varieties have a higher concentration than have large varieties; Dark and Booth[330] confirmed this, but even so it does not exclude the size/concentration variation within a given variety. Pepkowitz *et al.*[329] also found no relationship between "shape ratio" (width : length) and carotene concentration.

Carrots lifted and stored certainly maintain[325] and probably increase[324,331] their carotene content until they begin to sprout. Stored sweet potatoes "ripen" and increase their carotene content

* Recently confirmed by Booth, V. H. (1951) *J. Sci. Food. Agric.*, 8, 350.

by as much as 50 per cent.,[313,332] the rate and increase depending on the variety, and therefore, from this point of view there seems no objection to harvesting them early.[333]

An interesting recent development in the sweet potato-starch industry has been reported.[334] After extraction with water, the pulp and starch contain only 10 per cent. and " a trace " respectively of the total carotenoids, the remainder is in the supernatant liquor obtained by filtering off the pulp and removing the starch by centrifugation ; acidification of the liquor produces a coagulum which contains 46 per cent. of the total carotenoids although it represents only 2 per cent. by weight of the original sweet potato. The possibilities of this carotene protein concentrate in practical nutrition have yet to be explored.

In an extensive study of general cultural factors in their relation to carotene production in carrots, Booth and Dark[330] found that in order to reach their maximum concentration in the autumn carrots had to be sown before the end of May and, in the case of some high carotene-yielding varieties considerably before then. Other interesting points which emerged from this study were that summer sown carrots when harvested in late winter and early spring as " stecklings " have only one-third the normal carotene concentration, that thinning and " chitting " (pregermination on filter paper) had no effect on the carotene production of the roots, and that carrots produced from newly harvested seeds contain less carotene than do those produced in later seasons from the same seed.

AQUATIC PHANEROGAMS

Little work has been carried out on this group of plants ; Hey[335] reported that *rhodoxanthin* characterized in 1933 by Kuhn and Brockmann,[177] was first isolated from *Potamogeton natans* in 1893 by Monteverdi who, in collaboration with Lubimenko, obtained it crystalline in 1913. Hey, himself, investigated the carotenoid composition of the leaves of the Canadian pond weed *Elodea canadenis*, and apart from carotene isolated a new xanthophyll " *eloxanthin* ", $C_{40}K_{56}O_3$, m.p. 182·5–183° ($\lambda_{max,}$ CS_2, 444, 473, 502$m\mu$). Recently Karrer and Rutschmann[96] have suggested that eloxanthin is identical with lutein (xanthophyll) -5 : 6-epoxide (*see* p .17). It was first thought that no lutein was detected in the leaves of this plant, but later work has shown it to be present.[96]

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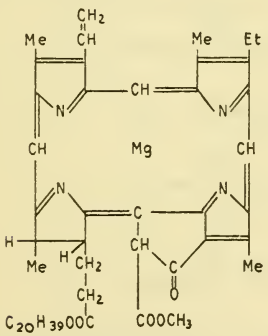
CHAPTER III

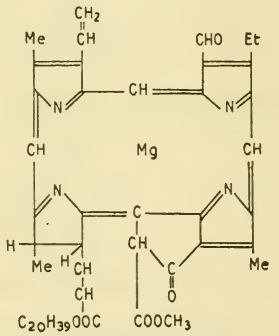
FORMATION AND FUNCTION OF CAROTENOIDS IN PHANEROGAMS

THEORIES OF FORMATION

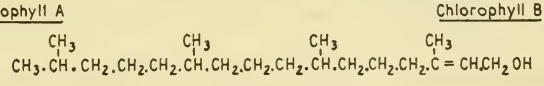
The site of formation of carotenoids in plant cells is by no means certain. Weinzinger[1] has investigated this from the cytological viewpoint but has produced no overwhelming evidence in favour of formation either in the chondriosomes or in the plastids. A point he does make which requires re-emphasis is that there is no *a priori* reason to assume that the site of accumulation of carotenoids is also the site of their formation. Assumptions of this type of reasoning misled for many years workers studying the conversion of β-carotene into vitamin A (*see* p. 275).



Chlorophyll A



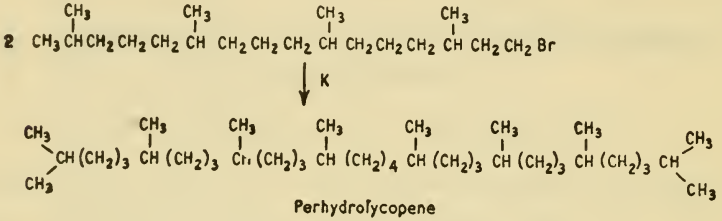
Chlorophyll B



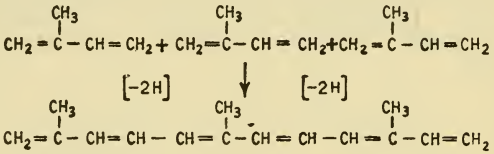
Phytol

As long ago as 1837 Berzelius,[2] who first extracted carotenoids from autumn leaves, considered that these pigments were breakdown products of chlorophylls, although in 1838 he found that they also occurred in green leaves. This suggestion has influenced the thoughts of many later scientists in their search for a theory of carotenoid formation in plants. More specifically Willstätter and Mieg[3] suggested

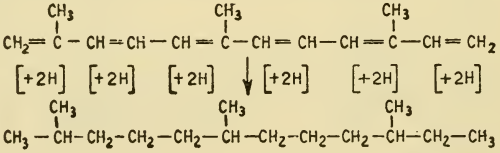
that carotenoids were formed from the phytyl residues occurring in both chlorophylls *a* and *b*. This was made plausible by the chemical conversion of phytol into perhydrolycopene[4] by the condensation of two molecules of dihydrophytyl bromide in the presence of potassium.[5]



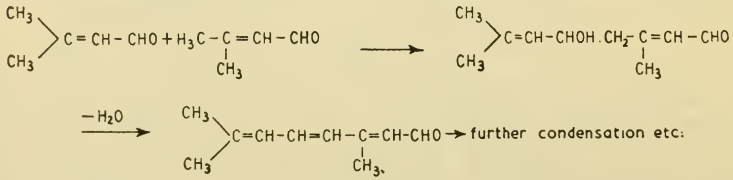
Other suggestions are that phytol and the carotenoids are derived from isoprene; the carotenoids being formed by addition and subsequent dehydrogenation,[6] viz.:



and phytol by addition followed by hydrogenation,[7] viz.:



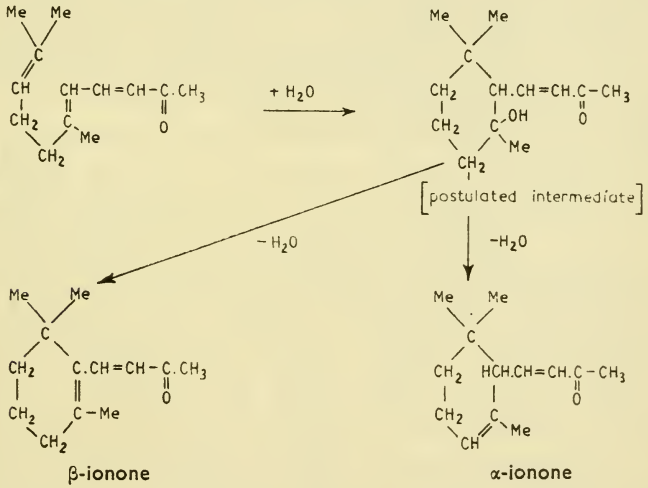
Karrer, Helfenstein and Widmer[5] suggest that rather than isoprene β-methyl crotonaldehyde may be the fundamental unit, the synthesis



being brought by an aldol type condensation followed by removal of water.

This has the merits of having a close analogy in the generally accepted hypothesis of the formation of fats from carbohydrates in mammals,[8] and further, it should be noted that Smedley[9] and Kuhn, and Grundmann and Trischmann[10] have obtained by chemical means long chain polyene aldehydes starting from crotonaldehyde.

Recent work by Bonner and Arreguin[11-13] on the related problem of the biogenesis of rubber indicates that, using the stem-culture technique, neither isoprene nor β-methylcrotonaldehyde was a precursor of rubber; nor were other obvious possibilities such as pyruvate and citrate. Acetone, acetate and acetoacetate (probably degraded to acetate) and glycerol were, however, active. Acetone and acetate can, theoretically condense to form β-methylcrotonic acid and it was found that this substance was active but not to the same extent as acetate and acetone. Acetate, acetone and β-methylcrotonic acid, but not glycerol, were also active in stimulating rubber synthesis in aseptic experiments with isolated stem fragments.[13]



Inherent in all these hypotheses of carotenogenesis is the assumption that lycopene is the parent carotenoid from which other carotenoids are formed by isomerization. Facts which militate against this assumption are that (1) lycopene is never detected even in minute traces in green material, (2) in the tomato the production of lycopene and carotene is probably carried out by two separate processes[14] (*see* p. 40) and (3) in the case of α- and β-carotene, at least, interconversion by asymmetrical isomerization does not appear to occur.[15] However,

it should be noted that ψ-ionone can be converted into a mixture of α- and β-ionones by the action of H_2SO_4.[16]

It must be emphasized then that little evidence exists in favour of any of the hypotheses outlined above and that conjecture has outstripped experimentation.

In considering the pattern of carotenoid formation it will be convenient to start with the seed and follow the production as the plant develops. Seeds contain small amounts of carotenoids, probably qualitatively the same as those in the corresponding leaves (*see* p. 42). Synthesis generally begins within 3–5 days of germination;[17,18] for example, although no increase in the carotene content of a soya bean occurs during the first 24 hours, 72 hours later a three-fold increase has occurred.[17] Wheat seedlings take rather longer (10 days) to treble their content[19] and in some cases there may be a slight drop at first until, coinciding with the appearance of the first leaf, there is a rapid synthesis.[20] The increase is not at the same rate in all parts of the seedling, for after 54 hours there is three times more carotene in the cotyledon than in the hypocotyl.[17] In a number of pulses and cereals the carotene content is approximately doubled 7 days after germination.[20A] The amount of pigment produced during the first 40 hours is proportional to the weight of the seed.[21]

Miller and Jablonski[22] have shown that the germination of grape fruit (*Citrus paradisi*) until the radicle was 1–2 in. long, increases the carotene content of the embryo from 3·36 to 44·8 mg. per 100 g. (dry wt.) and of the whole seed from 0·02 to 0·27 mg. per 100 g. (dry wt.).

Immediately following germination carotenoid synthesis precedes that of chlorophyll[18-23] but as the plant develops the formation of leaf carotenoids runs roughly parallel with that of the chlorophylls.[24-26] At the approach of maturity, however, the chlorophylls disappear before the carotenoids. These observations on immature plants in no way suggest conversion of chlorophylls into carotenoids and further, Guthrie[27] has shown that in the foliage of tomato and soya bean plants grown in the dark for several days, the carotenoid content remained constant although that of chlorophyll decreased. The significance of Beck's[23] claim that carotene is formed more readily from young than from old seeds is not yet obvious.

In ripening of fruit the possibility of an inter-conversion does arise, for on maturation the fruit chlorophylls disappear and the carotenoids accumulate. However, the chlorophylls destroyed do not liberate sufficient phytol to account for the carotenoids produced in ripened *Physalis alkekengi*[28] and *Lycopersicum* spp.[29] In the latter the chlorophyll-phytol concentration in the green fruit is only 1 mg. per 100 g. (dry

weight) whilst in the ripe fruit the lycopene concentration is 7·75 mg. per 100 g. dry weight. Further, Smith[30] has found that tomatoes produced in complete darkness possess no chlorophyll when unripe but do contain lycopene when mature. Miller, Winston and Schomer[31] found that the carotene content of the rinds of maturing oranges continues to increase after all the chlorophyll has disappeared.

There is thus no reason to believe that, in maturing fruit, any appreciable amount of carotene can be produced from chlorophyll; however, on the evidence so far available, there is also no reason to assume that phytol is not an *intermediate* in carotenoid formation, for the investigations just considered do not rule out the possibility that the plant calls on phytol reserves other than those provided by chlorophyll.

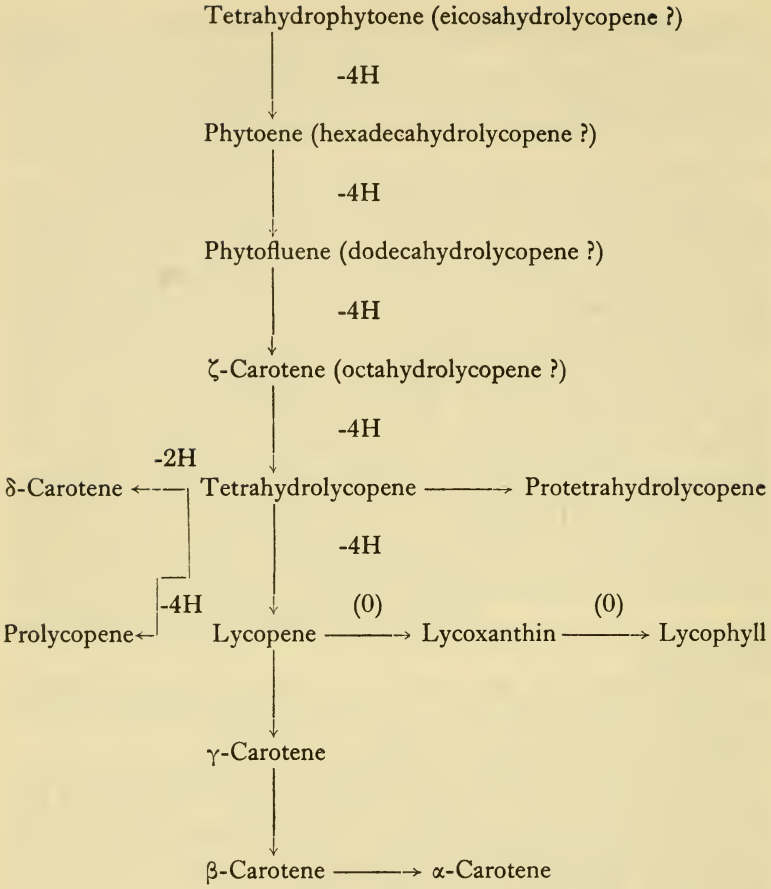
Possible intermediates in carotenoid synthesis in plants are the strongly fluorescent materials which can be separated from the carotenoids by chromatography. Those detected in green leaves by Strain[32,33] showed absorption spectra with " sharp fine structure " about 320 mμ. Phytofluene, a partly saturated fluorescing carotenoid, has been noted by Zechmeister and his colleagues[34-36] in fruit and petals (*see* p. 28) and has been suggested as a possible intermediate in carotenoid biogenesis. If this suggestion turns out to be true, then the route of carotenoid biogenesis in green leaves may be different from that in fruit and petals because phytofluene is never observed in green leaves.

It is important to note that the sky-blue fluorescing material associated with phytofluene in petals of *Tagetes erecta* is not a carotenoid derivative but α-terthienyl ($C_{12}H_8S_3$)[37].

Following their extensive investigations on carotenoid production in tomatoes, Porter and Lincoln[38] have proposed a scheme for the biogenesis of carotenoids in tomatoes which, in essence, involves the stepwise dehydrogenation (four H atoms at a time) of tetrahydrophytoene to lycopene which is then isomerized to α-, β-, and γ-carotenes and oxidized to lycoxanthin and lycophyll. The mechanism envisaged is shown on the next page.

This interesting hypothesis is based on genetical studies and on the fact that all these compounds do occur in tomatoes. The main objection which Porter and Lincoln have to combat is " may not the reactions go in exactly the opposite direction to that which they have suggested ? " In other words, may not lycopene (or α- and β-carotene, etc.) be the primary product which is then hydrogenated ?

Porter and Lincoln have carried out inheritance studies involving crosses of commercial varieties of tomatoes with high lycopene content with selections with low lycopene and high tetrahydrophytoene content



(Tangerine type). It was found that in each of these crosses the factors for the red colour (lycopene) are constant; Porter and Lincoln, therefore, continue their reasoning as follows: "Pigments and colourless polyenes of the Tangerine type are not present or present only in traces in the hybrids of the first cross. The lycopene content in the hybrids of the second cross is not diminished from the content of this compound in the red parent. Each of these crosses segregates in the F_2 into three red and one orange, or one yellow, respectively. The fact that the factors for lycopene formation are dominant in both of these crosses strongly suggests that the hydrogenation of lycopene to form

the compounds of the Tangerine type or tetrahydrophytoene does not occur. Instead the dominance of the factors for lycopene implies lycopene is formed from these compounds by dehydrogenation."

Three possible mechanisms of dehydrogenation of tetrahydrophytoene are considered : (*a*) different enzymes are necessary for each dehydrogenation step thus, on the basis of the one gene-one enzyme theory, this would involve a number of genes in the formation of lycopene. The development of selections which contain large amounts of one or more of the postulated intermediates (phytoene, phytofluene, ζ-carotene) is compatible with this hypothesis. (*b*) one gene controls the production of a single enzyme which can carry out all the dehydrogenations ; the formation of a strain producing predominantly one intermediate (say ζ-carotene) would be determined by a gene controlling the production of a specific hydrogen acceptor (in this case, one which will accept H from phytofluene), and (*c*) as it is known that two major genes R and T are necessary for lycopene formation (*see* p. 80) these might control the formation of two enzymes one carrying out α-β dehydrogenation and the other γ-δ dehydrogenation. Absence of gene T would stimulate production of colourless polyene pigments characteristic of the Tangerine type, whilst in the presence of T, phytoene would be converted into a compound containing 4 isolated double bonds which by action if the first enzyme would be converted into lycopene. Porter and Lincoln do not indicate which of these alternatives is their preference. The last suggestion is not in agreement with other investigators' conception of the function of the R and T genes (*see* p. 80).

Finally, the formation of α-, β-, γ- and δ- carotene from lycopene is explained as follows. Inheritance studies show that a single gene difference exists between selections which have principally lycopene and those which have principally β-carotene.[39] Although dominance of either is not exhibited in the first cross, it is assumed that, as the genetical evidence just outlined indicates the formation of lycopene from more highly saturated compounds, β-carotene is formed from lycopene. γ-carotene is intermediate in structure between lycopene and β-carotene, it is assumed to be an intermediate in the reaction. Finally, α-carotene is assumed to be formed from β-carotene and δ-carotene " by virtue of its structure "* is assumed to be formed from tetrahydrolycopene by loss of 2H. This hypothesis of Porter and Lincoln is most stimulating and whatever its fate in the light of future work, it will be of real value in accelerating the final elucidation of this fascinating problem.

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* *See* page 26.

It is perhaps relevant to indicate here that in a possibly related problem, the biogenesis of unsaturated fatty acids, the available evidence points away from the production of these acids by dehydrogenation of the saturated fatty acids.[40]

Nitrogen metabolism and carotenogenesis

A number of investigators have attempted to relate carotenoid production to nitrogen metabolism in the plant. A positive relationship exists between the crude protein of forages and their carotene content.[41-49] A similar relationship was demonstrated between carotene content and plant non-protein nitrogen,[50] (*see* Fig. 15). It is difficult to decide whether the results of these investigations indicate metabolic inter-relations or whether they are merely fortuitous owing to the fact that the concentration of all three factors are directly proportional to the growth of the plant. The loss of carotenoids from isolated leaves parallels the loss of protein.[50A]

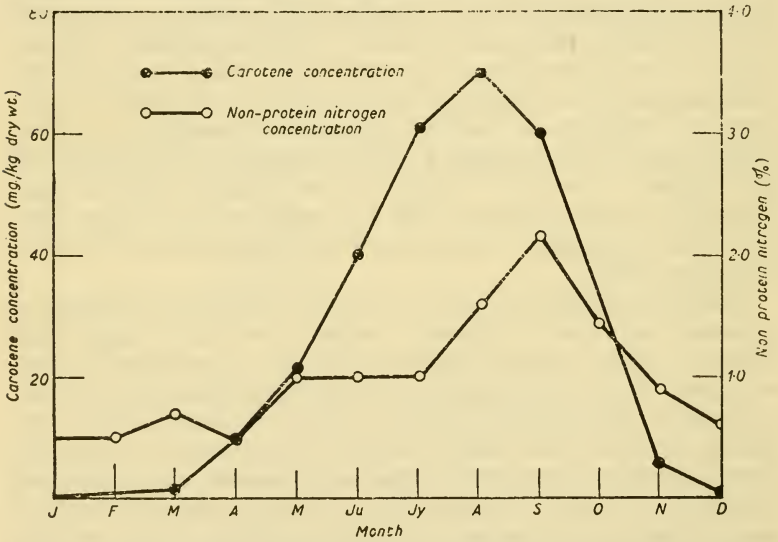


Fig. 15.—Showing the correlation between carotene concentration and non-protein nitrogen concentration in the developing carrot (*after Watkins, W. E. (1947) J. Agric. Res.*, **75**, *63*).

Widening the experimental approach, Barrensheen, Pany and Srb[51] followed changes in ammonia-N, amide-N, amino-acid-N and carotene in developing wheat seedlings. Whilst the amide and amino-acid-N remained constant, an increase in ammonia-N paralleled the

carotenoid increase. They consider that this indicates the probable formation of leaf carotenoids from deaminated amino acids (possibly leucine or valine) mobilized from the hydrolysis of some stored protein. The iodine values of benzene extracts of developing wheat seedlings were measured but no variations were recorded. This appears to rule out saturated lipids (? fatty acids) as a potential source of carotenoids—formed by dehydrogenation followed by subsequent methylation. Also against the idea of the formation of carotenoids by dehydrogenation of saturated lipids, is the recent work of Holman,[18] which demonstrates that the unsaturation of soya bean lipids decreases during germination.

Wilson[52] has attempted to correlate nitrogen metabolism and carotenoid production from the fact that the variation in the carotenoid levels in plants on dull and sunny days inversely follows the nitrite variation under these conditions. It is suggested that the accumulating nitrite oxidises the carotenoids to colourless compounds, for this oxidation rapidly occurs *in vitro*. The main objection to this suggestion is that the diurnal variation in carotenoid levels of plants is by no means fully established. Although recently Roberts[53] has produced what is, up to the time of writing, the best evidence that such a variation does occur.

General considerations

Two final general observations concerning carotenoid production in leaves can be noted before turning to the recent work of Bandurski: in box leaves carotenoid synthesis appears to be associated with increased lipid production and the disappearance of starch,[54] and barley seeds rich in nitrogen and aneurin (thiamin) produce plants giving a greater yield of carotenoids than do seeds less rich in these constituents.[55]

Studies with isolated tissues

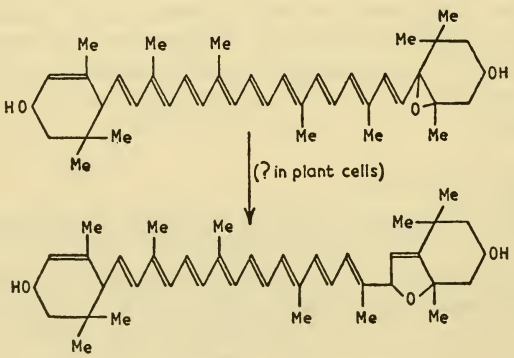
The study of carotenoid synthesis in detached bean leaves which has been carried out recently by Bandurski,[56] can be considered the first attempt to elucidate the fundamental biochemical problem concerning carotenogenesis in higher plants; even so this important investigation has only scratched the surface of the problem. Detached bean leaves cultured on a " 3-salt " medium and exposed to light can synthesize considerable amounts of carotenoids in 24 hours, much more than can leaves attached to their petioles. This difference is probably due to the fact that when a leaf is severed from its stem no translocation of food reserves can take place. Carotenoid formation

is stimulated by addition of sucrose or glucose to the medium and can also occur in the dark, but only to a limited extent (about 1/5 of that in light). This indicates a non-photosynthetic pathway. Inhibition of photosynthesis by culturing leaves in an atmosphere free from CO_2 or by the addition of hydroxylamine to the culture medium, reduced the synthesis of carotenoids almost to nil in the salt medium; synthesis was, however, resumed if glucose or sucrose was added to the medium. It seems from these experiments that carotenoid synthesis depends only indirectly on the presence of light, in so far as the pigments are produced from photosynthesised substrates.

Under Bandurski's experimental conditions, neither glycerol nor pyruvate can replace glucose or sucrose in stimulating pigment synthesis. Fluoride, but not sulphanilamide, inhibits synthesis from glucose.

Formation in petals

Knowledge concerning the biosynthesis of carotenoids in flowers is limited, although possible intermediates have been detected. Monkey flowers (*Mimulus longiflorus*) developed under natural conditions contain no *cis*-carotenoids, but those developed by keeping stems with buds in water for several days exposed only to diffuse light, produce considerable amounts of *pro*lycopene (*see* p. 30) and *pro*-γ-carotene (*see* p. 30) as well as other stereoisomers. These results suggested that *cis*-isomers might be precursors of the naturally-occurring *trans*-isomers.[57]



Karrer and his colleagues,[58,59] who have demonstrated the natural occurrence of 5 : 6- and 5 : 8- carotenoid epoxides in petals, and to a lesser extent in leaves (*see* p. 15), consider that the production of 5 : 6-epoxides from the parent carotenoid, which requires reagents such as

perbenzoic acid in the laboratory, may be achieved in plant cells by a H_2O_2-peroxidase system. They further consider that conditions in the plant are such as to bring about the isomerization of 5 : 6- epoxides. Thus lutein (xanthophyll)-5 : 6-epoxide may well be the precursor of flavoxanthin or chrysanthemaxanthin, shown on previous page.

With regard to this isomerization, it is brought about chemically by addition of traces of HCl to chloroform solutions of 5 : 6-epoxides ; previously, however, Strain[60] had suggested that the isomerization of carotenoids by plant acids observed chemically is prevented *in vivo* by the presence of plant organic bases.

INFLUENCE OF LIGHT

(i) *Etiolated seedlings*

Before discussing the effect of light on carotene synthesis by normally growing plants it will be interesting to consider the result of illuminating etiolated seedlings.

Grown under the most stringent conditions of light exclusion etiolated seedlings of maize, wheat, barley,[33] and sunflowers[61] contain carotenoids ; xanthophylls predominate and are similar to those obtained from the corresponding green leaves.[33,62] Strain[33] also demonstrated that xanthophylls in etiolated seedlings are very susceptible to oxidation by atmospheric oxygen, and this probably accounts for the contradictory findings previously reported. In his fundamental investigations Strain proved unequivocally the synthesis of carotenoids in the dark and a small but definite synthesis has also been demonstrated in detached bean leaves.[56] The production of carotenoids by a non-photosynthetic mechanism is also suggested by Holman's[18] work, which indicates that in germinating soya beans, carotenogenesis precedes chlorophyll formation.

On illuminating etiolated seedlings with blue or white light there is a transient drop in both carotenes and xanthophylls[63-66A] accompanied by a sharp rise in chlorophylls.[63] In red light Rudolph[63] claims that there is a steady increase in all pigments but that the chlorophylls increase more slowly ; Franck, however,[66A] found the same results with both red and blue light. From his experiments Rudolph was led to entertain the idea that carotenoids were precursors of the phytol necessary for the synthesis of chlorophyll ; but as Wald[67] observed " the later changes [in the growth of plants in light of different wavelengths] are complicated, perhaps due to the opening of new channels of production by photosynthesis."

Frank[66A] has constructed the action spectrum for the destruction of carotenoids and the synthesis of chlorophyll during the early stages of illumination of etiolated oat seedlings. They are identical and are similar to the absorption spectrum of a porphyrin. Frank puts forward the following stimulating suggestion: The porphyrin mediating in these reactions could be magnesium vinyl phaeophorphyrin, which by stimulating the conversion of carotene into (?) phytol is itself supplied with essential unit for its own conversion into magnesium vinyl phaeophorphyrinphytyl ester (protochlorophyll), which is probably the penultimate step in the biogenesis of chlorophyll. It will be interesting to see how this stands up to experimental attack, but it is important to note that Granick[66B] has already found that, in a mutant of the unicellular alga *Chlorella*, the conversion of magnesium vinyl phaeoporphyrin into protochlorophyll is accompanied by the disappearance of carotene when the alga is kept at room temperature; at 0°, however, carotene destruction still occurs although there is no concomitant increase in protochlorophyll.

Beck,[68] however, accepts Rudolph's suggestion and further considers that carotenoids may be precursors of auxin. When the effect of light on the synthesis of the carotenes and xanthophyll fractions is considered separately the results are somewhat contradictory. Whitmore has reported that the carotene concentration in bean leaves was greatest when plants were grown in green light, whilst the concentration of xanthophylls was greatest in either red light or in the dark. When the yields of xanthophylls were considered, it was noted that plants grown in the dark only produced half as much as did those grown in red light.[69]

Seybold and Egle,[70] Beck[71] and Nagel[72] have found that xanthophylls develop more quickly than carotenes when etiolated seedlings are developed in white light; on the other hand, Barrenscheen, Pany and Srb[51] indicated that in wheat seedlings the increase is mainly in the carotene fraction. This has recently been confirmed by Blaauw-Jansen, Komen and Thomas[73] who exposed 8-day seedlings of *Avena sativa* to light of varying intensities. At the beginning of the investigation the xanthophyll concentration was relatively high, but the amount did not increase on illumination. The amount of the carotene fraction did increase on illumination and the increase was proportional to the intensity of the illumination.

(ii) *Normally growing plants*

In normally growing plants most of the work which has been described has dealt with the effects of light on colour of developing

fruit and this has already been discussed (*see* p. 40). In the green tissues variation in intensity of light affects all pigments equally[74] and, unless the intensity is too great, carotenoid production is proportional to the light intensity.[70,74] There is, in practice, an optimal intensity of illumination; for example, in isolated bean leaves this is 600 foot-candles over the temperature range 25–35°.[56] Whether this is truly the case or whether it is the result of the interplay of thermal and photic effects is very difficult to decide; as has already been emphasised (*see* p. 40) the separation of these two effects is extremely difficult.

The outcome of varying the photoperiod is by no means clear cut. Very early experiments indicated a marked variation in colour of carrots exposed to different day lengths.[75] Murneek[76] stated that foliage of soya bean, cosmos, and salvia plants grown under 7 hours of daylight had higher carotene contents than had those grown under 14 hours of light. Barnes,[77] however, in a well-documented report concludes that a somewhat smaller variation in photoperiod (9–14 hours) has no effect on carotenoid production when conditions of temperature and moisture were optimal. More recent work by Roberts[53] suggests that pigmentation is altered by variation in the photoperiod, for branches of the same plant when grown under different photoperiods contain different amounts of carotenoids. Roberts, however, did not consider the age factor which operates from leaf to leaf (*see* p. 20). Photoperiod and temperature may be connected.[247]

EFFECT OF TEMPERATURE

Although seedlings grown at low temperatures may contain at least as much carotene as those grown at normal temperatures,[78] the existence of an optimal temperature range (60–70°) for the production of carotene in roots such as carrots[30,79] and beet[79] cannot be doubted. Carrots grown at lower temperatures are visibly less coloured owing to the absence of pigment from the peripheral cells.[30] Recently, using isolated bean leaves, Bandurski[56] found the temperature coefficient for carotenoid synthesis to be 2·9 in the dark and 1·4 in the light.

Work on the relation between temperature and ripening of fruit has already been discussed (*see* p. 40).

EFFECT OF SOIL NUTRIENTS

(i) *General*

A large literature exists on this subject and although a number of investigations have been insufficiently controlled to warrant the conclusions drawn, the general picture is reasonably clear. Short reviews

covering the literature up to 1941–2 have been provided by Virtanen[80] and by Maynard and Beeson[81].

The conclusions of the first workers in this field, Virtanen, von Hansen, and Saastamoinen[82] and Barnes,[77] have not been altered significantly by results of more recent investigations. They found that conditions which lead to the development of a healthy plant also favour maximal carotenoid formation. Plants cultivated under normal fertilizer conditions are not likely to be improved by addition of extra fertilizers;[77,80,83-86] reports to the contrary need rigid reinvestigation.[87-91] Recently Virtanen[92] has stated that the more rapidly a plant grows the greater its carotene concentration.

Variations in the supply of fertilizers below the normal do undoubtedly adversely affect carotenoid production.[89,93-98] In spite of this nutritional variation Bernstein, Hamner and Parks[96] suggest that carotenoid formation depends "far more" on environment and climatic factors than on the state of fertilization, for they found only little variation in carotene content of plants grown in different soils when the soils were contained in pots and subjected to identical environmental conditions. This point of view is upheld by Janes,[83] by Kemmerer and Fraps,[99] by Janes and Campbell,[100] and by Booth and Dark,[101] but Beck and Redman[102] consider any such environmental relationship indirect.

(ii) *Nitrogen*

Under artificial conditions the type of nitrogen supplement is of some importance. In sand cultures Virtanen *et al.*[92] consider that, in general, potassium nitrate is a better source than ammonium sulphate, but for pineapples[94] and tobacco leaves[103] NH'_4 is more effective than NO'_3. Both claims may be true, for Mapson and Cruickshank[104] found that the presence of ammonium sulphate, ammonium chloride, and ammonium phosphate decreased carotene production in cress germinating on ashless filter paper by as much as 60 per cent., whilst ammonium nitrate, ammonium bicarbonate, ammonium acetate, and ammonium succinate had no such effect. The depressant action of ammonium sulphate was reduced by sodium succinate, malate, or aspartate but not by the free acids. These acids together with acetic and nitric acids had no effect *per se* on carotene synthesis. Most significantly $(NH_4)_2SO_4$ did not inhibit synthesis of xanthophylls. Nagel[72] states that when extra nitrogen in the form of $NaNO_2$ is fed to tobacco plants there is an increase in the total carotenoid production but that, at the same time, there is a decrease in the xanthophyll/carotene ratio, indicating the preferential synthesis of carotenes.

(iii) *Phosphorus*

The position of phosphorus is well established; variations in soil phosphate levels have no direct effect on carotenoid production.[95-97, 105-107] Wynd and Noggle[108,109] have attempted to correlate carotenoid production in cereals with the various phosphorus fractions in the soil. At the moment their results are equivocal and difficult to interpret.

(iv) *Potassium*

There may be an optimum level of potassium fertilization required for formation of carotenoids because it is claimed that deficiency reduces,[96,110] a moderate addition improves,[105,106] and an excessive addition inhibits,[82,89,98,111] pigment production; it must be borne in mind, however, that there are further reports which state that potassium has no controlling effect.[93,95,97]

(v) *Sulphur*

There is one report that sulphur deficiency reduces carotenoid production.[96]

The discussion so far has been confined to leafy materials; when fruit is considered, very careful experiments have failed to reveal any effect of wide variations in macronutrients on the carotene content of tomatoes,[113] (but *cf.* Schupfan)[114] although the effect on growth and fruitfulness was marked.

(vi) *Micronutrients*

Detailed investigations by Lyon, Beeson and Ellis led to the conclusion that the following micronutrients play no part in controlling carotene formation in tomatoes. Manganese, copper, zinc, molybdenum and iron.[115] Lo and Chen,[116] however found that zinc but not nickel increased the carotene content of tomatoes.

In leafy material, and to a lesser degree in roots (carrots) reactions to micronutrient deficiencies have in some cases been noted. However, calcium,[95,106] manganese,[95,116A] molybdenum,[116] and aluminium,[116] have no effect, although there is one report that, in general, calcium deficiency in sand cultures reduces carotene synthesis.[107] Opinion is so far unanimous that magnesium deficiency decreases,[95,117,117A] and that addition of zinc,[116,118] increases carotenoid production. Recent work suggests that a magnesium-calcium balance is necessary for the optimal production of plant pigments; soya beans grown on a medium containing excess calcium compared with magnesium produce more carotenoids and less chlorophylls than do plants cultured under normal conditions. The situation is reversed when magnesium is in

excess.[119] Deficiency of iron reduces production in Swiss chard and pineapple,[94] but apparently has no effect on lettuce;[106] Lo[118] claimed that addition of nickel sulphate to the soil had some positive effect on the carotene content of plants.

Powers,[120] in a short report has claimed that the addition of boron to the soil in Oregon resulted in a 30 per cent. increase in the carotenoid content of lucerne (alfalfa); Beeson[80] claims to have confirmed this, but full details of these investigations are still awaited.

PHYSICO-CHEMICAL PROPERTIES OF SOIL AND CAROTENOID PRODUCTION

Wynd and Noggle[121] in an important study broke new ground in relating carotenoid formation in oat and rye leaves to the physico-chemical characteristics of the soil; they confirmed that the nitrogen content of the soil is the most important single factor. The base exchange capacity and percentage loss on ignition of the soil appears to affect carotene production but probably only because in base-saturated soils these values parallel the nitrogen content of the soils; similar considerations make it difficult to decide whether phosphorus, calcium, magnesium, CO''_3, or pH are important *per se*, although later work suggests that the amount of replaceable magnesium is important.[109] With regard to pH it has been claimed that increasing the soil pH increases carotene production,[82] and that carotene formation in cress grown in water culture was " rather closely " related to the pH of the cell sap.[104]

The important distinction to be drawn between yield and concentration is emphasised by Wynd and Noggle. A rich soil with a high degree of base saturation will produce a high yield of dry matter containing a high percentage of carotene, whereas soils with lower degrees of base saturation may produce a low yield of dry matter containing an equally high percentage of carotene. The dependence of yield on dry matter has been confirmed,[100,122] and this agrees with the earlier observation of Barnes[77] and the more recent work of Hunter, Kelley and Somers[91] that soil moisture has no effect on the carotene concentration of carrots when based on dry weights, but that on a wet weight basis the concentration was inversely proportional to the soil moisture owing to higher moisture content of the carrots grown on damp soils. This probably explains the claim that soil moisture influences the " colour " of carrots.[123] Drought *per se* has no great effect on carotene production.[124]

Wynd and Noggle found that, in Kansas, soil with the following characteristics produced crops with high yields of carotene :-base-exchange capacity 20 m.equiv., total replaceable base, 20 m.equiv., replaceable calcium 18 m.equiv., and replaceable magnesium 2 m. equiv., per 100 g. of soil; loss on ignition 4 per cent., and nitrogen content 0·09 per cent. Less than 15 m.equiv. of replaceable base and 0·08 per cent. of nitrogen render the soil unsatisfactory.

INHERITANCE STUDIES ON CAROTENOIDS

It was in 1920 that Steenbock and Boutwell[125] showed that maize with white endosperm was deficient in vitamin A activity; eight years later Hauge and Trost[126] indicated that this activity in maize was transmitted exclusively with the yellow endosperm. In recent years, with the identity of the carotenoids well established and their quantitative assay rendered comparatively simple with the use of spectrophotometers, considerable progress has been recorded. Johnson and Miller[127] have confirmed and extended the early work of Mangelsdorf and Fraps.[128] Using mature grain from 19 inbred lines they revealed a very close relationship between the number of dominant *Y* genes for yellow endosperm colour and both carotene and total carotenoid concentration. However, the carotenoid content of leaf tissue from white endosperm lines was slightly higher than that from yellow endorsperm lines; this strongly suggests that carotenoid formation in the leaf and formation (and/or storage) in the endosperm are independent processes.

Johnson and Miller[129] further studied the immediate effects of cross pollination on the carotenoid content of maize endosperms, and found large variations within 35 inbred lines; they obtained evidence that carotenoid inheritance is subject to the usual xenia effects. These variations within inbred lines have been generally confirmed by Porter, Strong, Brink and Neal,[130] by Emsweller, Burrell and Borthwick[131] and by Aurand, Miller, and Huber[132]. Porter *et al.*, however, consider that those variations are small compared with seasonal maturity factors. Emsweller *et al.* maintain that interbreeding does increase uniformity to some extent. Porter *et al.* also noted that when inbred strains are compared on the basis of the time required to reach a certain stage, those plants needing the longest time contained relatively and absolutely more carotenoids than those requiring shorter times. This is not considered to be due to any inherent increase in ability to elaborate carotenoids, but to the longer growing period;

the rate of carotenoid formation increases more quickly than does the development of the leaf. It has recently been shown that the carotene content of the crosses of yellow dent maize is always significantly related to the content of the parent strain.[132A]

Randolph and Hand[132B] studied the carotenoid content of pure yellow diploid maize carrying the three dominant genes *YYY* for yellow, and a derived tetraploid with double the number of genes, *YYYYYY*, for yellow. Doubling the number of chromosomes increased the carotene content of the maize by 40 per cent. Each carotenoid was increased to the same extent. This increase combined with the increased endosperm cell volume in the tetraploids (3·6 times) resulted in a five-fold increase in the amount of carotenoid per cell. It is considered that this increase is due to a cumulative action of the dominant genes for yellow endosperm colour, the amount of carotenoid elaborated per gene in the tetraploid being 2·5 times as great as that produced per gene in the diploid. On the other hand, doubling the number of chromosomes in white maize resulted in a decrease of 19 per cent. in carotenoid content, there being no cumulative gene action in this case. A somewhat similar investigation by Brunson and Peterson[133] showed that in maize there was a straight line relationship between carotene and zeaxanthin concentration and gene dosage. They confirmed a slight but consistent cumulative tendency with higher gene doses ; this action was most marked in the zeaxanthin fraction.

Webster, Brookes and Cross[133A] record a carotene content of 0·0192–0·0226 mg/g. for open-pollinated corn and 0·0170–0·0199 mg/g. for hybrids ; these differences are considered significant.

There is no such increase in the carotenoid content of tetraploid rye,[134] barley,[135] and some other plants,[136] compared with diploid types, although an increase has been reported in tetraploid wheat.[137]

In tomatoes there are three gene pairs controlling coloration, *Rr*, *Tt*, and *Yy*. *Rr* control the formation of lycopene and to a less extent carotene and the xanthophylls,[138] whilst *T* and *t* determine the spatial configuration of the carotenoids (principally lycopene) ; for example, the dominant *T* controls the production of all-*trans*-lycopene and the recessive *t* the production of poly-*cis*-lycopenes.[139] The genotypes of the red, yellow and tangerine tomatoes are thus considered to be, respectively, *RRTT*, *RRTt*, and *RRtt*.[140] Recent work has led Mackinney and Jenkins[141] to develop this idea and to conclude that in the absence of *R*, *T* is responsible for lycopene production only on a limited scale, whilst in the absence of *T*, *R* is responsible for large amounts of ζ-carotene, prolycopene and protetrahydrolycopene (poly-*cis*-carotene). The outstanding work of Porter and Lincoln[38] on cross-

breeding of tomatoes and selection for preferential production of single carotenoids has already been considered on p. 68. *Y* and *y* are to some extent responsible for the skin colour by controlling the production of an alkali-soluble, non-carotenoid pigment, which accumulates in the skin.[138]

VARIETAL DIFFERENCES

Considerable varietal differences in carotenoid content have been noted in forage grasses,[45] tobacco leaves,[142,142A] carrots,[143-145] maize,[131,132] oranges,[146] tomatoes,[38,147] peas,[148] mangoes,[149,150] and wheat.[151] Little or no differences are reported in red peppers,[133,152] beet,[145] and possibly also in peas.[145]

There is no doubt that varietal factors far outweigh environmental factors and the best chance of producing high carotenoid-containing crops is by breeding.[144,146,147] Kohler *et al.*[147] have by extensive crossing produced a tomato [Baltimore × F_1 × *L. hirsutum* P.I. 126445] containing 101 μg./g. of crude carotene (88 per cent. β-carotene) the value for the usual commercial tomato being about 6 μg./g. Perhaps the most interesting point about this is that the carotene may be

TABLE 11.—*Carotenoid Content of some varieties of Mango Fruit**

VARIETY	Amount mg./100 g. (wet weight)		
	β-Carotene	Neo- β-Carotene B	Lutein (xanthophyll)
Madras	0·86, 0·68	0	0·52, 0·48
Benares	1·83	0·101	3·96
Lucknow	2·90, 2·71	0·173	1·89, 1·76
Delhi-Large	0·58	0·05	1·544
Delhi-Small	0·48	0·02	0·79
Calcutta Fazli	2·45	0·26	1·39
Calcutta Langra	1·38	0·06	1·40
Tammoria or Saffron	2·63	0·11	3·32
Chonea	0·9	0·05	1·44
Sandhuri	1·03		1·25
Kalmi busehri	1·44		
Shujabadi	1·52		1·88
Lahore	0·40		0·96
Banarsi langra	1·30		2·7
Desi (Gola)	0·87		1·32
Saharanpuri	1·56		2·36

* From Sadana, J. C., and Ahmad, B. (1946), *Indian J. Med. Res.*, **34**, 69, and Chaudhary, M. T. (1950), *J. Sci. Food Agric.*, **1**, 173.

produced at the expense of lycopene for the total carotenoid concentration of this fruit is normal. Typical varietal differences are recorded in Table 11 for a fruit (mango) and in Table 12 a root (carrot); for similar data for peas (*see* p. 44). Further details on other species will be found in the Appendix (*see* p. 294).

TABLE 12.—*Carotene Content of different varieties of Carrots.**

VARIETY	Amount (mg./100g.)	
	Fresh wt.	Dry wt.
Amsterdam Forcing	4.2	31
Belgian Long Yellow	0.25	1.97
Belgian white	0.03	0.2
Burpee's Oxheart	2.96	31.4
Danvers Half Long	3.44	21.2
Danvers, Woodruff's Special	3.52	32.2
Early Golden Ball	1.91	22
Golden hart	4.2	42.9
Hutchinson	1.25	11
Imperator	2.8	24.6
Improved Long Orange	2.9	24.4
Nantes Half Long	3.0	26.8
Supreme Half Long	3.36	30.6
Tender sweet	4.3	37.4
Touchon	2.2	22.9

*From Harper, R. H. & Zscheile, F. P. (1945). Food Res., **10**, 84. For further values *see* Appendix I (p. 290).

PATHOLOGY

No systematic study of the relationship between carotenoid production and pathological conditions of plants has been reported, but a few isolated statements are available. Sullivan and Chilton[153] found that rust-infected white clover leaves are lower in carotene than are rust-free leaves by as much as 30 per cent. Leaf hopper (*Empoasca fabae*) damage can reduce the carotene content of lucerne by more than one half.[154,155] According to Ham and Tysdal,[156] the hoppers attack leaves showing the least yellowing, that is, those with the highest carotene content. Hamner[157] reaches the rather broad conclusion that any condition producing chlorosis of leaves will decrease their carotene content.

It is claimed that yellowing of box (*Buxus sempervivens*) leaves owing

to parasitization by fungi or insects is due to excessive carotenogenesis accompanied by excessive lipid production and a fall in starch content.[158] Proof of excessive carotenogenesis has, however, not been adequately presented.

Griffith, Valleau and Jeffrey[144] studied 18 varieties of tobacco plant but could find no relationship between carotene content and mosaic resistance factors.

It has been found recently that the pycnidial lesions on crab-apple leaves (*Malus ioensis*) infected with the common rust fungus *Gymnosporangium juniperi-virginianae*, contain γ-carotene to the extent of 34·5 per cent. of the total carotenes present. Unaffected regions of the leaves produce no γ-carotene.[159] (*See also* p. 108).

Booth and Dark[101] have noted that carrots infested with the larvae of the carrot fly (*Psila rosae*) have a slightly higher carotene concentration than have healthy roots. Recently it has been reported that treatment of kidney bean seedlings with 2 : 4 -dichlorophenoxyacetic acid reduces carotenoid synthesis.[160] Treatment of germinating seeds with streptomycin retards carotenoid synthesis,[161] whilst spraying with isopropylphenylcarbamate has no effect.[248]

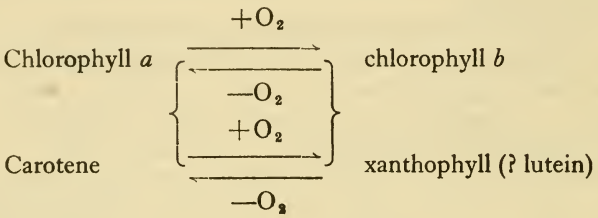
FUNCTION

Theories advanced to explain the function of carotenoids in plants are many but are generally based on one or two characteristic properties : (*a*) the ability to absorb oxygen and (*b*) the ability to absorb light energy in the blue region of the visible spectrum. Nevertheless, rigorous proof of any specific function is still lacking, but as will become apparent one theory appears much more plausible than any other. The problems of carotenoid function in photo-reception and photosynthesis have been critically reviewed by Wald[67] and Rabinowitch[162] respectively. Frey-Wyssling[163] considers carotenoids to be excretory products of unknown metabolites ; their insolubility in water removing them from active participation in the functioning of the cells ; later investigations have, however, shown that carotenoids are almost always solubilized by attachment to proteins[164] (*see* p. 6). Some of the more outré suggestions concerning carotenoid function, which will not be discussed here, have been summarized by Wenzinger.[1]

CAROTENOIDS IN OXIDATION-REDUCTION SYSTEMS

In theory carotenes and xanthophylls appear to be components of a perfect redox system and this is also true of chlorophylls *a* and *b*.

Arnaud[165] originally suggested that because carotene, a highly labile substance, remained stable in the leaf it must take part in some redox system. This idea was expanded in 1913 by Willstätter and Stoll[166] who suggested that as chlorophylls and carotenoids are so intimately connected in plants, they are probably involved in a coupled redox system thus :



Willstätter and Stoll[167] later carried out a series of experiments to test this hypothesis. They examined the relative concentration of the chloroplast pigments before and after exposure to extreme conditions of heat and light. One would expect changes in the ratios chlorophyll *a* : chlorophyll *b*, and carotenes : xanthophylls under such extreme conditions if the pigments were part of a balanced system. Generally, no such change in ratio occurred and when they did they could in no way be interpreted as implying interconversion.

(It should also be pointed out that the function of leaf carotenoids in a redox system would almost certainly involve an isomerization of a β-ionone residue to an α-ionone residue for β-carotene is the major carotene in leaves whilst lutein is the predominant xanthophyll ; such an isomerization has not yet been observed *in vivo*.) During the fading of leaves, although the total chlorophyll content diminishes rapidly, the ratio of chlorophyll *a* : chlorophyll *b* remains constant throughout ; the disappearance of chlorophylls is not accompanied by a rise in carotenoid content which remains constant until the extreme stages of necrosis are reached ; they do, however, undergo qualitative changes (*see* p. 19).

In 1938 Seybold and Egle,[168] investigating shade- and sun-plants found that the ratios xanthophylls : carotenes and chlorophyll *b* : chlorophyll *a* are both greater in shade plants. This work needs confirmation but as it stands it rules out a coupled redox system for the " oxidised " forms of both pigments appear to increase simultaneously. Recently, two Russian scientists, Sapozhnikov and Lopatkin[169] have taken up this problem and have claimed that under favourable photosynthetic conditions the carotene : xanthophyll ratio does increase ;

inhibition of the photosynthetic dark reaction (with narcotics, ether, etc.) causes a decrease in this ratio. In neither case does the total amount of carotenoids present alter.

Starch production in plants is, according to Seybold[170], generally associated with the presence of chlorophyll *b*. Investigating some members of the *Allium* family, which do not produce starch, he found a deficiency of chlorophyll *b*, but no significant variation from normal in the xanthophyll : carotene ratio although as would be expected the ratio chlorophyll *b* : chlorophyll *a* was very low. There was also no significant departure from normal in the total carotenoid or total chlorophyll content. Similarly, in developing mango fruit there is no correlation between carbohydrate synthesis and carotenoid synthesis.[171]

CAROTENOIDS AS OXYGEN TRANSPORTERS

This conception of carotenoid function also follows from Arnaud's[165] pioneer work in 1889 which demonstrated that carotenoids are easily autoxidisable. It was assumed that carotenoids form unstable peroxides which can transfer O_2 to other substances. The *in vitro* " pro-oxidant " activity of carotenoids appears to favour the assumption.[172-176] With this in mind the suggestions made at various times that carotene has a catalytic function in the binding of oxygen in plants can be appreciated ;[177,178] however, it must also be emphasized that all these suggestions are speculative and that no experimental evidence is available. Further, as Rabinowitch[162] has pointed out the reverse process of liberation of oxygen during photosynthesis is equally feasible. Lazar[179] claims that carotenoids stimulate root formation and general development of plants in cultures of *Impatiens balsamina*. If this is confirmed the action may well be related to oxygen transfer, for he later found that carotene and sucrose produced a response similar to that of glucose + oxygen.[180] Giroud[181] maintains that carotene has an antioxidant rather than a pro-oxidant action ; he claims that it is not fortuitous that plants richest in carotenoids are also richest in ascorbic acid, for the ascorbic acid is protected by the carotenoids. There is, however, no real evidence that this suggested correlation between vitamin C and carotenoid levels is a reality.[148] Taking a viewpoint similar to that of Giroud, Hérisset[182] considers that the carotenoids act by inhibiting plant and animal oxidases.

An apparently insuperable objection in the way of theories based on the autoxidisibility of carotenoids is their undoubted stability *in situ* in the plant chloroplast compared with instability in extracted solutions.

The reason for this stability is that probably the carotenoids exist as protein complexes in the same way as does chlorophyll,[183] they may even be attached to the same protein as is chlorophyll to form what Lubimenko[183] called "natural chlorophyll" (*see also* Smith).[184] Although it has not yet been isolated, this carotene protein complex is more stable than are carotene solutions; further examples of carotenoproteins will be dealt with in later sections of this book (*see* p. 171).

Chailakhyan[184A] has recently examined the effect of introducing lanolin containing 0·2% of carotene into incisions made in the stems of *Chrysanthemum*, *Perilla* and *Rudbuckia* spp. In *Perilla* and *Rudbeckia* growth of the main stem was retarded but side runners developed abnormally well. In *Chrysanthemum* there was decreased growth of the main stem (less leaf-tier spacing) accompanied by the development of thicker, stiffer, and darker leaves. Powdered saffron (rich in carotenoids, including carotene) had the same action, whilst a chlorophyll preparation, obtained from nettles, was inactive.

CAROTENOIDS IN PHOTOSYNTHESIS

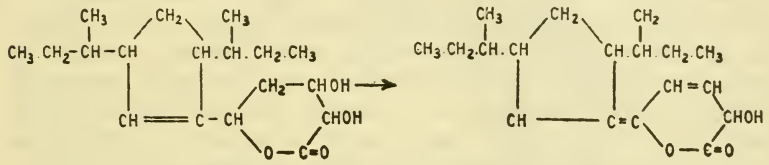
As long ago as 1844 Draper[185] showed that the blue and violet regions of the spectrum (in which chlorophyll action is minimal) are almost completely ineffective in producing photosynthesis as measured by oxygen liberation and Willstätter and Stoll[167] have stated categorically that carotenoids play no part in photosynthesis. Engelmann[186] using a different technique, came to the opposite conclusion. He irradiated plant material with the visible spectrum and noted the regions of the leaf corresponding to definite wave-lengths, at which mobile aerobic bacteria accumulated, these accumulations denoting areas of rapid oxygen evolution. The variations which occurred in the points of maximum accumulations when materials of different colour were used led him to conclude that pigments other than chlorophylls (*i.e.*, carotenoids) enter into the photosynthetic mechanism.

It is clear that carotenoids cannot play a primary role in photosynthesis for no case has been recorded of their ability to promote photosynthesis in the absence of chlorophyll; Rabinowitch[162] believes that carotenoids participate in photosynthesis by transferring their excitation energy to chlorophyll:

$$CAR + h\nu \longrightarrow CAR^*$$

$$CAR^* + CHL \longrightarrow CHL^* + CAR$$

There is nothing inherently improbable in this suggestion and such a sensitization seems to occur in the case of the fluorescence of chlorophyll in green algae and diatoms[187] (*see* p. 141). A relevant chemical experiment was carried out by Karrer and Straus,[188] who found that colloidal solutions of carotene sensitized the autoxidation of benzidine. Of even more interest is the recent demonstration by Kögl and Schuringa[189] that β-carotene, α-carotene, and lycopene sensitize the photochemical inactivation of the plant hormone auxin-a-lactone by converting it into lumiauxin.



As might be expected the efficiency of this action is maximal at the wavelength corresponding to the main peak of the absorption spectra of the carotenes. Whether this inactivation occurs *in vivo* remains to be demonstrated.

The implications in the important study of Frank[190] must not be overlooked. She has shown that in etiolated *Avena* seedlings carotenoids do not act as filters for blue light, and, as first postulated by Noack[190A], suggests that the carotenoids are located behind the protochlorophyll in the plastids.

In the aquatic plant *Potamogeton fluitans*, submerged and shaded leaves are green; on exposure to strong light they become red owing to the replacement of chlorophyll in the plastids by carotenoids;[191] this is reversed on removal of the light source. It is claimed that this observation, which requires confirmation, demonstrates the protective action of carotenoids when photosynthesis is inhibited by strong light.

Hérisset has recently made the somewhat startling claim that mixed solutions of carotene and chlorophyll stimulate photosynthesis, aldehydes being produced in the presence of CO_2.

Neither pigment alone possesses this property.[191A]

CAROTENOIDS IN PHOTOKINETIC RESPONSES

Wald[67] considers the primary function of carotenoids in plants to be the stimulation of photokinetic responses such as phototropic

bending and chloroplast migrations. He bases this generalization on three main considerations, (1) photosensitive structures contain carotenoids, (2) photokinetic action spectra* correspond closely to the absorption spectra of known carotenoids and (3) no other pigment occurring in the photosensitive structures possesses a comparable spectrum.

Blaauw[192] was the first to measure the action spectrum of the phototropic bending of the oat (*Avena*) seedling, but it was not until 1930 that the suggestion was put forward that the mediator might be a chromolipid.[193] Since then, Voerkel,[194] Castle[195] and Bünning[196] have confirmed and developed his suggestion. Went[197] has examined the spectral sensitivity in pea seedlings of leaf growth, inhibition of stem growth, and phototropic bending; only the latter response is maximal when the seedlings are irradiated with blue light, *i.e.*, in the spectral region where light absorption by carotenoids is maximal, the first two responses are minimal under these conditions. This does not imply that phototropic bending is the only photo-reaction into which the carotenoids enter, for Bottelier[198] has shown that the action spectrum for protoplasmic streaming in the epidermal cells of *Avena* coleoptiles is very similar to that for phototropic bending.

It should be noted, however, that the action spectra for the production of spikes in barley and for the initiation of flowering in soya beans are identical, with minima in the spectral region 450–480 mμ.[199] These results strongly suggest that carotenoids do not play any part in these processes.

Wald's third point that no other pigment in the photosensitive structure possesses a comparable spectrum needs reconsideration in the light of recent work by Galston. Riboflavin occurs throughout the *Avena* coleoptile[200] and Galston and Baker[200] state that *in vivo* measurements of action spectra are not sufficiently precise to distinguish between β-carotene and riboflavin both of which absorb light maximally around 450 mμ. Re-examining the spectral data of Haig,[201] they concluded that the "tip reaction" in *Avena* probably involves a carotenoid and the "base reaction" probably riboflavin. If, as Kögl and Schuringa[189] suggest, β-carotene controls phototropic responses by sensitizing the photo-inactivation of auxin-a lactone, then there exists evidence that riboflavin can act in an analogous manner. It has been shown that riboflavin can inactivate indole acetic acid which has claims to be considered a naturally-occurring auxin[202,203]. Relevant

* Action spectra are constructed by plotting the reciprocal of the energy at different wavelengths required to elicit a constant response, against these wave-lengths.

to this problem is the recent finding of Bandurski and Galston[204] that an albino mutant of *Zea mais*, which contains no carotene but normal amounts of riboflavin, responds phototropically almost as well as the normal strain. For a full and stimulating discussion of this problem the reader is referred to a recent article by Galston.[205]

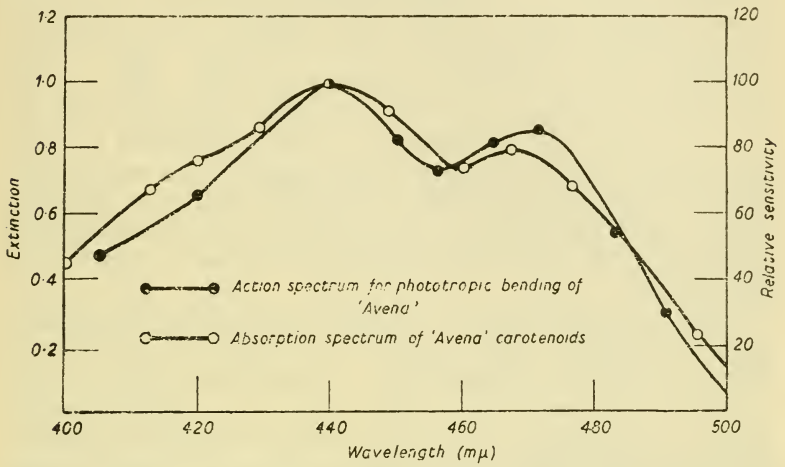


Fig. 16.—Showing the similarity between the absorption spectrum of the carotenoids extracted from *Avena* seedlings and the action spectrum for the phototropic bending of these seedlings (*after Johnston, E. S. (1934)* Smithsonian Inst. Pub. Misc. Coll. **92,** No. 11., *and Wald, G. (1943)* Vitamins & Hormones, **1,** *208*).

Tauc[206] has shown that illumination of the epicotyl of *Vicia faba* produces a potential difference between the illuminated and non-illuminated side. The action spectrum for the photo-electric effect is very similar to the absorption spectrum of β-carotene and this, together with the fact that the effect is most marked in the regions of carotenoid accumulation inclines Tauc to the view that carotenoids mediate in this response.

CAROTENOIDS AND REPRODUCTION

It will be noted in later chapters that there is considerable circumstantial evidence that carotenoids have a part to play in the reproduction of cryptogams and of various animal species ; this possibility must now be considered in the phanerogams. The whole problem of carotenoids and reproduction has recently been discussed.[207]

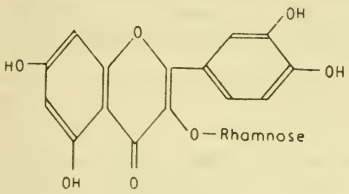
There is ample evidence (quoted in the previous chapter) that, at maturity, the carotenoid concentration in the green parts of plants begins to decline. It has been suggested that this is due to mobilization of carotenoids into the reproductive structures of the plant.[208] Evidence in favour of this mobilization is very meagre, and it has, in fact, been denied.[209] Further, it should be noted that in the case of maize (corn) this decline was still evident in plants in which pollination was eliminated by covering the ear shoots with paper bags.[130] One possibility is that at maturity the synthetic mechanism rather than the final product, is diverted to the flowers and then to the developing fruit. It is well known that carotenoids are rapidly synthesized in many developing fruit (*see* p. 39), but it has only recently been reported that the carotene concentration of the anthers and petals of a number of plants (Californian poppy, jasmine, pumpkin, and St. John's Wort) increases through the budding period and reaches a maximum at flowering.[210]

Zhukovskii and Medvedev[211] consider that microsporogenesis is intimately connected with carotenoid metabolism for they claim to have demonstrated histologically that carotenoids and lipids are concentrated in the tapetum of the pollen sac and that, during formation of pollen, both migrate into the developing pollen grains, young pollen grains being white whilst mature grains are yellow. This work does not, however, eliminate the possibility that carotenoids are produced *in situ* in the pollen grains. A somewhat related observation is that pollen tube growth on an agar medium is stimulated by the addition of carotene to the medium.[212]

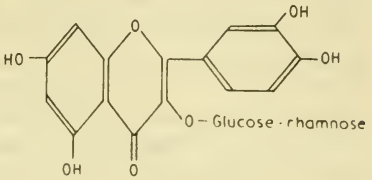
Deleano and Dick[213] have shown that the amount of carotene per leaf is greater in fully developed male crackwillow (*Salix fragilis*) than in fully developed female trees; the concentration in both cases, is however, the same. It seems then that the increased carotene content of male leaves is merely a reflection of their larger size and has no significance for carotenoids *per se*.

The function of carotenoids in pollen, if any, cannot be universal because not all pollen contains carotenoids (*see* p. 52), and in this connection, the recent work of Kuhn and Löw[214] on *Forsythia intermedia* Zabel (*F. suspensa* × *F. viridissima*) is relevant. This plant is self-sterile, fertilization only taking place by cross pollination of the R°-type (short-styled flowers with long filamented anthers) and the R^{+} type (long-styled flowers with short filamented anthers). Kuhn and Löw found no qualitative or quantitative differences between the carotenoids in the petals of the two types. No mention is made of the carotenoids in the two pollens and this is rather disappointing because they found

important differences in the glucosides present in the pollens. R° pollen contains quercitrin, whilst R^{+} pollen contains rutin.



Quercitrin



Rutin

Moewus[215] has developed the biological aspect of this problem and reports that rutin and quercitrin are pollination inhibitors. Cross-fertilization is effective because pollen from long-styled flowers contains a quercitrin splitting enzyme and the short-styled pollen a rutin-splitting enzyme; cross-pollination allows fertilization by hydrolyzing the pollination inhibitors. The inhibition caused by rutin and quercitrin can be destroyed by H_3BO_4, when the two types of *Forsythia* become self-fertile. Confirmation of this novel report is eagerly awaited.

Recently, Schwartzenbach[215A] has described a technique for measuring the effect of various carotenoids on the pollination of *Cyclamen persicum*. The carotenoids examined fell into three groups: those which stimulated, those which retarded and those which had no effect on pollination. A consideration of these results reveals that each group contains a heterogeneous collection of carotenoids and that no structural criterion of activity emerges from this study.

DESTRUCTION OF CAROTENOIDS IN PLANTS

The well known fact that a preliminary blanching of harvested plant materials prevents oxidation of carotenoids indicates that the process is enzymic in nature. Similar oxidations have been achieved photochemically, and it is difficult to say finally whether *in vivo* two separate processes occur or whether light plays a part in a fundamental enzymic process. Bernstein and Thompson[216] consider that two separate processes occur.

(A) The Photochemical Process. In 1933 Meyer[217] found that chlorophyll was an activating agent in the photochemical destruction of (*inter alia*) lycopene. There the matter rested for 10 years until in 1943 Pepkowitz[218] made an important contribution to this aspect of

carotenoid metabolism when he found that the photochemical destruction of carotene solutions *in vitro* occurs in the presence of chlorophyll which is directly involved and does not act merely as a catalyst; sodium cyanide partly inhibits this reaction. It is interesting that about the same time it was noticed that carotene inhibits the photodestruction of chlorophyll.[219] *In vivo*, however, chlorophyll does not apparently take part in the photochemical destruction of carotenoids, for Bernstein and Thompson[216] showed that photodestruction is as great in etiolated leaves as in green leaves. Photodestruction occurs equally well in all regions of the spectrum and is influenced by atmospheric oxygen. Between oxygen concentrations of 0·5 and 20 per cent. destruction is proportional to the oxygen concentration; between 0·5 % and 0·02 % no change in destruction rate is noted, probably owing to the replacement of atmospheric oxygen by some cellular constituent. Below 0·02 % destruction ceases.

(B) Enzymic Destruction. Enzymic degradation of carotenoids occurs in plant tissue when the pigments and/or enzymes are liberated by destroying the cells by maceration.[220] Strain[33] found that, apart from blanching, this oxidation could be inhibited by small amounts of zinc dust, magnesium oxide, sodium hydroxide, ammonium hydroxide, lead acetate and mercuric chloride; cyanide is also an inhibitor.[219] Mitchell and Hauge[220] consider that the enzyme responsible is lipoxidase (*vide infra*) and that cell permeability controls oxidation in the intact plant, for, as just stated, oxidation is very rapid when the cells are ruptured by freezing or when the plant wilts. The occurrence of natural protective substances in the intact cell should not be overlooked, for Weier[221] found that if blanched carrots were leached with cold water prior to storage the stability of the carotenoids was considerably reduced, indicating the presence of a protective substance soluble in cold water after liberation from the cells by blanching; this has recently been confirmed.[221A]

Mitchell and Hauge[222] found that the increased oxidative action in sunlight is not completely explained by the possible catalytic effect of chlorophyll, but may be due to two other factors, (*a*) increased transpiration due to the opening of stomata in light, which would produce more rapid wilting, and (*b*) production of leaf temperatures above that of the surrounding air, which would result in both accelerated wilting and increased enzymic activity. The effect of temperature on carotene destruction has been more recently examined by Bernstein and Thompson,[216] who showed that between 4° and 25° the temperature coefficient (Q_{10}) for the enzymic destruction is 1·6–1·7. Griffith and Thompson[221A] have shown that in lucerne leaves, the sunlight-sensitized destruction

is 7–8 per cent. of the total pigment present, whilst enzymic destruction amounts to 27–28 per cent. With stems different values were obtained, sunlight accounting for about the same loss as with leaves whilst the enzymic destruction increased to as much as 70 per cent.

LIPOXIDASE

The enzyme responsible for carotenoid oxidation in plants is probably *lipoxidase*,[223] which has been the subject of a number of recent investigations.

In 1939 Sumner and Dounce[223] demonstrated that a carotene solution in oil is rapidly bleached (oxidised) by an enzyme present in soya beans and other legumes. Later Sumner and Sumner[224] and Sumner and Smith[225] noted that xanthophylls and bixin were similarly oxidized, that a necessary adjuvant was an unsaturated fat, which was simultaneously converted into a peroxide, and that the optimum pH and temperature were 6·5 and 40–45° respectively. Reinvestigating the problem, Tauber[226] considered the enzyme to act directly on the unsaturated fatty acid and the carotene to be indirectly oxidized by the unstable peroxides so formed. He thus renamed the enzyme "*unsaturated fat oxidase*." Süllmann[227] and Mikhlin and Pshennova[228] reached conclusions similar to those of Tauber but pointed out that the enzyme was identical with *lipoxidase* first described in 1932 by André and Hou.[229] Süllmann also found that the enzyme had no prosthetic group and that, when a neutral fat was the substrate, lecithin and α-tocopherol acted as inhibitors; on the other hand lecithin was without effect when a free fatty acid was the substrate.

Strain[230,231] considered that only fats containing a $-HC=CH.(CH_2)_7.C=O$ group with a *cis*-configuration were substrates for lipoxidase. Recently Holman and his colleagues,[232,233] whilst confirming the necessity for the presence of a *cis*-configuration in the substrate, found that the position of the ethylenic double bond is not critical for arachidonic acid [$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3$, COOH] is oxidized at the same rate as are linoleic and linolenic acids [$CH_3.(CH_2)_4.CH=CH.CH_2.CH=CH.(CH_2)_7.$ COOH and $CH_3.CH_2.CH=CH.CH_2.CH=CH.CH_2.CH=CH.(CH_2)_7COOH$, respectively.] They consider that the structural necessity for lipoxidase activity is the methylene interrupted doubly unsaturated system, $-CH=CH.CH_2.CH=CH-$, for conjugated unsaturated systems are not attacked.[233,234] Pigments other than carotenoids (*e.g.*, chlorophylls *a* and *b* and haem) can also be inductively oxidized by lipoxidase.[231,235]

Sumner[236] does not accept the view that the fat peroxides, produced as intermediates in the oxidation of the unsaturated fatty acids, oxidize the carotenoids, for he found that oxidation by fatty acid peroxides does not take place in the absence of the enzyme. He takes the view that to effect oxidation of carotenoids the enzymic peroxidation of the fat must actually be in progress. Oxidation is thus probably the result of transfer of oxygen from an unstable intermediate formed during the oxidation of the unsaturated fat, for in the presence of carotene the rate of fat peroxidation is diminished. Extending this work Holman[237] found that the conjugated dienes, produced during the oxidation of unsaturated fatty acids[238,239] are reduced in the presence of carotenoids, and he thus considers that the oxidation of these pigments is a consequence of their interruption of the chain of reactions by which the unsaturated fats are oxidized. A full discussion of the whole problem is given by Bergström and Holman.[240]

Kies[241] has recently isolated a crystalline polypeptide from Soya beans which activates lipoxidase ; a very similar compound also occurs in gum arabic.

A number of methods for determining lipoxidase activity have been devised ;[225,227,242-244] they are based on the measurement of the rate of destruction of carotene under standard conditions and are effective only within rather narrow limits. In legume seeds the level of activity varies from 1·9 " units " in Lima beans to 60 " units " in Mandalay Soya beans.[242] Lipoxidase has recently been crystallized[245] and analysed for its constituent amino-acids.[246]

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CHAPTER IV

CAROTENOIDS IN PLANTS

Cryptogams

(A) *BRYOPHYTA AND PTERIDOPHYTA*

No systematic investigations have been carried out on the carotenoids of two of the main groups of cryptogams, the Bryophyta and the Pteridophyta. Kohl,[1] in his pioneer work undertaken at the turn of the century, reported the presence of carotenoids in the following members of these groups : *Marchantia polymorpha*, *Catharinea undulata*, *Funaria hygrometrica*, *Adianthum* spp., *Trichomanes radicans*, *Selaginella mortensii* and *S. krauseana*.

By implication, the moss *Funaria* probably contains carotenoids, for the energy required to produce constant chloroplast orientation is least in the region of the spectrum (blue) where carotenoids absorb light maximally.[2]

Two Pteridophyta, *Equisetum* spp. and *Selaginella* are reported to contain rhodoxanthin[3, 4, 5] whilst bracken (*Pteridium aquilinum*) contains rather less β-carotene than is usual in green leaves.[6] Carotenoids are present in the spores of the Pteridophyta.[7]

(B) *THALLOPHYTA*

The *Thallophyta*, which contain the sub-groups, Fungi, Algae, Lichens, and Bacteria, have, with the exception of Lichens, attracted much more attention and, therefore, knowledge of the carotenoids of this group is so considerable that the sub-groups will be treated separately.

LICHENS

Kohl[1] reported carotenoids in the lichen *Baeomyces roseus* and, by implication they occur in a number of short lichens because these were vitamin A-active when fed to rats[8] (*see* p. 27 for a discussion of vitamin A-active carotenoids). The lichen *Roccela montagnei*, which grows on sandal-wood trees, contains 28–40 mg. of carotene per 100 g. dry wt. and a little cryptoxanthin.[8A] Apart from these pieces of information nothing is known of lichen carotenoids.

FUNGI

In considering fungal carotenoids it will soon become clear that this group differs from phanerogam carotenoids in four main respects :

(1) Carotenoids are often completely absent from fungi.

(2) β-Carotene is by no means such a common fungal carotenoid as it is in Phanerogams.

(3) Characteristic carotenoids are often present in fungi which are acidic in character, being similar to astaxanthin. No such pigments occur in Phanerogams.

(4) The characteristic xanthophyll of higher plants—lutein—has never been detected in fungi and the other phanerogam xanthophylls only rarely.

Whilst bearing these striking differences in mind it should be noted that the Phalloidaceae resemble the higher plants in one respect, that is in having their carotenoids grouped round the nucleus of the chromoplasts.[9]

Before discussing the carotenoids in the various classes of fungi, it will be convenient to point out here that carotenoids have been looked for in a number of fungi and found absent. The species concerned are listed in Table 13. It is appreciated that in all probability many other fungi do not produce carotenoids but this list is confined to those species which have been specifically investigated from this point of view.

The pioneer work of Kohl[1] and Zopf[10] also indicated that carotenoids were probably present in a number of species which have never been further examined using modern techniques. These species are listed in Table 14.

Table 13

Fungi from which Carotenoids have been shown to be absent

Agaricus (Telamoria) armillatus[1]
Agaricus laceatus[1]
**Alternaria solani*[2]
Amanita muscaria[1]
Amanita pantherina[1]
Arthonia spp.[1]
Ascobolus furfuraceus[6]
Bachospora dryma[1]
Bacidia muscorum[1]
Biatora fungidula[1]
Bilimbia melaena[1]
Boletus luridus[1]
Boletus scaber[1]
Buellia spp.[1]
Cladonia coccifera[1]
Clavaria ternica[1]
Claviceps spp.[1]
Cortinarius bulliardi[1]
Cortinarius violaceus[1]
Daedalea flavida[3]
**Fusarium lycopersici*[2]
**Fusarium moniforme*[2]
**Fusarium oxysporium*[2]
Ganoderma (Formes) lucidus[3]
Gomphidius glutinosus[1]
Gomphidius viscidus[1]
**Helminthosporium sativum*[2]
Helvella esculenta
Hydnum ferrugineum[1]
Hydnum repandum[1]
Hygrophorus coccineus[1]
Hygrophorus conicus[1]
Hygrophorus punicens[1]
Lactarius deliciosus[1]
Lecidea spp.[1]
Lenzites subferruginea[3]
Nephoroma lusitanica[1]
Oidium violaceum[1]
Paxillus atrotomentosus[1]
Penicilliopsis clavariaeformis[1]
Peziza aeruginosa[1]
Peziza echinospora[1]
Peziza sanguinea[1]
Phragmidium violaceum[1] (†)
Pichia spp.[4]
Polyporus grammocephalus[3]
Polyporus luzonensis[3]
Polyporus rubidus[3]
Polyporus zonalis[3]
Polystictus hirsutus[3]
Polystictus sanguineus[3]
Polystictus versicolor[3]
Polystictus xanthopus[3]
Pullularia spp.[4]
**Rhizoctonia solani*[2]
Rhizopogon rubescens[1]
Russula alutacea[1]
Russula aurata[1]
Russula emetica[1]
Russula integra[1]
Saccobolus violaceus[1]
Sarcogyme pruinosa[1]
Taphrina deformans[2]
Thalloidima candidum[1]
Thelephorus spp.[1]
**Thielavia terricola*[2]
Torulopsis lipofera[4]
Torulopsis luteola[4]
Torulopsis pulcherrima[4, 5]
Trametes persooni[3]
Trametes versatilis[3]
Zygosaccharomyces spp.[4]

* Only vitamin A-active carotenoids are absent from these species: inactive carotenoids may possibly be present.

† See also Table 14.

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TABLE 14

Fungi in which early workers[1,2] *have reported the presence of carotenoids, but which have not recently been investigated*

Ascobolus spp. (not *A. furfuraceus*[3])
Calocerca cornea
Calocerca palmata
Calocerca viscosa
Chytridium spp.
Coleosporium pulsatilla
Ditiola radicata
Leotia lubrica
Lycogola flavofuscum
Melampsora aecidioides
Melampsora salicis capreae
Nectria cinnabarina
Peziza aurantia
Peziza (*Lachnum*) *bicolor*
Peziza (*Lachnea*) *scutellata*
Phragmidium violaceum (†)
Pilobolus crystallinus
Pilobolus kleinii
Pilobolus oedipus
Polystigma ochraceum (*fulvum*)
Puccinia coronata
Saccharomyces (spp.)
Sphaerostilbe coccaphili
Spathularia flavida
Stemonitis spp.
Triphragmium ulmariae
Uredo (*Coleosporium*) *euphrasie*
Uromyces alchemille

† See also Table 13.

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A review on fungal carotenoids has recently appeared.[11]

MYXOMYCETES

Lederer,[12] who has carried out a considerable amount of important work on fungal carotenoids, extracted two pigments from *Lycogala epidendrom.* Although not definitely confirmed these pigments appear to be rhodoviolascin (*see* p. 121) and torulene (*see* p. 105).

PHYCOMYCETES

Carotene has been detected in the spore-bearing cells of *Phycomyces blakesleeanus*,[13,14] *Pilobolus kleinii*[15] and *Mucor hiemalis.*[16] In *Phycomyces* Schopfer and Jung[13] claim that carotene is the β-isomer " because of its marked vitamin A activity," whilst Castle[14] considered it to be α-carotene. Karrer and Krause-Voith,[17] however, state that the main pigment is β-carotene which is accompanied by a little α-carotene. This has been confirmed by Bernhard and Albrecht[18] and Garton, Goodwin and Lijinsky.[19,20] Bernhard and Albrecht[18] also reported the presence of lycopene in traces and Schopfer and Grob[21] state that 5 carotenoids occur in their strain (Z) of *Phycomyces.* Goodwin[22] has recently confirmed the presence of lycopene and has shown

that neurosporene (? tetrahydrolycopene, *see* p. 28), γ-carotene, and ζ-carotene are also present in small amounts; phytofluene and phytoene, the colourless partly saturated carotenoids, are also present. A material with an absorption band having a maximum at 256 mμ. occurs in association with phytoene, it remains to be decided whether this is a carotenoid or not. Phytoene has not previously been reported in fungi.

Differential accumulation of carotenoids has been observed in the sexual forms of some Phycomycetes. Lendner (quoted by Satina and Blakeslee)[23] first noted in 1918 that the (+) strain of *Mucor hiemalis* contained more pigment than the (—). This was confirmed by Satina and Blakeslee[21] and Chodat and Schopfer[16] and extended to *Phycomyces blakesleeanus* by Schopfer.[24] Garton *et al.*,[19,20] however, found that their (—) strain of *Phycomyces blakesleeanus* always contained about twice as much as their (+) strain, irrespective of many variations in cultural conditions. Important results were obtained by Emerson and Fox[25] using various species of the aquatic phycomycete *Allomyces*. *Allomyces* spp. can be divided into two types, (*a*) Euallomyces, which show marked morphological alteration of generations, and (*b*) Cystogenes which produce cysts but go through no obvious sexual phase. Two cystogenes were examined, *A. cystogena* and *A. moniliformis*; the former synthesizes no carotenoids whilst the latter produces γ-carotene and distributes it widely and indiscriminately in sporangia, hyphae and spores. The asexual and female plants of the Euallomyces, *A. arbuscula*, *A. javanicus* and *A. macrogyna*, synthesize no carotenoids whilst the male forms produce γ-carotene and store it specifically in the gametangia in the oil droplets of the cytoplasm, the pigment persisting in the gametes after emergence from the gametangia. Traces of β-carotene also occur alongside γ-carotene, but no xanthophylls were ever detected.

Ascomycetes

As early as 1892[26] Zopf recognized two separate carotenoids in *Polystigma rubrum*; this has been confirmed by Lederer,[12] who considers that one is possibly lycoxanthin (*see* p. 32); the second pigment in an acidic carotenoid with ill-defined absorption bands having maxima at 516 and 485 mμ. in light petroleum and 550 and 515 mμ. in CS_2. Kohl's[1] very early spectroscopic data on *Nectria cinnabarina* suggests that it may produce similar pigments.

Neurospora crassa, both the wild type and a non-conidiating mutant 580, produce a complex mixture of carotenoids most of which are epiphasic. The four major epiphasic pigments were obtained crystalline

and were identified as lycopene, γ-carotene, rhodoviolascin (= spirilloxanthin, *see* p. 121) and neurosporene (? = tetrahydrolycopene, *see* p. 28). Only β-carotene, of the four minor epiphasic carotenoids was unequivocally identified, but the other three were probably lycoxanthin (or rhodopin), α-carotene and rhodopurpurene (*see* p. 121). The major constituent of the hypophasic pigment fraction of *Neurospora crassa* is an unidentified acidic carotenoid. Phytofluene is also present.[27]

Heim[9] has reported the presence of unidentified carotenoids in the following Discomycetes, *Sarcoscypha coccinea*, *Peziza aurantia*, *Melastiza miniata*, *Anthracobia melaloma*, and *Humaria* spp.

An unidentified morel, "Hongro de San Juan" (*Boletus* spp.) collected in Guatemala contained 0·005 mg. of carotene per 100 g. (wet weight).[27A]

Basidiomycetes

Towards the end of the last century Müller[28] and Bertrand and Poirault[29] noted carotenoids (lipochromes), often in crystalline form, in uredospores. Bachmann (quoted by Lederer)[12] considered the pigment to be carotene, but a thorough investigation of two species, *Puccinia coronifera* and *Coleosporium senecionis*, by Lederer[14] revealed the presence of α-, β- and γ-carotene in both fungi. The former also produced an acidic carotenoid, which was not examined in detail but appeared similar to the acid pigment, torularhodin, produced by *Rhodotorula* spp. (*see* p. 106).

Heim[9] has observed carotenoid crystals in the cytoplasm of *Mutinus caninus*, *M. bambusinus*, *Lysurus hexagonus* and of a *Beudocolus* from Madagascar. In *M. caninus*, orange droplets also occur but this is apparently more characteristic of the Ascomycetes.

The single carotenoid of *Tremella mesenterica* is β-carotene, whilst in the related *Aleuria aurantia* a complex mixture exists in which β- and γ-carotenes, rubixanthin (probably), and an unidentified pigment are present[12]. The difficulty Lederer experienced in extracting the carotenoids from *A. aurantia* should be noted; the dry material must be treated with water to burst the cells before the pigment can be extracted even with acetone.

Willstaedt[30] has investigated the carotenoid distribution in certain species of *Cantharellus*; *C. cibarius* contains chiefly β-carotene but also some α-carotene, a little lycopene and γ- and δ-carotenes, the concentration of β-carotene being 4 mg. per 100 g. (fresh wt.). *C. lutescens* and *C. infundibiliformis*, on the other hand, synthesize appreciable amounts of lycopene but no β-carotene. A pigment tentatively identified as β-apo-8′-carotenal was also detected in *C. infundibiliformis*.

The apo-carotenes were first produced by Karrer and his school[31] by controlled oxidation of naturally-occurring carotenoids and this is the first time that they have been reported to occur naturally.

It is important, therefore, that *C. infundibiliformis* should be re-investigated in the light of present knowledge, because Willstaedt's pigment may turn out to be either an epoxide or a pigment similar to neurosporene.

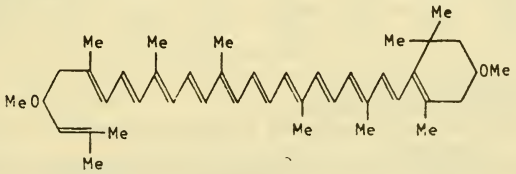
When the general properties of Willstaedt's pigment are considered in the light of present knowledge, it appears much more likely to be neurosporene than β-apo-8′-carotenal.

Recently Haxo[30A] has examined the related species *C. cinnabarinus*. Of the complex mixture of pigments obtained only two were identified with known carotenoids, viz., β-carotene and phytofluene. Two (III and VI) of the remainder were obtained chromatographically pure but not crystallized; they were adsorbed just above lycopene and β-carotene respectively. A third was obtained crystalline and found to be a new xanthophyll; it was named canthaxanthin (*see* Table 6).

Schizomycetes

Of the two families of anascoporogenous yeasts the Rhodotorulaceae contain carotenoids and the Torulopsidaceae do not.[32] Zopf[33] was the first to notice carotenoids in the red yeasts and in 1916 Chapman[34] noted that the absorption spectrum of the carotenoid extracted from red yeast was different from that of carotene. The more recent investigations of Lederer[12,35] on three strains of *Rhodotorula rubra* have to a considerable extent elucidated the carotenoid composition of this yeast. Each strain contained the same 4 pigments:

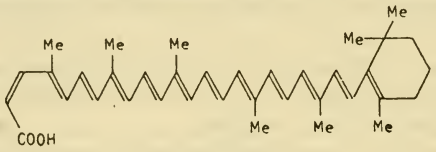
(1) an acidic pigment ($\lambda\lambda_{max.}$ 583, 545, 500 mμ. in CS_2),
(2) β-carotene,
(3) torulene,
(4) an unstable carotene which could not be examined in detail.



Torulene

Lederer considers torulene to be 3 : 3′-dimethoxy-γ-carotene but he emphasizes that this is by no means proved. It is inactive as a vitamin A precursor.[36,37]

Karrer and Rutschman[38,39] isolated the acidic material and named it *torularhodin* ($C_{37}H_{48}O_2$). It contains one carboxyl group and thus does not owe its acidic character to keto enol tautomerism. Further investigations showed that torularhodin had vitamin A activity and the following structure is suggested :[39]



Torularhodin

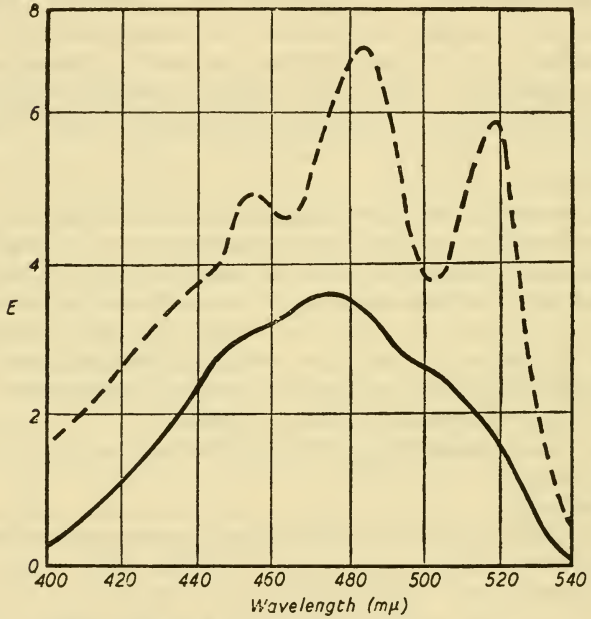


Fig. 17.—The absorption spectra of
(*a*) Torulene in hexane - - - - - -
(*b*) The acidic carotenoid present in *Neurospora crassa*, in light petroleum. ———
(*a*) *redrawn from Lederer, E. (1938)* Bull Soc. Chim. Biol., **20,** *554.*
(*b*) *redrawn from Haxo, F. (1949)* Arch. Biochem, **20,** *400.*

Torularhodin was the main pigment present in Karrer's cultures and also the main component of the carotenoids extracted from *R. sanniei* by Fromageot and Tschang,[40] who detected γ-*carotene* and

lycopene in traces as well as greater amounts of β-carotene and torulene. The amounts these workers obtained from 1 g. of dry material were :

2,900 μg. of torularhodin
143 μg. of torulene
10 μg. of β-carotene.

Neither Fink and Zenger[41] nor Bonner, Sandoval, Tang and Zechmeister[42] could, however, detect the acidic pigment in more than minute amounts in their cultures of *R. rubra*. Bonner *et al.*, who examined a number of mutant strains of *R. rubra*, always found torulene as the main pigment associated with smaller amounts of β- and γ-carotene ; two unidentified pigments first thought to be possibly β-carotene-5 : 6, 5′ : 6′-diepoxide[43] and auroxanthin[44] but now known to be neurosporene and ζ-carotene,[27] as well as the reduced carotenoid *phytofluene* were also detected. For spectroscopic data on torulene and torularhodin *see* Table 15 and Fig. 17.

TABLE 15.—*Characteristic Fungal Carotenoids**

Pigment	m.p.	Absorption spectra maxima (mμ.)		
		Carbon disulphide	light petroleum	Chloroform
Torulene[1,2]	185°	563-5, 520-5, 488-91		539, 501, 469
Torularhodin[2]	201-203° (decomp.)	582, 541, 502	537, 501, 467	554, 515, 483
Neurosporene[3] *see also* Tetrahydrolycopene)	124°	502·5, 470·5, 439·5	470, 441·5	
Acid carotenoid[3] from *Neurospora crassa*	—	512-514	516, 482	
Pigment III } from *C.*	—	—	520, 470	462, 405
Pigment VI } *cinnabarinus*[4]	—	494	—	455
Canthaxanthin	218°	500	—	462

* Pigments first reported in other organisms but also present in fungi are not recorded here.

REFERENCES TO TABLE 15.

1. LEDERER, E. (1938), *Bull. Soc. Chim. Biol.*, **20**, 611.
2. KARRER, P., and RUTSCHMANN, J. (1946), *Helv. Chim. Acta*, **29**, 355.
3. HAXO, F. (1949), *Arch. Biochem.*, **20**, 400.
4. HAXO, F. (1950), *Bot. Gaz.*, **112**, 228.

The report that *R. glutinis*[45] has a vitamin A activity of 5–10 i.u. per gram of dry weight, is consistent with the presence of β-carotene in similar amounts to those reported for *R. torula*.

In the case of *Sporobolomyces roseus* and *Sp. salmonicolor*, Lederer[1,2,3,5] could find no β-carotene, but only torulene and torularhodin. However, in some new *Sporobolomyces* species β-carotene occurs to the extent of 23 μg. per g., the corresponding values for torulene and torularhodin being 92 and 41·7 μg./g.

Apparently in the Rhodotorulaceae the carotenoids are not secreted in the fat globules but are dissolved in the pericapsular fat.[4,6] The qualitative distribution of carotenoids in fungi is set out in Table 16.

TABLE 16.—*The Qualitative Distribution of Carotenoids in Fungi*

PIGMENT	α-carotene	β-carotene	γ-carotene	δ-carotene	ζ-carotene	lycopene	neurosporene	phytofluene	phytoene	torulene	rubixanthin	rhodoviolascin	lycoxanthin (or rhodoplin)	rhodopurpurene	torularhodin	REFERENCE
Aleuria aurantia	+	+														1
Allomyces arbuscula			+													2
Allomyces javanicus		+	+													2
Allomyces macrogyna			+													2
Allomyces moniliformis			+													2
Cantharellus cibarius	+	+	+	+		+										3
*Cantharellus cinnabarinus**		+						+								12
Cantharellus infundibiliformis						+	?									3
Cantharellus lutescens						+										3
Coleosporium senecionis	+		+							+	+					1
Dacromyces stillatus		+	+						+	+						13
Gymnosporangium juniperi-virginianae	+	+	+													14
Lycogola epidendron										+		+				1
Neurospora crassa	+	+	+			+	+	+				+	+	+		4
Phycomyces blakesleeanus	+	+	+		+	+	+	+	+							5, 6, 7, 8, 9
Pilobolus kleinii		+														14
Polystigma rubrum													+			1
Puccinia coronifera	+	+	+												+	1
Rhodotorula rubra	+	+			?		?	+		+					+	1, 10, 11
Rhodotorula sanniei		+								+					+	10
Sporobolomyces roseus		+								+						1
Sporobolomyces salmonicolor		+								+						1
Tremella mesenterica		+														1

* also canthaxanthin

REFERENCES TO TABLE 16.

1. LEDERER, E. (1938), *Bull. Soc. chim. Biol.*, **20**, 611.
2. EMERSON, R., and FOX, D. L. (1940), *Proc. Roy. Soc.*, London, **128B**, 275.
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7. BERNHARD, K., and ALBRECHT, H. (1948), *Helv. chim. Acta*, **31**, 2214.
8. GARTON, G. A., GOODWIN, T. W., and LIJINSKY, W. (1951), *Biochem. J.*, **48**, 154.
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12. HAXO, F. (1950), *Bot. Gaz.*, **112**, 228.
13. GOODWIN, T. W. (1951), *Unpublished work.*
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15. BÜNNING, E. (1937), *Planta*, **26**, 719.

Formation

The possible relationship between chlorophyll and carotenoid formation in the higher plants has been discussed in the previous chapter. This possibility is ruled out in dealing with fungi which are characterized by the absence of chlorophylls.

In *Rhodotorula rubra* a typical pattern of pigmentation was noted which could be divided into three distinct phases: (*a*) a period of active synthesis leading to maximal carotenoid concentration; (*b*) a period of persistence when no changes take place and finally (*c*) a period during which the pigmentation gradually disappears. Pigment formation is stimulated and the onset of the third phase delayed indefinitely when oleic acid is added to the medium; on the other hand, the addition of ammonium sulphoricinoleate inhibits carotenogenesis and accelerates the appearance of the third phase.[46,47]

Garton *et al.*[19,20] have noted the same general pattern in *Phycomyces blakesleeanus* but found that the first phase, that of active

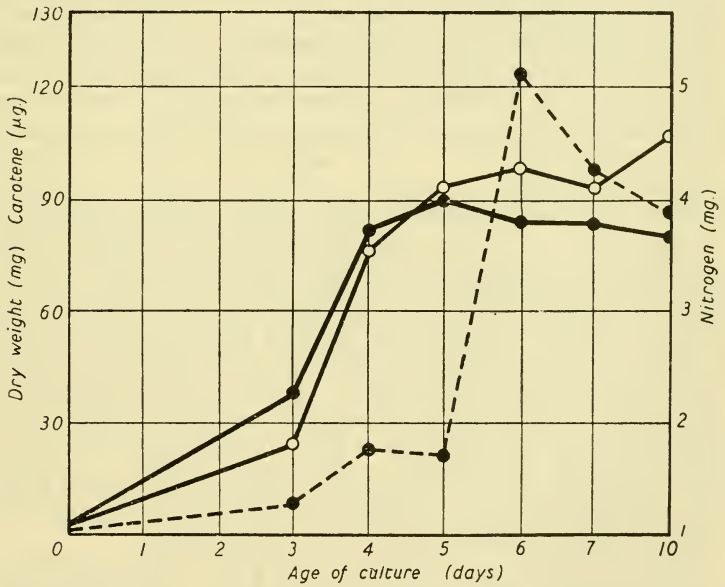


Fig. 18.—The rate of carotene synthesis in *Phycomyces blakesleeanus*, compared with growth (dry wt. production and nitrogen assimilation). (*From Goodwin, T. W., and Willmer, J. S. (1952) Biochem. J.*, in the press.)

●- - - - - - - - ● β-Carotene.
○———————○ Dry wt. production.
●———————● Nitrogen assimilation.

synthesis, itself can be divided into two sub-phases. In the first sub-phase (the first 3–5 days of growth) β-carotene synthesis is very slow compared with general growth and lipid synthesis; during the next 2–3 days, however, the rate of pigment formation is very rapid, this coincides with the cessation of fungal growth.[48] From 8–20 days the amount of pigment present remains constant and thereafter it begins to disappear until in a 36-day old culture, very little carotene remains. This series of events is illustrated in Fig. 18.

Effect of the Carbon Source. Variations in carbon source affect carotenogenesis differently in different fungi. Glycerol, for example, is the most effective single carbon source for pigment production in *Rhodotorula sanniei* although a mixture of lactic acid and glucose is equally effective. Glucose alone does not support pigmentation for, in conjunction with gelatin as a nitrogen source, it will initiate but not maintain pigmentation and a colourless yeast is produced. This form, however, has not lost its ability to synthesize carotenoids for when transferred to an adequate medium it assumes its original colour.[40]

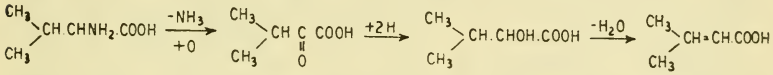
Maltose and glucose are equally effective as carbon sources for carotenogenesis in *Phycomyces blakesleeanus*, but xylose and fructose are considerably less effective although equally good in promoting general growth and lipogenesis. Lactose was ineffective merely because the fungus does not grow on a medium containing this carbohydrate as its sole carbon source.[19,20] In the hands of Garton *et al.*[20] glycerol was inactive for the same reason, but it should be noted that Schopfer[49] found his strain of *Phycomyces* to grow reasonably well on glycerol.

Schopfer and Grob[21] have recently shown that *Phycomyces* grows only slightly and produces no carotene when cultured on a medium containing ammonium lactate as the sole carbon and nitrogen source. If sodium acetate is added to this, medium growth is much improved and some carotene is synthesized. This is taken to indicate that acetate is a primary precursor of carotene. A direct demonstration of the incorporation of acetate into the carotenoid molecule has been obtained by Schopfer and his colleagues[48A] using acetate labelled with C^{14} in either the methyl or the carboxyl group. When the acetate was labelled in the carboxyl group the activity of the carotene was twice that of the carotene when the labelling was in the methyl group.

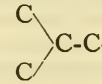
Glover, Goodwin and Lijinsky[48B] using 2-^{14}C-CH_3COOH have recently confirmed that acetate is utilized in the synthesis of carotene; they did not, however, obtain any evidence that the incorporation was in any way specific. The original experiments of Grob, Poretti, von Muralt and Schopfer[48A] can also be interpreted in this way, for the activity of the carotene should be higher when the methyl group

rather than when the carboxyl group of the acetate is labelled, because if acetate alone were required for the synthesis condensations would probably eliminate some carboxyl atoms. Recent experiments have shown that the addition of 2-^{14}C-acetate to *Phycomyces* metabolizing glucose, results in rapid evolution of active CO_2, suggesting randomization of the label between the methyl and carboxyl of acetate.

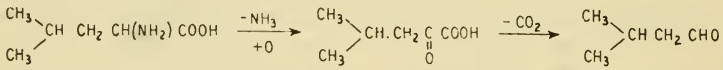
Effect of altering the Nitrogen Source in the Medium. Schopfer[50] reported that glycine and asparagine were equally effective in promoting carotene synthesis in *Phycomyces*, but that ammonium nitrate was better than either. Garton *et al.*[19,20] reinvestigated these compounds in detail and also tested valine, leucine, *iso*leucine and alanine as well as ammonium acetate. In media containing one of these substances (at a level of 0·034 per cent. of nitrogen) and 3 per cent. of glucose, carotenogenesis was essentially the same in all cases except in the medium containing glycine: this amino acid stimulated carotene synthesis, producing mycelia containing 200 mg./100g. dry wt. of carotene compared with the usual level of 120–140 mg./ 100 g. dry wt. It is interesting to note that valine under these conditions which could, theoretically, give rise to β-methylcrotonic acid, a possible repeating unit in carotenoid synthesis (*see* p. 64) in this manner:



was inactive. Leucine, which could also be considered to be able to provide an active residue, was also without effect.

Recent experiments by Goodwin and Lijinsky[51] have shown that in media containing insufficient glucose for optimal carotene production, but sufficient for reasonable mycelial growth, leucine and valine stimulated carotenogenesis by as much as 400 per cent. compared with asparagine or glycine (Fig. 19). Leucine is more effective than valine. It appears from these experiments that normally (on a medium containing adequate glucose, 3 per cent.) *Phycomyces* utilizes the products of glucose fermentation for carotene synthesis (for the pigment first appears in quantity only after the mycelium is fully grown and in the mycelial layer in contact with the medium where the conditions must be nearly completely anaerobic)[52] and that on a low glucose medium (1 per cent.), these being in short supply, the fungus utilizes the deaminated products of leucine and valine as the building unit. This repeating unit must thus have a 5-carbon skeleton with the following configuration:—  and it is easy to visualize how this can

be supplied by valine, which has this structure. Leucine is a 6-C compound and must lose one carbon atom, probably by decarboxylation ; this would yield after deamination, *iso*valeraldehyde, and it is interesting to recall in this connection the classical work of Ehrlich in 1911 (quoted by Foster)[53] which demonstrated that leucine is the specific precursor of the *iso*valeraldehyde which occurs in the fusel oil produced during yeast fermentation. The mechanism is as follows :



A similar type of decarboxylation also occurs during leucine metaboliam in animal tissues.[54] At the time of writing, the results of preliminary experiments[48B] have indicated that these postulated intermediates may stimulate carotogenesis.

Although these experiments indicate that carotene is probably synthesised *via* a 5-carbon unit, the main features of the intermediary steps in its biogenesis remain to be solved. Perhaps the two most important problems needing solution before very much progress can be made are these : (1) Do four 5-carbon units combine " head to tail " to form a C_{20} compound which then dimerises " tail to tail ", or do two 5-carbon units first react " tail to tail " and the molecule is then built up by " head to tail " condensation at either end of this 10-carbon unit : (2) Is the 5-carbon unit saturated (? isovaleraldehyde) or unsaturated (? β-methylcrotonaldehyde).

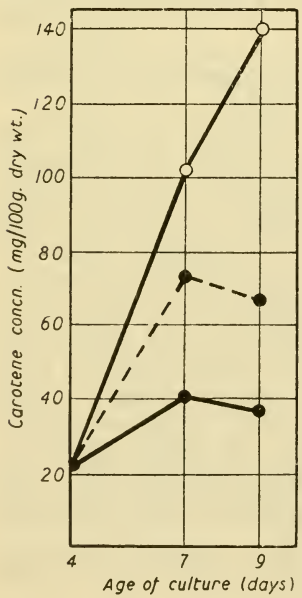


Fig. 19.—The % of β-carotene produced by *Phycomyces blakesleeanus* transferred to a medium containing 1% glucose and either *L*-valine, *L*-leucine, or *L*-asparagine as nitrogen source. (*From Goodwin, T. W., and Lijinsky, W.(1951) Biochem, J.*, **50**, 268.)
leucine.
valine.
asparagine.

If a well-formed mycelial mat of *Phycomyces* is placed on a medium containing only leucine or valine but no glucose, little or no carotene synthesis takes place :[52] this is taken to mean that the energy required for absorption of the amino acids into the cells and for condensation and/or dehydrogenation of the repeating units must come from the dissimilation of glucose.

Méry[55] describes qualitative experiments which suggest that tyrosine stimulates considerably both lipogenesis and carotenogenesis in red yeasts.

Significance of the Carbon : Nitrogen Ratio in Carotenogenesis. Schopfer's pioneer experiments on carotene production in *Mucor hiemalis* and *Phycomyces blakesleeanus* indicated that the carbon/nitrogen ratio (C/N) is an important factor controlling the extent of carotenogenesis, the higher the ratio the more carotene produced.[24,50] Similar suggestions have been put forward to explain the quantitative aspects of lipogenesis in micro-organisms (*see* Kleinzeller[56] for a review). Garton *et al.*[19,20] found that the C/N ration can often assume a spurious significance when applied to carotenogenesis. Provided enough nitrogen is available to allow maximal mycelial growth, the controlling factor is not the C/N ratio but the amount of assimilable carbon available after growth has finished. An example will make this clear : the concentration of carotene in a mycelium containing sufficient nitrogen for maximal growth (0·2 per cent. asparagine) and just sufficient carbon (1·5 per cent. glucose), is 20–30 mg./100 g. (dry wt.). When the asparagine concentration is kept constant and the glucose concentration increased to 2·5 per cent. the carotene concentration increases to 120–150 mg./100 g. If, however, the glucose concentration is kept at 2·5 per cent. increasing the nitrogen concentration to 1·0 per cent (*i.e.*, decreasing the C/N ratio) does not reduce the carotene concentration, which remains at 120–150 mg./100 g.

As previously stated Goodwin and Willmer[48] have found that carotenogenesis only proceeds rapidly when the mycelial mat is fully formed, *i.e.*, when nitrogen assimilation has stopped. It was at first found difficult to reconcile these results with the previous results of Garton *et al.*,[19,20] who noted that when fully grown mats were transferred to a medium containing glucose but no nitrogen no carotene synthesis occurred ; from these experiments it was tentatively concluded that the fungus had to be metabolizing exogenous nitrogen in order to synthesize carotene. It has now been shown that the failure to produce carotene in the absence of nitrogen in the original experiments was due to the fact that the medium used was buffered at pH 7. Using non-buffered media fully grown mats will synthesize carotene

when transferred to a medium containing glucose but no nitrogen, the concentration of carotene in mycelia growing on a N-free medium being greater than that in mycelia on a medium containing nitrogen.

Effect of Light. Deventer (quoted by Zechmeister)[57] found that light was necessary for carotene formation by *Neurospora sitophilla.* Haxo,[27] on the other hand, found that light was not essential for carotenoid synthesis in *N. crassa* but that it did stimulate synthesis considerably, the spectral region between 520 and 580 mμ. being the most effective. Schopfer[24] stated that blue light was necessary for carotene synthesis by *Phycomyces blakesleeanus*, for none was produced in the dark or in red light. Garton *et al.*[19,20] could not reproduce Schopfer's original observations, and the results of many experiments indicated that *Phycomyces* cultured in the dark always produced about one half as much carotene as did cultures grown in the light. Further, the wavelength of the light used appeared to be of little importance; as long as some light was falling on the cultures full carotene production occurred. Recently Schopfer has informed us that his original analytical methods were such that small amounts of carotene in his dark cultures may have gone unobserved.[58] Furthermore, as he points out there is always the possibility that the *Phycomyces* of 1951 does not behave in the same way as the strain of 1934.

Although stimulating the production of coloured carotenoids, light did not effect the synthesis of phytofluene in *Neurospora crassa*;[27] in *Phycomyces* on the other hand, synthesis of all the carotenoid components appears to be equally affected by light.[22]

It has been reported that the pigments of *Microcerca coccophila* (Hypocreacea), which is very probably a carotenoid, is produced in the conidia in light and darkness, but in the mycelia only in the light.[59]

Effect of Temperature, Oxygen Tension, pH, and other Factors. According to Fromageot and Tschang[40] pigmentation in *R. sanniei* remains qualitatively the same within the temperature limits 14–28° and the pH limits 5·2–7·6. Luteraan and Dieng[60] claim that pigment (? carotenoid, *see* below) formation in *Saccharomyces cerevisiae* depends on the O_2 tension of the enviroment and Méry[55] noticed a similar effect in *R. gracilis.*

It has been stated by Luteraan and Dieng[60] that exposure of *Rhodotorula* spp. to the vapours of camphor, terpineol or menthol results in the decolouration of the yeast within 24–48 hours. If these cultures are then washed free from the terpenes and transferred to a new medium, rapid and abnormal pigmentation and growth take place. *Saccharomyces cerevisiae*, which normally does not produce carotenoids takes on a red tinge when treated in this way; it has been assumed

that this coloration is due to the production of a carotenoid but no proof of this has been supplied. Addition of steroids has no effect on pigmentation in either *Rhodotorula* or *Saccharomyces*, but oxygenation stimulates pigment production in the latter.

Luteraan and Choay[47] report that when an unspecified *Penicillium* is cultured on a medium completely free from aneurin, it produces a considerable amount of carotenoids in place of the usual non-carotenoid pigment. Full details of this investigation are not yet available.

Goodwin and Willmer[48] have shown that *Phycomyces* produces very much less carotene on a medium buffered at pH 7 than on media held at lower pH values.

TABLE 17

The effect of Diphenylamine (1/30,000) *on the Carotenoid Production by* Phycomyces blakesleeanus.

Carotenoid	Standard Medium (without DPA added)		Standard Medium (with DPA added)	
	Amount per flask (μg.)	% of Total	Amount per flask (μg.)	% of Total
Phytoene	78	11·1	—*	—*
Phytofluene	15	2·1	80	50·3
α-Carotene	5	0·7	nil	0
β-Carotene	586	83·7	25	15·7
ζ-Carotene	17 (5)	2·4	47	29·6
γ-Carotene	17 (3)	2·4	nil	29·6
Neurosporene	17 (4)	2·4	7	4·4
Lycopene	17 (5)	2·4	nil	4·4
TOTAL	701		159	

* The exact increase in phytoene is difficult to measure in presence of DPA.

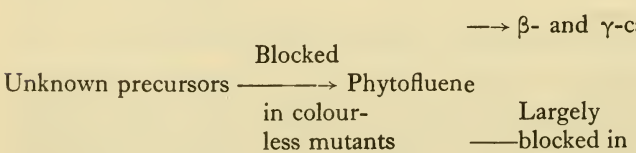
Inhibition Studies. Turian,[61] developing the original qualitative observations of Kharasch, Conway, and Bloom[62] that chromogenesis in many bacteria and fungi is inhibited in the presence of diphenylamine, recently showed that this substance, at a concentration of about

1/20,000–1/35,000, inhibits carotenoid production in the bacterium *Mycobact. phlei* (*see* p. 126). Goodwin [22] has investigated the effect of diphenylamine on carotenogenesis in *Phycomyces* and found that whilst the production of α-, β- and γ-carotenes and lycopene were almost completely inhibited, that of the more saturated carotenoids, phytofluene, phytoene, ζ-carotene and neurosporene is considerably increased (*see* Table 17). These observations suggest two possibilities (*a*) that β-carotene and lycopene may be built up *via* saturated intermediates, the inhibition by diphenlyamine occurring thus :

The second possibility (*b*) is that the more saturated carotenoids are produced by an independent pathway from the same common precursor as the α-, β- and γ-carotenes and lycopene ; blocking of the latter synthesis would then leave more of the common precursor for conversion into phytoene, etc. Much further work is required before a decision can be made as to which possibility is the correct one.

The mode of action of diphenylamine remains obscure ; an investigation into a large number of compounds known to exhibit various types of inhibition has not revealed any with the same action on *Phycomyces* as diphenylamine. [62A] The recent observation of Turian [62B] that in *Mycobact. phlei*, phenol has the same action as diphenylamine, does not hold with *Phycomyces* ; neither does his finding that dinitrophenol enhances carotenogenesis (*see also* p. 126). Streptomycin inhibits carotene synthesis in *Phycomyces*. [62A].

Mutant Studies. Only one investigation on carotenogenesis in fungi has been reported in which mutant strains were examined. Bonner *et al.* [42] used 7 ultra-violet mutants of *Rhodotorula rubra* ; the quantitative distribution of carotenoids in these mutants are recorded in Table 18. These results prompted Bonner *et al.* to propose the following scheme of carotenoid synthesis in *Rhodotorula* :



It is obvious, as Bonner *et al.* fully appreciated, that is only one of a number of possible interpretations of their results.

After $2\frac{1}{2}$ years of monthly transfers of these mutants on agar, their phytofluene content had markedly diminished whilst that of the other carotenoids had not altered.[63]

TABLE 18

Quantitative distribution of Carotenoids in various ultra-violet mutants of Torula rubra (from Bonner, J., Sandoval, A., Tang, Y. W., and Zechmeister, L. (1946) *Arch. Biochem.*, **10**, 113)

Mutant No.	Colour	Amount of carotenoid (mg./100 g. dried yeast)					
		Torulene	Neurosporene	γ-carotene	ζ-carotene	β-carotene	Phytofluene
original	red	5·2	0·40	0·74	nil	0·76	0·63
original	red	5·2	0·16	0·55	nil	0·71	0·75
VII	red	6·4	0·44	2·1	nil	1·3	1·1
IV	red	6·6	0·56	3·1	nil	2·4	1·2
VI	red	6·0	0·51	2·2	nil	2·0	·3
II	brownish-orange	0·3	0·88	2·9	0·60	0·95	0·75
I	pale-orange	0·16	0·22	0·8	nil	0·64	1·0
III	Colourless	0·02	nil	nil	nil	nil	nil
V	Colourless	nil	nil	nil	nil	nil	nil

FUNCTION

(*a*) *In photokinetic responses.* Most work which has been carried out on the function of carotenoids in fungi points to their taking part in photokinetic responses.[64]

As in the case of the oat seedling (*see* p. 88) Blaauw[65] made the pioneer measurements of the action spectrum of phototropic bending of a fungus, *Phycomyces nitens*. Later, Castle[14] found that action spectrum of *P. blakesleeanus* had a maximum at about 440 mμ., and this has been confirmed.[66,67] By measuring photoelectrically the difference in light absorption between pigmented and colourless zones, Bünning[68] obtained an absorption curve very similar to the action spectrum, with

two maxima at 445 mμ. and 485 mμ. ; further, the pigment extracted from the coloured cells proved to be β-carotene.

Although the investigations just discussed are a good indication that in fungi, as well as in higher plants, carotenoids may function by mediating in photo-kinetic responses, the implications of Galston's work must not be overlooked. It has already been stated that Galston[69,70] showed that action spectra measurements are not sufficiently precise to distinguish between riboflavin and a carotenoid as the mediator in the phototropic response of the higher plants (*see* p. 88); as 96 per cent. of the auxin activity of *Phycomyces* is due to indole acetic acid,[71] it is possible that riboflavin is the mediator in this fungus for it can sensitize the photodestruction of indole acetic acid.[72] Further, *Phycomyces blakesleeanus* grown in a medium containing diphenylamine and thus containing only about 1–2 per cent. of its usual amount of carotene, is strongly phototropic ;[73] this amount of carotene may, of course, be sufficient for phototropic action.

Against the suggestion that riboflavin is the mediator is the fact that auxin (both indole acetic acid and auxin a) is not necessary for the growth of *Phycomyces*.[74] and also no evidence has been yet presented to indicate that riboflavin occurs in the photosensitive regions of the fungus.

(*b*) *In reproduction.* The work of Emerson and Fox,[25] in which the preferential accumulation of γ-carotene in the male gametes of *Allomyces* was described, strongly suggests a function in the sexual processes. Up to the present, however, no specific function in reproduction has been demonstrated.

BACTERIA*

A large number of bacteria have been examined for carotenoids but only a fraction of the pigments have been completely identified. However, the following general conclusions can reasonably be drawn :

(i) As is the case of fungi, β-carotene is by no means ubiquitous ;

(ii) Lutein (xanthophyll) is conspicuous by its absence. It has been reported only in *Mycobacterium phlei*[75] (this is disputed)[76] and *Micrococcus tetragenus*.[77]

(iii) Many carotenogenic bacteria are characterized by specific carotenoids, mostly xanthophylls. These xanthophylls sometimes occur in the form of methyl ethers (*cf.* torulene) ;

* The nomenclature used in this section is that of Bergey—Manual of Determinative Bacteriology, 6th Ed. (1948, London, Ballière, Tindall, and Cox.)

(iv) Most modern investigations have failed to reveal the presence of acidic carotenoids in the majority of bacteria examined ; this is in contrast with the situation obtaining in the carotenoid-containing fungi ;

(v) Chargaff and Lederer's claim[78] that γ-carotene is probably the typical bacterial carotene cannot, in the light of modern investigations, be upheld (*see* Table 20).

It was previously thought that anaerobes do not synthesize carotenoids[79,80] but, as will be discussed later, *Rhodospirillum rubrum* has more recently been found to produce considerable amounts of carotenoids anaerobically.

Schizomycetes

Coccaceae. No β-carotene has been detected in *Sarcina lutea* in which the specific carotene *sarcinene* was first detected ;[81] also occurring in this organism is a xanthophyll with spectral properties very similar to those of sarcinene ; it has been called sarcinaxanthin.[82,83,84] Takeda and Ohta[84] obtained 3·4 mg. of sarcinaxanthin from 385 g. of dried organisms ; its behaviour in the partition test indicates that it contained only one hydroxyl group. *S. aurantiaca* probably contains β-carotene[80,85] and *lycopene.*[86]

Sobin and Stahly[85] have detected spectrographically two new xanthophylls in *S. lutea*, and one in *S. aurantiaca* which may also contain zeaxanthin.[81] *S. flavia* contains one, and *Micrococcus luteus* both the xanthophylls elaborated by *S. lutea.* According to Nakamura[84] *S. lutea* contains a xanthophyllic (? sarcinaxanthin) ester. δ-carotene and *rubixanthin* were detected in every strain of *Staphylococcus aureus* examined by Sobin and Stahly,[85] but they could not detect zeaxanthin which had previously been reported in *Staph. aureus.*[81] γ-carotene, lycopene, rubixanthin, lutein (xanthophyll) and rhodoxanthin are stated to be present in *Micrococcus tetragenus.* The type of pigment varies according to the type of culture. The yellow type contains lutein, the mucoid-pink type lycopene, and the pink type rhodoxanthin ; the pink-yellow type and brown type, on the other hand, appear to contain γ-carotene and rubixanthin.[77]

Bacteriaceae

In 1893 Lankester[87] found a red pigment which he named *bacteriopurpurin* in *Bact. rubescens.* Molisch[88] reinvestigated this pigment and found that it had two components, α- and β-bacteriopurpurin. *Flavobacterium arborescens* contains five pigments, one of which is probably

sarcinene (*see* p. 119), and one probably α-bacteriopurpurin. [85] Petter [59] claimed that α- and β-bacteriopurpurin occurred in *B. halobium*, but Lederer [12] found only one form (α-) in his cultures of the same organism. Bacteriopurpurin is probably demethylated rhodoviolascin, a pigment to be discussed shortly (*see* p. 121). *B. mycoides* contains a carotenoid identical with rhodopin [85] (*see* p. 121). *F. esteroaromaticum*, *F. suaveoleus*, and *F. fecale* all contain one xanthophyllic carotenoid, this is identical in the three organisms and is probably unique. Similarly *F. sulphureum* contains a specific carotenoid, this time a hydrocarbon ; recent work, however, suggests that it may be either neurosporene or ζ-carotene (*see* p. 26). *Cellulomonas flavigena* produces two xanthophylls probably identical with those elaborated by *S. lutea*. [85]

Enterobacteriaceae

Two members of this family have been examined ; spectroscopic measurements indicate that *Erwinia lathyri* and *E. ananas* contain single and distinct carotenoids. [85]

Bacillaceae

β-carotene and *γ-carotene* have been detected in *B. lombardo-pellegrini* and *B. grasberger* ; the latter organism also probably contains *lycopene*. [80]

Actinomycetales

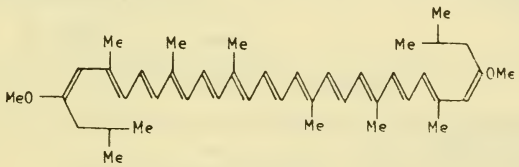
Mycobacteriaceae. In this group, apart from *leprotene*, a dehydro-β-carotene devoid of vitamin A activity [90-92] isolated from *Mycobact. phlei* and *M. leprae*, no specific carotenoids had been reported up to 1950. Very recently, however, Turian [93,94] has obtained an acidic carotenoid from *Mycobact. phlei* which has properties similar to astaxanthin but with an absorption band with a maximum at a much lower wavelength than astaxanthin (*see* Table 19). Turian considers that this is the pigment that Ingraham and Steenbock [75] in their early investigation took to be a quinone similar to phthiocol ; he proposes the name chrysophlein for this new pigment. Goodwin [95] in a preliminary investigation could find no chrysophlein in his strain of *Mycobact. phlei*, but did observe the presence of a pigment which had properties somewhat similar to that of chrysophlein in a culture kindly supplied by Turian. Goodwin also found phytofluene in *Mycobact. phlei*. [95] β-carotene has been detected in *Corynebact. carotenii* [75] and *M. lacticola* [95] and β-carotene and γ-carotene in *M. phlei*. [78,80,89] Xanthophylls are rare but *M. phlei* may contain *lutein* (xanthophyll),

zeaxanthin, and *cryptoxanthin*;[78,95] *M. lacticola*[96] produces an acidic carotenoid (probably astaxanthin) or a neutral xanthyphyllic pigment according to the constituents of the culture medium. Recently a wartime Japanese report has become available which indicates that the soyama B strain of *M. tuberculosis* grown on Santon's synthetic medium contains either β-carotene of leprotene, probably the latter.[97] Lederer[97A] has recently found leprotene in *Mycobact. bruynoghe* and *adant.*

THIOBACTERIALES

Rhodobacteriaceae. The first modern investigation of the carotenoids of the sulphur-containing bacteria was carried out by Lévy, Teissier and Wurmser[98] in 1925; they obtained from *Chromatium okenii* a carotenoid identical with *α-bacteriopurpurin.* It occurred in the bacterium as a brown chromoprotein soluble in dilute NaCl solution. These workers also showed that the *bacterioerythrin* of Archichovsky[99] is in all probability α-bacteriopurpurin. Karrer and his colleagues[100-102] investigated *Rhodovibrio*, *Rhodobacillus* and *Thiocystis* and described five new pigments: *flavorhodene*, *rhodopurpurene*, *rhodoviolascin*, *rhodopin*, and *rhodovibrin*; β-carotene is the only common carotenoid present in these organisms and even so it is only a very minor constituent.

Rhodoviolascin ($C_{42}H_{60}O_2$) probably has the following structure:



Rhodoviolascin

Rhodopin ($C_{40}H_{58}O$) is a monohydroxycarotenoid of undetermined structure. Rhodovibrin contains two oxygen atoms, but only one hydroxyl group, and is methoxyl free. Rhodopurpurene ($C_{40}H_{56\text{ or }58}$) and flavorhodene are hydrocarbons of indefinite structure, the former may be identical with lycopene and the latter with either ε-carotene or neurosporene, more probably owing to its adsorption properties, with the former. (*see* p. 28) Fig. 20 gives the absorption spectra of some of these pigments.

ATHIOBACTERIALES

Van Niel and Smith[103] isolated a carotenoid which they called *spirilloxanthin* from *Rhodospirillum rubrum.* More extended investigations of this pigment have led Polgár, van Niel and Zechmeister[104]

to conclude that there is "good reason for admitting" that spirilloxanthin is identical with Karrer's rhodoviolascin. Data on the properties of bacterial carotenoids and their qualitative distribution are recorded in Tables 19, 20.

TABLE 19.—*Properties of Bacterial Carotenoids*

NAME	m.p.	Absorption maxima in mμ.		
		light petroleum	Carbon disulphide	Chloroform
*Sarcinene[1]	—	415, 440, 469		
†Sarcinaxanthin[2]	149–150°	415, 440, 469	464, 494	423, 451, 480
†Xanthophyll[3] from *Sarcina lutea*		— — —	466 499	451, 480
‡Flavorhodene[4, 5]	111–113°	442, 470	472, 503	453, 482
§Rhodopurpurene[4, 5]	162°	472, 502	479, 511, 550	458, 487, 527
Rhodopin[4, 5]	171°	470, 470, 501	478, 508, 547	453, 486, 521
Rhodovibrin[4, 5]	168°		517, 556	
Rhodoviolascin[4, 5] (=Spirilloxanthin)	218°		496, 530, 473.5	476, 507, 544
*†α-Bacteriopurpurin[6]	—	460, 495, 528 (in methanol)	498, 532, 571	
††β-Bacteriopurpurin[7]	—	452, 482, 502 (in methanol)		
Leprotene[8]	198–200°	425, 452, 484	477, 499, 517	428, 460, 495
Xanthophyll from *Flavobact. esteroaromaticum, F. suaveolens,* & *F. faecale.*[3]	—		453, 482, 513	460, 513
*Carotene from *F. sulphureum*[3]	—		437, 466, 437	451, 481
Xanthophyll from *Erwinia laythri*[2]	—		478, 513	458, 485
Xanthophyll from *E. ananas*[2]	—		474, 508	460, 493
Chrysophlein[9, 10]	—	452	487	—

Heavy figures relate to references below.

* The probable identity of these with neurosporene cannot be ignored.
† These may be identical.
‡ May be identical with ε-carotene.
§ May be identical with lycopene.
*† α-Bacteriopurpurin is probably one of Karrer's rhodo-carotenoids.
†† β-Bacteriopurpurin is probably identical with rhodoviolascin.

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Volk and Pennington[105] detected β-carotene, rhodopin, rhodovibrin and probably rhodoviolascin (spirilloxanthin) in *Rhodomicrobium vannielii*; *cis*-isomers of rhodopin and rhodovibrin are also reported, but complete evidence of their identification is still lacking.

According to Mahdihassan[111] symbiotic carotenoid production occurs in *Cicadella viridis*; two types of bacteria occur, one type producing sarcinene and the other β-carotene.

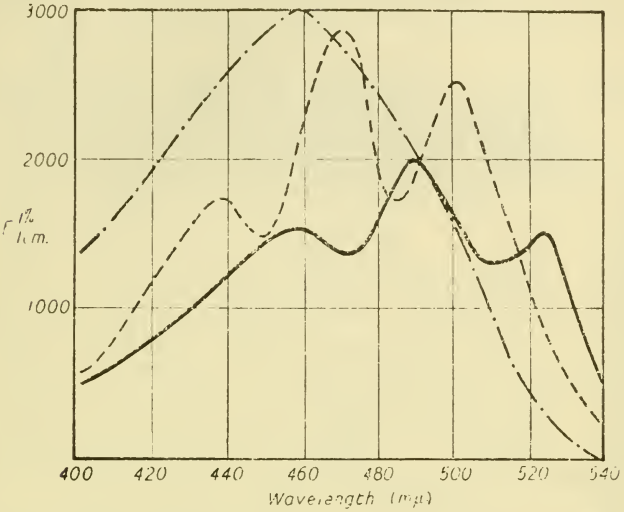

Fig. 20.—The absorption spectra of
(*a*) rhodopin in ethanol. - - - - - - - -
(*b*) rhodoviolascin in hexane. ————
(c) chrysophlein in ethanol. - — - — - — -
(*a*) and (*b*) *redrawn from Karrer, P., and Solmssen, U.* (*1943*) Helv. chim. Acta, 26, 118.
(c) *redrawn from Turian, G.* (*1950*) Helv. chim. Acta, **133**, 1303
Note.—The $E_{1\,cm.}^{1\%}$ scale does not apply to chrysophlein, which has not yet been obtained crystalline.

FORMATION IN BACTERIA

(i) *Carbon and Nitrogen sources in media*

Ingraham, Fred and Steenbock[107] found that glycerol was the best carbon source for carotenoid production in bacteria; this is in agreement with the finding on fungi (*see* p. 110). Studying *Mycobact. phlei* in detail, Ingraham and Steenbock[75] demonstrated that this bacterium utilizes *iso*propanol and ethylene glycol almost as well as it does glycerol. Variations in the nitrogen source, by replacing asparagine by ammonia, urea, or peptone had no effect on pigment production providing the pH of the medium was controlled. *Flavobact. arborescens* produced more carotenoids on a liquid medium containing glycerol or glucose than on an agar medium. Haas and

TABLE 20.—*The qualitative distribution of Carotenoids in Bacteria*

NAME	α-carotene	β-carotene	γ-carotene	δ-carotene	Lycopene	Corralin	Leprotene	Rhodopin	Capsanthin	Astaxanthin	Cryptoxanthin	Lutein	Rhodoviolascin	Rhodopurpurin	Rhodovibrin	Flavorhodene	Zeaxanthin	Sarcinene	Sarcinoxanthin	Rubixanthin	Rhodoxanthin	α-bacteriopurpurin	β-bacteriopurpurin	chrysophlein	REFERENCE
Bacillus grasberger		+	+	+	+				?																1
Bacillus lombardo-pellegrini ..		+	+																						1
Bacterium halobium ..																						+	?		30, 31
Bacterium mycoides ..								+																	26
Bacterium rubescens ..																						+	+		28, 29
Chromatium okenii ..																							+		33
Corynebacterium spp.		+																							2
Corynebact. carotenii		+																							3, 4, 5
Flavobacterium arborescens																		+				+			36
Micrococcus erythromyxa										?															6
Micrococcus rhodochrous										+															6
Micrococcus tetragenus			+		+							+								+	+				27
Mycobacterium bruynoghe and adant.							+																		36
Mycobacterium lacticola ..		+								+															7
Mycobacterium leprae ..							+																		8, 9, 10
Mycobacterium phlei * ..	+	+	+				+				+	+												+	32, 37
Mycobact. tuberculosis		?					?																		35
Rhodomicrobium vannielii ..		+											?	+	+										34
Rhodovbrio spp. ..		?						+					+	+	+	+									14, 15, 16
Rhodospirillum rubrum † ..													+												20, 21, 22
Sarcina aurantiaca		+			?												+								17, 18
Sarcina lutea ..																		+	+						11, 18, 19,
Staphylococcus pyrogenes aureus ..				+													+			+					6, 11, 26
Strepotothrix corallinus						+																			15
Thiocystis spp. ..	+	+			+			+					+	+	+	+									23, 24, 25

* also phytofluene (37). † no phytofluene (37).

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Bushnell[96] found more deep-seated changes when the constitution of the medium was altered. On nutrient agar, carotenes and neutral xanthophylls were produced by *Mycobact. lacticola*, whilst on mineral oil media, carotenes and astaxanthin, but no neutral xanthophylls, appeared. The absence of neutral xanthophylls in the second case suggests that astaxanthin is formed directly from carotenes, in the same way as ketones or polyketones are the primary oxidation products of synthetic paraffins on which *Aspergillus versicolor* is growing.[108] Cultures or other species of *Mycobacterium* (*M. phlei*, *M. leprae*, and *M. smegmatis*) produced pigment when grown on agar, but none when cultured on hydrocarbon oils.[96] Addition of olive oil to the medium increased the carotenoid production of *Mycobact. phlei* and the vapours of pine oil decreased it [109], whilst the presence of β-carotene and lutein (egg yolk pigment) inhibited growth but stimulated carotenogenesis.

(ii) *Mineral Constituents*

Ingraham and Steenbock's[76] investigations indicated that the

amount of pigment produced by *Mycobact. phlei* is conditioned by the mineral constituents of the medium. On a synthetic glucose-asparagine medium production is stimulated by the addition of Fe $\cdot\cdot\cdot$ and reduced by K $\cdot$ or PO_4'''. When glucose is replaced by glycerol the situation is altered; K $\cdot$ has no effect, whilst PO_4''', Fe $\cdot\cdot\cdot$ and Cu $\cdot\cdot$. all decrease pigment formation. Turian[110] has recently broadly confirmed Ingraham and Steenbock's observations, but has separated chromogenesis (carotenogenesis) from plasmogenesis; using Ingraham and Steenbock's basal medium, he found that Fe $\cdot\cdot\cdot$ stimulated both reactions but Mn $\cdot\cdot$ specifically stimulated carotenogenesis. It is considered that the redox properties of these two metal ions are concerned in their action.

(iii) *Inhibition Studies*

Turian[61] has recently shown that diphenylamine added to the culture medium at levels of 1/20,000–1/35,000, inhibits carotenoid synthesis without inhibiting growth; this inhibition is reversed by Fe $\cdot\cdot\cdot$ (0·1 mg./100 g.) but not by other ions. The effect of Fe $\cdot\cdot\cdot$ may be of biological importance (as Turian is inclined to believe) or it may be due to the fact that diphenylamine is rapidly oxidized in the presence of Fe $\cdot\cdot\cdot$. Diphenylamine is considered to act by inhibiting oxidation (dehydrogenation) of the colourless carotenoids (phytofluene, etc.) which may be precursors of the "true" carotenoids As stated on p. 115, Goodwin[22] has shown that the colourless carotenoids do accumulate and the coloured carotenoids disappear when the fungus *Phycomyces blakesleeanus* is grown on a medium containing diphenylamine, but emphasized that this does not prove that phytofluene and phytoene are precursors of β-carotene, etc.

Recently Turian[62B] has shown that phenol (1/2,000–1/5,000) also retards carotenogenesis in *Mycobact. phlei*; resorcinol is about 2–3 times less active whilst α-naphthylamine, thiourea, KCN, salicylaldoxime are almost inactive. Dinitrophenol (10^{-6}), on the other hand, stimulates the production of carotenoids.

The carotene formation in *Rhodobacillus palustris* is independent of the iron content of the medium.[110]

(iv) *Effect of Light, Temperature, etc.*

The physical conditions affecting growth of *Sarcina aurantiaca* have been investigated by Reader.[65] This organism grew better at 20° than at 37°, and although the yield of carotenoids was higher at 20°, the concentrations were identical at both temperatures. The pH limits

for growth were 5·30–9·43 with maximum production occurring at pH 7·15. There was no difference in pigmentation of cultures grown either in the dark or diffuse daylight. Bright light bleached coloured cultures but the ability to produce pigment was not lost because pigmentation was normal when subcultures of these bleached colonies were transferred to the dark. Ingraham and Steenbock[77] found that light had no appreciable effect on carotenoid formation in *Mycobact. phlei*, and that carotenoids were produced by the *Mycobact. phlei* when cultured at room temperature but not when the temperature was raised to 37°. O_2 reduces whilst light stimulates carotenogenesis in *R. rubrum*.[95]

(v) *General*

Ingraham and Baumann[77] have stated that storage and utilization of carotenoids and lipids run parallel in *Mycobact. phlei*.

Recently a group of American workers[114] have prepared a saline extract of a chromogenic strain of *Staph. aureus* which stimulates pigment (carotenoid) production in non-chromogenic strains of the same bacterium. This extract does not give the colour reactions of proteins but does give a slight positive test for pentose.

In two paratubercle bacilli, *B. lombardo-pellegrini* and *B. boquet*, the degree of pigmentation (presumably mainly due to carotenoids, (*see* p. 124) is directly proportional to the aneurin content of the medium.[112A] It should perhaps be noted that in these organisms aneurin can be replaced by its pyrimidine moiety (2-methyl-4-amino-5-aminomethylpyrimidine) for they have the ability to synthesize the thiozole part of aneurin.

In spite of all the work reported here it is obvious that the mechanism of carotenoid formation in bacteria is just as obscure as in other organisms, but Karrer *et al.*[55] have suggested that formation from asparagine and malic acid involves the conversion of these substrates into β-methylcrotonaldehyde, which is a possible precursor of carotenoids (*see* p. 64). No experimental support for this suggestion has yet been forthcoming.

FUNCTION OF CAROTENOIDS IN BACTERIA

Very little work has been carried out in an effort to define the function of bacterial carotenoids. A possible role in photosynthesis appears to have been ruled out in the case of the purple bacteria[113] including *Spirillum rubrum*,[114] although French[115] has isolated, from a number of photosynthetic bacteria, a protein complex *photosynthin*, which

contains both chlorophyll and carotenoids, and Sapozhinkov[116] claims that during photosynthetic reduction in the purple sulphur-containing bacteria, carotene is converted into " xanthophyll " (but *see* p. 121). Phototactic responses may, however, be mediated through carotenoids in *R. rubrum*, according to a suggestion of French.*[115] Recent work by Manten[117] and Thomas[118] has shown that this suggestion is to a certain extent true, although Manten states that bacteriochlorophyll plays an important part in this response. Thomas has also found that the wavelengths of maximal efficiency for photosynthesis are the same as those for maximal phototaxis, *viz.* 590, 525, 490, and 460 mμ; whether this by implication indicates that carotenoids do play a part in bacterial photosynthesis remains obscure.

Carotenoids may protect *Sarcina lutea* and *S. aurantiaca* against the adverse effects of ultra violet rays.[118A]

Exogenous carotene may be important both as a growth inhibitor and a growth promoter according to contradictory claims put forward by Darzins[109] and Vasileva[119] respectively. The former found that β-carotene inhibits the *in vitro* growth of the paratubercle bacillus and the latter claimed that it stimulated the growth of abdominal typhus bacteria. Darzins also noted an increase in the acid fastness of the tubercle baculli grown on carotene containing media. This was due to the elevation of the melting points of their constituent fats. Lutz[112A] has also considered the carotenoid content of *Mycobact. Phlei* strains in relation to their acid-fastness.

Luteraan, Champean and Choay[120] have speculated on the possible role of carotenoids in the respiration of micro-organisms and recent American work has shown that carotenoids can replace sodium acetate in the nutrition of lactic acid bacteria.[121]

Haas, Yenzi and Bushnell[112] claim that the failure to deplete human subjects of vitamin A was due to intestinal synthesis of carotenoids. In a comprehensive investigation with which the author was concerned[113] this claim could not be substantiated; bacterial cultures from faeces of humans on a vitamin A deficient diet produced small amounts of a very unstable pigment soluble in light petroleum but which was not β-carotene.

ALGAE

The carotenoids of the algae are not only interesting in themselves, but also because of the rôle they play as precursors of vitamin A in marine animals. Large numbers of workers have been interested

* This has recently been demonstrated.[208]

in algal carotenoids but apart from the pioneer work of Kylin[124] a considerable amount of the detailed knowledge has come from Heilbron's laboratories. Comprehensive reviews of algal pigments have been published by Heilbron himself[125] and his colleague A. H. Cook.[126] These have been of considerable value in compiling this section. From the point of view of this book it is considered better to discuss the carotenoids according to the general classification described by Fritsch,[127] rather than to discuss fresh water and marine algae separately. Little is gained by adopting the latter procedure.

Chlorophyceae

The green algae resemble biochemically the green leaves of higher plants much more closely than do any other class of algae. Carter, Heilbron and Lythgoe[128] found β-*carotene* and *lutein* (xanthophyll) to be the predominating carotenoids. Kylin[129] found the same distribution in *Enteromorpha intestinalis*, *E. compressa* and *Cladophora rupestris*, and *Oedogonium* spp.[124,129]; small amounts of α-*carotene* were also occasionally encountered. The same distribution also exists in *Cladophora glomerata*[130]. The xanthophylls accompanying lutein (xanthophyll) are probably not so varied as those associated with lutein in the higher plants, but *violaxanthin* has been found in *E. intestinalis*, *C. rupestris*,[129] *C. glomerata*[130] and *Vaucheria hamata*;[131] *zeaxanthin* in *E. intestinalis* and *C. rupestris*[129] and *taraxanthin* in *Cladophora sauteri*, *Nitella opaca*, and *Oedogonium* species.[131] Luetin-5 : 6-epoxide occurs in *Cladophora glomerata*.[130]

A number of Chlorophyceae depart from this general picture. *Trentepohlia aurea* yields a single pigment only, first noted as *haematochrome* by Cohn[132] in 1861, which is now known to be β-carotene ;[125,133] this alga is one of the richest sources of β-carotene known for it comprises 0·2 per cent. of the dry weight as compared with about 0·05 per cent. of the dry weight of leaves of higher plants. *Haematococcus pluvialis* is an even more interesting exception for it contains astaxanthin,[134] hitherto considered a characteristic carotenoid of invertebrates, especially crustacea (*see* p. 168); this pigment was originally termed *euglenarhodone*[135]. Three astaxathin esters occur in *H. pluvialis* two mono- and one di-esters. The fatty acid associated with the di-ester and one of the mono-esters has an empirical formula $C_{16}H_{30}O_2$, and that with the other ester $C_{18}H_{34}O_2$; α- and β-carotene and lutein are also present. Astaxanthin has also been found, together with β-carotene, in *Protosiphon botryoides* and *Brachiomonas simplex*.[136] The presence of *fucoxanthin* in *Zygnema pectinatum*[125] is extremely surprising and may be due to contamination by diatoms, of which group fucoxanthin

is the characteristic carotenoid (*see* p. 132). Fucoxanthin is certainly absent from *Chlorella vulgaris.*[137]

The recent work of Karrer and his colleagues[138] on *Chara ceratophylla* and on *Nitella syncarpa* is most interesting for they find the carotenoid distribution to be differential. The vegetative parts contain principally α- and β-carotene, whereas the antherida contain mostly *γ-carotene* together with small amounts of *lutein* (xanthophyll) and still smaller amounts of β-carotene. Strain[139] has stated that α- carotene is the major carotene of certain Siphonales and that ε-carotene (*see* p. 137) occurs in *Bryopsis corticulans.* He also reports the presence of a carotenoid very similar to, but chromatographically different from, fucoxanthin ; about 50 per cent. of this pigment, which has been named *siphonaxanthin*, occurs as an ester which has been named *siphonein.*

Carotenoids occur in *Lyngbya perelegans.*[140]

Seybold and Egle[141] have investigated quantitatively the carotene and xanthophyll fractions of a number of Chlorophyceae. Representative values, obtained on *Ulva lactuca*, were 4·4 and 21·8 mg. per 100 g. wet weight, of carotenes and xanthophylls respectively (*see also* Table 22). For all the species examined the xanthophylls : carotenes ratio was between 5 : 1 and 6 : 1. The same ratio was obtained for fresh water algae.[142]

Xanthophyceae

Little is known of the carotenoids of this class of alga. Poulton[143] indicated the presence of an hydroxy carotenoid which gave a blue colour with concentrated HCl. Carter *et al.*[128] were able to examine only one member of this group, the mud alga *Botrydium granulatum.* *β-carotene* and Poulton's carotenoid were detected ; there is good reason to believe that the latter is *flavoxanthin*, for it had the same absorption spectrum as a sample of flavoxanthin isolated from green leaves.[144] The only other pigment giving the same type of colour reaction with HCl is violaxanthin which has a very different absorption spectrum (Table 2).

β-carotene has been detected in the fresh water *Tribonema bombycinum* which also contains a number of xanthophylls none of which appears to be a known pigment.[145]

Bacillariophyceae (Diatoms)

In nearly all diatoms examined *β-carotene* has been identified (*see e.g.* Strain, Manning and Hardin).[145] An apparently new carotene, *ε-carotene*, has been detected in *Nitzschia closterium* and *Navicula*

torquatum.[146] This pigment, which has the same absorption spectrum as neurosporene (*see* p. 107), is much less strongly adsorbed on alumina than the latter. It is possible that if neurosporene is accepted as being tetrahydrolycopene, ε-carotene is tetrahydrocarotene.

The position of the xanthophylls in diatoms is by no means clear. Seybold and Egle[134] and Heilbron[125] have repeatedly established the presence of *lutein* (xanthophyll) and *fucoxanthin* in diatoms. Pace[147] agrees with the presence of lutein but considers the other xanthophylls which are present to be xanthophylls characteristic of the higher plants. He claims to have detected *isolutein*, *cryptoxanthin* and, possibly, *zeaxanthin* and *violaxanthin* " b ". A similar claim has been made by Handke.[148] Strain[149] considers this false and he and his colleagues claim the existence of *diatoxanthin* and *diadinoxanthin*, two specific xanthophylls very similar to, but apparently quite distinct from zeaxanthin and lutein respectively.[145] These pigments have not yet been isolated. The possibility does exist that they are *cis*-isomers of lutein and zeaxanthin. Strain *et al.* also consider that in diatoms there are several normally-occurring fucoxanthins, (*e.g.* fucoxanthins a, b, and c,[146] the latter two have recently been renamed *neo*fucoxanthins A and B[145]) and deny that they are either artefacts or the results of post-mortem changes (*see* p. 133). All the pigments observed by Strain *et al.* occurred in all diatoms examined : *viz. Navicula torquatum*, *Isthmia nervosa*, *Nitzschia closterium*, *N. palea*, *Stephanopyxis turris* and *Thalassiosira gravida.* Wassink and Kersten[137] have found carotene, fucoxanthin, and possibly diatoxanthin in *Nitzchia dissipata* ; fucoxanthin exists as a protein complex with an absorption maximum at 500 mμ. The change from brown to green which this alga undergoes on boiling is explained by denaturation of the protein complex. The absorption spectra of boiled and unboiled diatoms are, however, only slightly different.[137]

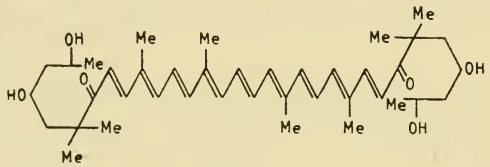
Chrysophyceae

The only work recorded is that by Heilbron[125] who examined the carotenoids of a mixture of three algae, *Apistonema carteri*, *Thallochrysis litoralis* and *Gloeschysis maritima.* β-*carotene*, *lutein* (xanthophyll), and *fucoxanthin* were isolated. These pigments were also isolated by Heilbron's group from *Nitzschia closterium* (above) and this similarity is added proof for Pascher's[150] thesis that the *Chrysophyceae* and *Bacillariophyceae* are closely related and probably derived from a common ancestry. Pascher also considers that the *Xanthophyceae* are closely related to these two classes but it will be remembered that the carotenoids of a typical member of *Xanthophyceae* are different.

However, more work is necessary before pigment differences can be put forward as an argument to dissociate the *Xanthophyceae* from the two other groups.

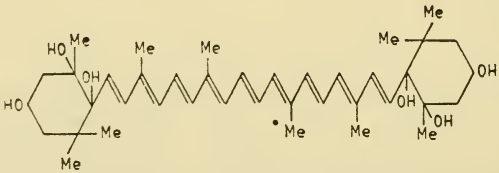
Phaeophyceae

The brown algae comprise the largest class of algae and they vary considerably in size and colour. β-*carotene* is present in all species which so far have been studied. The characteristic carotenoid of this species is *fucoxanthin* which is as universal as is β-carotene but occurs in much greater amounts. Fucoxanthin was first isolated in 1914 by Willstätter and Page,[151] but its structure is still in doubt. Heilbron and Phipers favour :



but Strain[139] points out that as this is 5 : 5′-dihydroxycapsorubin, its absorption spectrum should differ little if any from the parent capsorubin ; this is not so (*see* Table 4). Neither does the spectrum of fucoxanthin correspond with that of β-carotenone, which contains the same chromophoric system ;[152] other reasons why this structure of fucoxanthin must be rejected are given by Karrer and Jucker.[153] The absorption spectrum of fucoxanthin and its isomer neofucoxanthin are drawn in Fig. 21.

With little evidence Retrovsky[154] has suggested the following structure for fucoxanthin ;



Heilbron and Phipers examined a large number of brown algae, choosing those as diverse as possible in their habits. In spite of this diversity the uniformity of pigment distribution was remarkable, fucoxanthin was always the main carotenoid. Lutein (xanthophyll) was detected in small amounts only in the smaller members and was absent from the larger algae. Kylin,[124] during his first investigations

concluded that two fucoxanthins existed in the *Phaeophyceae*, but later[129] he agreed with Heilbron and Phipers that one of his pigments was an oxidative artefact. Recently Strain and his colleagues[145,146] have reopened the question, and as previously noted, they claim that two labile isomers exist in addition to fucoxanthin itself. These they have named neofucoxanthin A and neofucoxanthin B according to Zechmeister's nomenclature (*see* p. 10); the status of these pigments is still obscure (*see* p. 131). They also noted the presence of diatoxanthin, diadinoxanthin, violaxanthin and probably flavoxanthin.

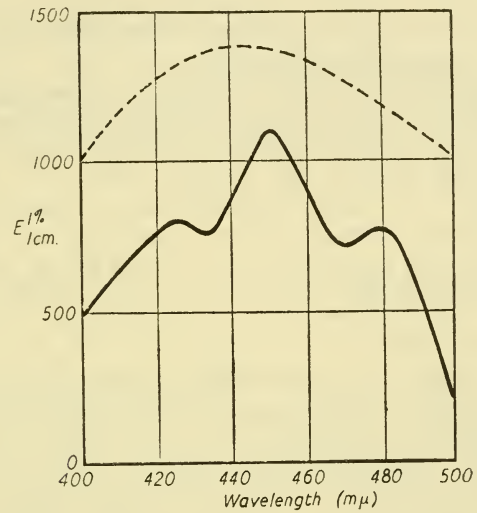


Fig. 21.—The absorption spectrum of (*a*) Fucoxanthin in hexane. (————) (*Redrawn from Karrer, P., and Würgler, E. (1943)* Helv. chim. Acta, **26**, 117, (*b*) Neofucoxanthin B (*from Strain, H. H., Manning, W. M., and Hardin, G. (1944)* Biol. Bull.Woods Hole, **84**, 169). (- - - - - - - - - - -)

Further confusion arose following the examination of dried *Fucus vesiculosus* by Heilbron and Phipers.[131] They found, rather surprisingly, no fucoxanthin but zeaxanthin instead, and concluded this was a post mortem change. Kylin,[155] however, claims that both zeaxanthin and violaxanthin are present in fresh *F. vesiculosus*; the failure to detect them in fresh material is considered to be due to the overwhelming preponderance of fucoxanthin; Kylin goes on to claim that in dead material fucoxanthin is preferentially oxidized, thus unmasking zeaxanthin.

Heilbron's[125] objections to Kylin's explanation are: (*a*) the absence

of a known analogous situation (in algae) of preferential oxidation of carotenoids, and (*b*) the absence of zeaxanthin from fresh gathered *Bacillariophyceae*. It should be noted, however, that strong support for Kylin's thesis comes from the fact that Karrer and Strong[156] have isolated crystalline zeaxanthin from the brown alga *Halysersis polypodioides*, and that in fading green leaves, zeaxanthin is relatively the most stable carotenoid (*see* p. 23).

Differential carotenoid distribution has been noted in *F. serratus*, *F. vesiculosus* and *Ascophyllum nodosum*[125,157]. The bright orange yellow of the male gametes is due almost entirely to β-carotene and the olive green of the ova to a mixture of fucoxanthin and chlorophyll. This will be discussed later (*see* p. 147).

Seybold and Egle's[141] quantitative study of *Fucus* and *Laminaria* species emphasises the fact that fucoxanthin is the predominating pigment; it occurs in concentrations 5–8 times greater than does β-carotene, which itself is more abundant than the "xanthophylls-not-fucoxanthin." The mean values obtained on *Fucus* and *Laminaria* were, for carotenes, xanthophylls not fucoxanthin, and fucoxanthin, respectively, 3·8, 2·5, and 13·8, and 1·5, 0·7 and 8·6 mg. per 100 g. wet weight (*see also* Table 22).

Rhodophyceae

The earlier work of Kylin[124,158] has been extended and to a great extent confirmed by Carter, Heilbron and Lythgoe.[128] *β-carotene* is always present; considerable amounts of *α-carotene* are present in some species (*e.g. Ceramium rubrum*) whilst in others (*e.g. Polysiphonia nigrescens*, it does not exist.[129] The most striking finding after examining numerous members of the seven orders of this class was the complete absence of any characteristic carotenoid; lutein (xanthophyll) is the principal xanthophyllic pigment and *taraxanthin* is generally present.[128] Only one member of this group was found which contained *fucoxanthin*, this is *Polysiphonia nigrescens*. No explanation of this is apparent at the moment for there is no morphological abnormality about this alga.

The quantitative distribution of carotenoids in the Rhodophyceae is very similar to that in the Chlorophyceae[141] (Table 22).

Dinophyceae (Peridinieae)

β-carotene is the main hydrocarbon carotenoid present in this group. An apparently characteristic pigment, *peridinin*, was detected in *Peridinium* spp. by Kylin.[124] A further examination[159] of this pigment has led to the suggestion that it is identical with the xanthophyll, *sulcatoxanthin*, first isolated in 1935 from *Anemonia sulcata*[160]

(*see* p. 158). Closer investigation of the fresh water *Peridinium cinctum* has revealed the presence of two further xanthophylls, *diadinoxanthin* (*see* p. 131) and a specific pigment *dinoxanthin*, very similar in properties to violaxanthin. Three isomers, neoperidinin (neosulcatoxanthin ?), neodiadinoxanthin and neodinoxanthin, were also detected but may possibly be artefacts.[145]

According to Scheer[161] *Prorocentrum micans* contains about 0·025 per cent. of carotenoids (dry weight) of which about 10 per cent. is carotene.

Chloromonadineae

Little is known about the carotenoids which occur in this small class of fresh water algae. Fritsch[127] states that the bright green tint of their discoid chromatophores is due to an excess of " xanthophyll " and Cook[126] notes that " colour tests " indicate the presence of xanthophylls.

Euglenineae

The carotenoids of this class are located in the stigma or eye spot. Kylin[124] noted 3 modifications of " red haematochrome " in *Euglena* species. β-*carotene*, *lutein* (xanthophyll) and *zeaxanthin* are probably present but the main pigment is that isolated by Tischer[162] which he called *euglenarhodone*. Recently Kuhn, Stene and Sörensen,[163] and Tischer himself,[164] have proved the identity of euglenarhodone and *astaxanthin* (*see* p. 170). This discovery of astaxanthin in plants is most striking, (it has also been noted in *H. pluvialis*) for until recently it had been considered the typical marine animal carotenoid. This well emphasises the fact that the flagellates, to quote Heilbron,[125] " bridge the gap between the vegetable and the animal kingdom." Heilbron has little doubt that the pigment which is located in the eye spots[165] of nearly all the motile cells of the flagellates will turn out to be astaxanthin.

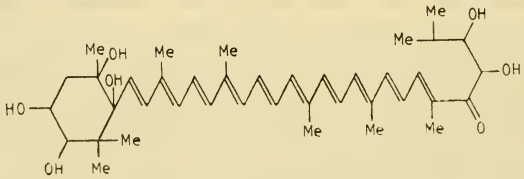
Cyanophyceae (Myxophyceae)

In 1843 Kraus and Millardet[166] were first to note the presence of a carotenoid in this group and called it *phycoxanthin*. Thirty years later Kraus[167] claimed that it was not identical with any carotenoid of the higher plants. Sorby[168] confirmed this but also found carotene present. The first modern investigation into an alga of this class was carried out on *Calothrix scopulorum* by Kylin[124]; apart from carotene he separated three other pigments, *myxorhodin*-α, *myxorhodin*-β, and

calorhodin. Heilbron, Lythgoe and Phipers[169] isolated crystalline β-*carotene*, *lutein* (xanthophyll) and a new pigment *myxoxanthin* from the fresh-water alga *Rivularia nitida*. This new carotenoid is not specific to *Rivularia nitida* and was soon isolated from *Rivularia atra* (marine) as well as from *Oscillatoria rubescens* (fresh water).[170] Owing to the large amounts of *O. rubescens* available this was examined in detail and another pigment *myxoxanthophyll* was isolated. Reinvestigation of *O. rubescens* by Karrer and Rutschmann[171] have confirmed Heilbron and Lythgoe's observation and small amounts of an acidic carotenoid *oscillaxanthin* and of zeaxanthin, which were not observed by Heilbron and Lythgoe, were also isolated. The relation between these pigments and those of Kylin has not yet been established.

Tischer[172,173] isolated from *Aphanizomenon flos-aquae* four carotenoids, aphanin, aphanicin, aphanizophyll and flavacin. Aphanin and myxoxanthin are now known to be the same pigment and to be identical with echinenone,[174,175] first isolated from sea urchins by Lederer;[176] this being so the name echinenone should be retained and the others abandoned. For details of the properties of echinenone *see* p. 163.

Karrer and Rutschmann consider that myxoxanthophyll has the following structure:



but emphasize that this is by no means certain. The most obvious objection to this structure is on spectroscopic grounds. It has a chromophoric system of eleven congugated double bonds compared with twelve in echinenone (or thirteen according to Karrer and Rutschmann), but exhibits an absorption spectrum with its main band at a slightly higher wavelength in ethanol (470 and 471 mμ. respectively) and at a much higher wavelength in chloroform (484 and 473 mμ. respectively); one would have expected the bands to have maxima much lower than this. Aphanizophyll is very similar to myxoxanthophyll, if not identical with it; Heilbron[125] considers their identity possible, but Tischer[172] does not agree.

Aphanicin is thought to be a di-carotenoid, *i.e.*, two aphanin (echinenone) molecules joined by an ether bridge. Further work is required before this suggestion can be fully accepted. Flavacin is, from its

absorption spectrum and chromatographic properties very probably ζ-carotene. Karrer and Jucker[3 1] suggest that it might be mutatochrome but it appears less strongly adsorbed on alumina than mutatochrome.

Recently Manten[1 1 9] reported that *Tolypothrix distorta* var. *symplocoides* contains β-carotene and echinenone (myxoxanthin).

Cryptophyceae

Nothing apparently is known of the carotenoid contribution to the pigmentation of this class of algae, Fritsch states that they show very diverse pigmentation which is commonly some shade of brown.[1 2]

The properties of algal carotenoids and their quantitative and qualitative distribution are given in Tables, 21, 22, 23.

Table 21.—*Characteristic Algal Carotenoids*

Name	m.p.	Absorption maxima (mμ.)	
		Ethanol	CS_2
Euglenarhodone[1] = Astaxanthin (*see* p. 172)			
ε-Carotene[2]		418, 442, 471	
Diatoxanthin[3]		453, 481	
Diadinoxanthin[3]		448, 478	
Fucoxanthin[4]	166–167°	459, 484	445, 477, 510
Peridinin[5] = Sulcatoxanthin (*see* Table 24, p. 158)			
Dinoxanthin[3]		441·5, 471	
Myxoxanthin[6, 7, 8] (= Echinenone)			
Myxoxanthophyll[6, 8] ..	169–170°	445, 471, 503	
Oscillaxanthin[9]		464, 496, 531*	494, 528, 568
Aphanin[10] = Myxoxanthin = Echinenone (*see* Table 26, p. 165)			
Aphanicin	195°	457, 491·5*	494, 533
Aphanizophyll ? Myxoxanthophyll	172–173°	444, 475, 507*	
Flavacin (? = ζ-carotene, (*see* Table 4)	155°		459, 489·5
Siphonaxanthin[11] ..		455	

* Actually measured in methanol.

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1. Tischer, J. (1944), *Hoppe-Seyl. Z.*, **281**, 143.
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TABLE 22

*Carotenoid Content of some Marine Algae**

SPECIES	AMOUNT					
	mg./100g.wet. wt.			mg./100g. dry wt.		
	Carotene	Xanthophylls†	Fucoxanthin	Carotene	Xanthophylls†	Fucoxanthin
CHLOROPHYCEAE						
Chaetomorpha linum ..	6	45		47·6	355	
Chaetomorpha melagonium	9	80		40·7	365	
Cladophora arcta ..	4·4	32·5		12·6	94	
Cladophora rupestris ..	14·3	75·1		36·3	191	
Cladophora sericea ..	7·9	49·2		31·0	194	
Enteromorpha compressa	5·6	31·0		30·4	168	
Enteromorpha linza ..	4·0	29·9		15·9	117	
Ulva lactuca	4·4	21·8		15·8	77·1	
PHAEOPHYCEAE						
Ascophyllum nodosum .. (brown)	2·5	2·1	8·7	6·8	5·8	23·8
Ascophyllum nodosum .. (yellow)	1·2	0·7	3·1	3·5	2·1	9·0
Desmarestia aculeata ..	2·9	—	23·7	11·0	—	90·0
Dictyota dichotoma ..	4·8	5·5	22·0	28·3	32·5	130·0
Fucus platycarpus ..	3·7	1·8	10·3	15·2	7·2	42·0
Fucus serratus	4·0	2·0	14·4	16·0	8·4	58·4
Fucus vesiculosus ..	3·8	3·8	16·8	14·0	14·0	58·9
Halidrys siliquosa ..	2·3	1·5	11·0	10·5	7·1	51·0
Himanthalia lorea ..	1·1	1·0	4·8	4·7	4·2	20·0
Laminaria digitata ..	1·6	0·6	7·9	8·8	3·6	41·4
Laminaria hyperborea ..	1·4	0·7	8·1	8·5	4·5	46·9
Laminaria saccharina ..	1·4	0·9	9·8	7·2	4·2	46·0
RHODOPHYCEAE						
Ahnfeltia plicata ..	5·6	21·0		15·4	58·2	
Antithammion plumula ..	1·5	19·8		4·2	68·5	
Brogniartella byssoides ..	4·0	11·3		17·9	50·0	
Ceramium rubrum ..	4·4	21·2		27·2	131·0	
Chondrus crispus (green)	2·4	13·6		9·3	51·9	
Chondrus crispus (red) ..	3·6	12·9		13·7	48·5	
Chylocladia clavellosa ..	2·8	4·8		23·8	41·0	
Corallina officinalis ..	2·9	5·4		4·4	8·0	
Delesseria alata	6·7	22·3		20·1	67·7	
Delesseria sanguinea ..	1·9	7·8		6·6	28·0	
Furcellaria fastigiata ..	2·1	5·6		8·9	23·9	
Halarachnion ligulatum	1·8	7·4		15·7	63·9	
Phyllophora brodiaei ..	2·9	13·2		8·0	36·0	
Plocamium coccineum ..	3·0	14·5		16·9	18·2 (?)	
Plumaria elegans ..	2·7	15·7		11·2	65	
Polyides rotundus ..	2·8	5·6		9·8	19·7	
Porphyra laciniata ..	3·6–10·2	16·3–79		24·9–28·5	100–177	
Rhodomela subfusca ..	0·4–4·0	4·5–22		1·9–21·2	22·5–112	

* From Seybold, A., and Egle, K. (1938), *Jahrb. wiss. Botan.*, **86**, 50.
† Not fucoxanthin.

COMPARISON OF HIGHER PLANTS WITH ALGAE

Algae in common with the higher plants invariably contain β-carotene mixed with varying proportions of α-carotene. Lutein (xanthophyll) also appears to be universally distributed, but is much

less abundant, other xanthophylls which occur in the higher plants, however, occur rarely in algae and then only in traces ; some investigators claiming that they never occur. Instead, new and specific xanthophylls are generally found. Of these, when the algal distribution over the world's surface is considered, fucoxanthin is probably the most abundant naturally-occurring carotenoid.

When the relative amounts of carotenes and xanthophylls in the vegetative regions of the plants are considered, there are no well-marked differences between the higher plants and the algae and no single value can be cited as characteristic of either group. The usual xanthophylls : carotenes ratio for higher land plants varies from 4 to 9 with a value of perhaps 15 for some alpine plants. These values are typical of those found for algae. Ratios between 5 and (?) 50 were obtained for 12 green fresh water algae, between 3·4 and 8·3 for fresh water *Rhodophyceae* varying in colour from green to reddish brown, and 5·4, 6·0 and 11·4 for 3 flagellates.[159]

FORMATION

(i) *Effect of Carbon and Nitrogen Sources*

As in the case of higher plants very little is known of carotenoid formation in algae. Interesting contributions have come from Chodat[177] and Chodat and Haag,[178] who consider carotenogenesis to be of two types :

(*a*) spontaneous carotenogenesis arising during growth on a normal medium owing to the genetical disposition of the algae.

(*b*) excessive carotenogenesis induced by the medium owing to carbon-nitrogen imbalance.

Chodat argues that if nitrogen is in excess all (or most) of the carbon is used up in protein synthesis and there is none available for fat and carotenoid synthesis. On the other hand if carbon is in excess, there is plenty to spare for fat and carotenoid synthesis. Adequate proof of this interesting hypothesis is yet to be presented, but it should be noted that in fungi it is the amount of assimilable carbon which is the controlling factor, the C/N ratio having only limited significance.[19,20] In this connection Lwoff and Lwoff[165] have shown that carotenoid synthesis by *Haematococcus pluvialis*, growing on a medium containing asparagine or peptone, is stimulated by the addition of sodium acetate although it is independent of its concentration in the medium.

More recently Wenzinger[179] has shown that carotenoid synthesis in *Dictyococcus cinnabarinus* is much increased when the nitrogen source [$Ca(NO_3)_2$] is reduced by one third, *i.e.*, when the C/N ratio is increased ; unfortunately, iron which is needed by this alga for

growth was also removed at the same time. So the extra carotenoid formation might, as Wenzinger admits, also be due to failure of the alga to synthesize chlorophyll in the absence of iron, the precursors of chlorophyll being diverted into carotene production. It must be admitted however, that in any case the experimental evidence provided is by no means compelling.

(ii) *Mineral Constituents*

Haag[180] claims that in algae, a deficiency of magnesium sulphate or of PO_4''' causes an accumulation of carotenoids before death supervenes. The following colour changes were noted, green ——→ red ——→ white (death). No supporting quantitative data were provided and, as in the case of fading leaves, the disappearance of chlorophylls may only be unmasking the carotenoids already present.

Fox and Sargent[181] found that the flagellate *Dunaliella salina* when cultured in saturated saline (25 per cent.) produced only β-carotene ; in solutions ½ to 2/3 saturated, much less β-carotene was synthesized and considerable amounts of chlorophyll made their appearance. Spoehr and Milner,[182] in an investigation not mainly concerned with carotenoid metabolism, noted that in *Chlorella pyrenoidosa* there was a decreasing carotenoid production with increasing " R " values ; the change was, however, much less marked than with chlorophyll. (The " R " value measures the degree of reduction of the carbon tissues ; *see* the original report for full details of the calculation of " R.")

As algae develop, the carotenoid content also increases ; this was demonstrated in the case of plankton of the North Sea, principally *Rhizosolenia styliformis* and *Biddulphia sirensis*.[183]

(iii) *Action of Light, Temperature and Oxygen*

A survey of more recent work indicates that whilst light is not essential for carotenoid production, it may have some qualitative effects.

The absence of light does not prevent carotenoid production in *Polytoma uvella*, *Euglena gracilis*, *Astasia* spp.[166] and *Dictyococcus cinnabarinus*.[179]

Variations in the depth of water from which the algae were sampled revealed only minor changes in their carotenoid content.[184,209] Fritsch,[127] however, states that the lipochromes of *Trentepohlia* normally mask the green colour of the latter's chloroplasts, but that in shaded situations these pigments may be almost completely lacking, so that the growths appear green.

Nitzschia closterium grown in " white " light (snow-white fluorescent lamps) contained relatively less diadinoxanthin than did cultures

grown in red light (neon tubes); the relative amounts of the other xanthophylls produced were unchanged.[145] It was noted in the same labotatory that *Chlorella pyrenoidosa* cultured in low intensity illumination contained more α- than β-carotene, whilst cultures produced under high illumination reversed the situation.[185]

Nothing is known of the effect of temperature on carotenogenesis in algae. Oxygen appears to be necessary expecially for production of xanthophylls,[180] but the evidence so far available is not compelling.

FUNCTION OF CAROTENOIDS IN ALGAE

Photosynthesis

It has previously been noted that in the higher plants carotenoids do not take part directly in photosynthesis but act, if at all, by passing on their absorbed energy to chlorophyll (*see* p. 86). The most recent investigations indicate that the same situation exists in algae.

The first investigations were carried out on *Chlorella* by Warburg and Negelein.[186] They concluded that the carotenoids were used in photosynthesis at very low efficiency. Montfort,[187,188] using brown marine algae, Emerson and Lewis[189,190] using *Chroococcus*, Dutton and Manning[191,192] using *Nitzschia closterium*, and Wassink and Kersten[137] using *Nitzschia* spp. reached the same conclusions.

Criticism has been levelled at the earlier work of Warburg and Negelein[186] and of Montfort,[188] but there seems no doubt that the situation has been accurately described by Dutton and Manning[191] and by Wassink and Kersten.[137]

Dutton and Manning found that, if photosynthesis produced by irradiating the alga with light of wave-length 496 mμ is ascribed completely to chlorophyll, the situation arises that the quantum efficiency of the process is over 100 per cent.; thus the mediation of the carotenoids was very strongly indicated. More recently these workers have shown that this light absorbed by *N. closterium* reappeared as chlorophyll fluorescence.[192] Wassink and Kersten[137] came to a similar conclusion and also demonstrated that the energy is transferred to chlorophyll without any wastage. They consider that a chlorophyll-fucoxanthin-protein complex exists in the plant (See also Tanada[210]).

Recently Blinks and his colleagues,[193-195] using *Coilodesme* spp. have confirmed the positive but minor role played by carotenoids in the photosynthetic processes in the brown algae and have extended these observations to the green alga *Ulva* spp. The situation is, however, different in the case of the red algae: the light absorbed by carotenoids, and indeed by chlorophylls, is not utilised for photosynthesis

TABLE 23.—*The Qualitative Distribution of Carotenoids in Algae*

Species	α-carotene	β-carotene	ε-carotene	γ-carotene	Lycopene	Siphonaxanthin	Taraxanthin	Violaxanthin	Haematoxanthin	Lutein	Echinenone (= Aphanin = Myxoxanthin)	Aphanicin	Aphanizophyll	Flavacin	Myxoxanthophyll	Oscillaxanthin	Zeaxanthin	Fucoxanthin	Sulcatoxanthin	Astaxanthin	Diadinoxanthin	Cryptoxanthin	Dinoxanthin	Isolutein	Diatoxanthin	Reference No.
Ahnfeltia plicata		+								+																5
Antithamnion plumula		+																								44
Aphanizomenon flos-aquae		+								+	+	+	+	+												1, 36
Apistonema carteri		+								+								+								1, 5
Ascophyllum nodosum		+																+								5, 24
Botrydium granulatum		+					+																			5
Brachiomonas simplex		+																								32
Brogniartella byssoides		+																								44
Bryopsis corticulans		+				+					+				+											33
Calothrix scopulorum		+								+																1, 2, 3
Ceramium rubrum		+					+			+	+															5, 21, 22, 31
Chaetomorpha linum		+																								6
Chaetomorpha melagonium		+			+																					6
Chara ceratophylla		+		+						+								+								5
Chorda filum		+																								5
Chondrus crispus		+																								5
Chylocladia clavellosa		+																								6
Cladophora arcta		+						+		+																6
Cladophora rupestris		+					+			+																22
Cladophora sauteri		+																								5, 25
Cladophora sericea		+																								6
Cladostephus spongiosus		+																								5
Corallina officinalis		+								+								+								5, 31
Cytoseira osmundacea		+						+										+								34
Delesseria alata		+								+																6
Delesseria sanguinea		+								+																6
Desmarestia aculeata		+								+								+								6
Dictyota dichotoma		+								+								+								5, 6, 23
Dilsea edulis		+								+								+								5, 21, 22
Ectocarpus siliculosus		+								+								+								22
Ectocarpus tomentosus		+								+																5
Egregia menziesii		+								+																34
Euglena pelionbolcensis		+								+										+						8, 16
Enteromorpha compressa		+								+																5, 6

TABLE 23.—*The Qualitative Distribution of Carotenoids in Algae*—contd.

Species	α-carotene	β-carotene	ε-carotene	γ-carotene	Lycopene	Siphonaxanthin	Taraxanthin	Violaxanthin	Haematoxanthin	Lutein	Echinenone (= Aphanin = Myxoxanthin)	Aphanicin	Aphanizophyll	Flavacin	Myxoxanthophyll	Oscillaxanthin	Zeaxanthin	Fucoxanthin	Sulcatoxanthin	Astaxanthin	Diadinoxanthin	Cryptoxanthin	Dinoxanthin	Isolutein	Diatoxanthin	Reference No.
Enteromorpha intestinalis	+	++								+																2, 21
Fucus ceranoides								+										+								5
Fucus furcatus		++																								34
Fucus platycarpus		++																								6
Fucus serratus		++						+		+							+	+								22, 23, 26
Fucus vesiculosus		++						+		++																5, 6, 22, 24, 28
Furcellaria fastigiata		+								+							+	+								6
Gelidium corneum		+								+																5
Gigartina stellata		+								+																5
Glerochrysis maritima		+							+	+							+	+		+						8, 14, 15, 16, 39
Haematococcus pluvialis																										6
Halarachnion ligulatum		+															+	+								6
Halidrys siliquosa		+															+	+								24, 30
Haliseris polypodioides		+																+								29, 30
Halidrys siliquosa		+						+										+								34
Hesperophycus harveyanus		+																								6
Himanthalia lorea		+																							+	34
Ishnia nervosa		+																								6
Laminaria anderson		+								+								+								34
Laminaria digitata		+						+										+								5, 6, 22, 23, 29
Laminaria hyperborea		+																								6
Laminaria saccharina		+																								6
Lemania mamillosa		+																								5
Myrocystis integrifolia		+						+		+									+							34
Nitella opaca		+						+		+								+								5
Nereocystis pyrifera		+			+													+								34
Nitella syncarpa		+		+																						18
Nitzschia closterium		+								+								+			++	++		?	++	6, 7, 11, 12, 13, 38
Nitzschia palea		+																			+					34
Nitzschia dissipata		+																								35
Navicula torquatum		+	+							+								+				+			+	10, 34
Oedogonium spp.	++	+					+			+																5, 24, 25
Oscillatoria spp.	.	+									+															9

TABLE 23.—*The Qualitative Distribution of Carotenoids in Algae*—contd.

Species	α-carotene	β-carotene	ε-carotene	γ-carotene	Lycopene	Siphonaxanthin	Taraxanthin	Violaxanthin	Haematoxanthin	Lutein	Echinenone (= Aphanin = Myxoxanthin)	Aphanicin	Aphanizophyll	Flavacin	Myxoxanthophyll	Oscillaxanthin	Zeaxanthin	Fucoxanthin	Sulcatoxanthin	Astaxanthin	Diadinoxanthin	Cryptoxanthin	Dinoxanthin	Isolutein	Diatoxanthin	Reference No.
Oscillatoria rubescens		+						+		+	+				+	+	+									3, 4
Pelvetiopsis limitata																			+						+	34
Perâtinum cinctum																		+			+					6, 34
Phyllobium naegelii																										5
Phyllophora brodiaei		+								+																6
Phyllophora membranifolia		+								+																5
Plocamium cocineum		+								+																5, 6
Pilayella littoralis		+						+		+								+								5, 24, 32
Plumaria elegans																										6
Polyides rotundus		+								+								+								5, 21, 31
Polysiphonia fastigiata		+								+																5
Polysiphonia nigrescens		+								+								+								5, 21, 31
Porphyra laciniata																										6
Porphyra umbilicalis																										5
Prorocentrum micans										+										+						36
Protosiphon botryoides																										32
Pterygophera californica		+						+		+								+								34
Rhodomela subfusca		+								+																6
Rhodymenia palmata		+								+	+				+			+								5, 25
Rivularia atra		+								+																5
Rivularia nitida		+								+																5
Sphacellaria cirrhosa		+								+	+							+			+					34
Stephanopyxis turris		+								+								+								5
Stypocaulon plicata		+								+								+			+					34
Thalassiosira gravida		+								+																5
Thallochrysis litoralis		+								+		+														37
Tolypothrix distorta v. symplocoides	+	+								+						+	+									19, 20
Trentepohlia aurea	+	+								+						+	+									19, 20
Trentepohlia jolithus		+								+																17
Trentepohlia umbrina		+								+																34
Tribonema bombycinum		+								+																5, 6, 21, 23
Ulva lactuca		+								+																5
Vaucheria hamata		+						+		+																5
Zygnema pectinatum		+								+																5

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in *Delesseria* spp., *Porphyra* spp. (including *P. nereocystis*, *P. naidum*, and *P. perforata*) or *Schizymeria*. The pigments concerned in photosynthesis in these algae belong to the phycobilin group. These findings have still to be confirmed. In this connection, however, it should be noted that Arnold and Oppenheimer[195A] found that the internal transfer to chlorophyll of the light energy absorbed by pyocyanin can account for the photo-synthetic effect of the latter.

Photokinesis

Although Engelmann[196] has claimed that the photosensitive zone is a colourless region just anterior to the stigma, and Luntz[197] has stated that it is impossible to claim more than that the photosensitive structure occurs in the anterior third (which contains the stigma) of the organism, it is now virtually certain that phototactic orientation of algae is mediated through the stigma. The carotenoids are concentrated in the eye spot of the flagellates and the action spectrum for a number of

Eulgena species[198] is almost identical with the most recently determined absorption spectrum of astaxanthin in petroleum ether.[134,199] Mast[198] also found that the action spectra of *Phacus triqueter*, *Trachelomonas euchlora*, and *Gonium* species were very similar to those of *Euglena* species. In the case of *Chalmydomonas* the action spectrum maximum was shifted from 474 mμ. to 504 mμ., and in *Pandorina*, *Eudorina*, and *Spondylomorum*, was shifted even further to about 534 mμ. Chemical investigations of the latter species have not been reported, but if astaxanthin is present its possible occurrence as a protein complex similar to that which has been reported in lobster eggs and shell (*see* p. 170), could to quote Wald,[64] " easily meet the most extreme requirements of prostistan action spectra." It must, however, be emphasised that action spectra and absorption spectra are not directly comparable.[200,201] The former are represented on the basis of an equal energy spectrum and the latter on the basis of an equal quantum intensity spectrum. Recalculation of action spectra in terms of equal quanta will result in a shift of $\lambda_{max.}$ to shorter wave-lengths (about 10 mμ.).[201] Carotenoid-protein complexes need not, therefore, be invoked to account for action spectra with $\lambda_{max.}$ only slightly higher than $\lambda_{max.}$ of the free carotenoid, but postulation of such complexes are necessary to explain action spectra in cases where the wave-length displacement is large. Galston's[69] recent criticisms concerning the accuracy of measurement of action spectra in higher plants (*see* p. 88) must also be borne in mind in this connection.

Luntz[197] has obtained maximal sensitivity (minimal threshold for phototactic orientation) at 492 mμ. for *Eudorina elegans* and *Volvox minor*. Using a different technique involving the measurement of times required at various wave-lengths of an equal energy spectrum, in contrast to measuring directly the energy required, to elicit a standard response, Laurens and Hooker[202] obtained maximal responses at about 494 mμ. for *Volvox globator*.

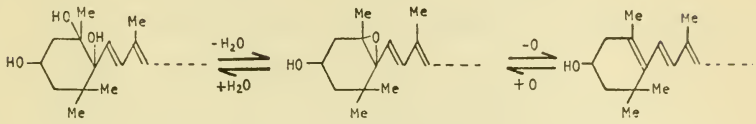
Manten[117] has shown that the photic orientation in the trichomes of *Tolypothrix distorta* v. *symplocoides*, which is phototropic in nature, is in all probability mediated through β-carotene.

An observation which may prove of extreme importance is that the slow but marked chromatic adaptation to its surroundings by *Chlorella* is due to changes in its carotenoid composition.[203]

Function in Redox Systems

Retrovsky[154] suggests that fucoxanthin, together with violaxanthin and zeaxanthin, plays a part in the redox systems in algae. This is

theoretically possible only if the structure of fucoxanthin is as he suggests (*see* p. 132). The mechanism would then be :



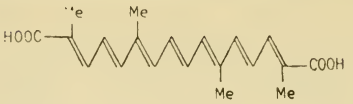
SEXUAL FUNCTION AND CAROTENOIDS

The differential distribution of carotenoids in the sex organs of many algae has already been noted. (*see* p. 134). Heilbron [124] suggests that β-carotene, the pigment in the male gametes of *Fucus serratus* and *F. vesiculosus*, may control the motility of the spermatozoa. Under the stimulus of light and in the presence of oxygen and a specific enzyme β-carotene may be converted into crocetin dimethylester which could then function as a gamete stimulator. [204]

It was noted in 1938 by Kuhn, Moewus and Jerchel [205] that washed cells of the unicellular flagellate *Chlamydomonas eugametos* became motile when either exposed to light, or kept in the dark but supplemented with sugar and oxygen, or kept in the dark and treated with filtrates of motile cells. Addition of larger amounts of filtrates which had been exposed to light for a short period stimulated the formation of female gametes ; if the filtrates were exposed for longer periods before addition, male gametes were formed. The essential step was irradiation, for non-irradiated filtrates were completely inactive. It eventually transpired that similar differential gametogenesis could be brought about by the addition of appropriate mixtures of labile (*cis*-) and stable (*trans*-) forms of crocetin dimethylester. A 3 : 1 mixture of the *cis*- and the *trans*- forms stimulated female and a 1 : 3 mixture stimulated male gametogenesis. [204] As *cis*-crocetin can be converted into the *trans*- form by irradiation, it is concluded that these isomers are the substances produced by irradiating the *Chlamydomonas* cell filtrates. The photolabile precursor of crocetin has not yet been identified but the structure of crocetin indicates that theoretically it could be formed by the degradation of a C_{40} carotenoid.

Crocetin has not been dealt with in detail in this book which is primarily concerned with C_{40} carotenoids (*i.e.*, those containing 8

isoprene residues). It is sufficient here to give its structure which has been elucidated by Karrer and his colleagues :[153]



Crocetin

No confirmation of the striking reports of Kuhn and Moewus[204] has yet appeared, but it has been reported that Smith[206] is having difficulty in reproducing some of their findings.

It has recently been claimed that sporogenesis is intimately connected with the extraplastidic carotenoids (occurring in the fat droplets), for " interaction " between sporogenic tissue and these carotenoids has been observed at all stages of growth in a variety of spore-bearing plants.[207] Just what the " interaction " is, is not at the moment clear.

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PART II

ANIMAL CAROTENOIDS

CHAPTER V

MARINE INVERTEBRATES

Having discussed the plant carotenoids starting with the higher land plants and ending with the lower aquatic plants, it will be convenient to discuss animal carotenoids in the reverse order, beginning with marine invertebrates.

As the discussion proceeds it will be clear that the carotenoids of marine invertebrates are of two types :

(*a*) those derived directly or indirectly from plants eaten as food, and

(*b*) those, characteristic of the animal species, which are produced either by altering alimentary carotenoids or *de novo*. It is by no means proved which of these processes does, in fact, occur, but, from evidence obtained with higher animals, it is extremely unlikely that carotenoids are produced *de novo* in invertebrates.

Lederer[1] has provided a complete account of the knowledge of animal carotenoids up to 1935. This monograph is extremely useful because of the detailed critical discussion of much of the early work.

PROTOZOA

According to Fox,[2] phytozoa give rise to the conspicuous yellow, orange, and red colours observed in rain ponds, lakes, salt ponds, sea patches and in snow. Chetton, Lwoff and Parat[3] state that parasitic infusorians acquire carotenoid pigmentation by eating the eyes of certain crustacea.

METAZOA

Porifera (Sponges)

The pioneer investigators in the field of animal pigments, Krukenberg and McMunn, detected carotenoids in a number of sponges which are listed by Lederer.[1] Lönnberg[4] has detected carotenoids in *Halichondria panicea*, *Suberites ficus*, *Dysidea fragilis* and *Axinella rugosa*.

Although in recent reports there are noted some occasional divergences the interesting point emerges that in the sponges the carotenes

preponderate, whilst, as will become apparent as the chapter proceeds, in most invertebrates xanthophylls preponderate. *Suberites domnucula* and *Ficulina ficus* yield a complex mixture of hydrocarbons, containing α-, β-, and γ-*carotenes*, *lycopene*, and *torulene*.[5] Karrer and Solmssen[6] isolated astaxanthin (*see* p. 168) from *Axinella crista-galli* and Drumm[7,8] and his colleagues α-, β-, and γ-carotene and a pigment probably identical with echinenone (*see* p. 164), the characteristic carotenoid of echinoderms, from the red sponge *Hymeniacidon sanguinea*; astaxanthin was, however, not present.

Coelenterata

Fox and Pantin[9] have produced an authoritative review of all types of pigments occurring in the coelenterata and the reader is referred to this for detailed information.

Carotenoids are the main source of colour in this family, and it was in the siphonophore *Velella spirans* that the first carotenoid protein complexes were noted.[10,11] As early as 1881 Merejkowski[12] recognised "tetronerythrin" and "zooerythrin" in *Actinia equina* (*mesembryanthemum*) *Aiptasia* spp., *Cereactis* spp., and various hydrozoa. Apart from a few observations by Schultze[13] further reports were lacking and in 1922 Palmer[14] could say with some justification that there was no definite evidence of carotenoids in coelenterates. Since then interest has been stimulated and in a long series of papers, Lönnberg[15,16] has at least indicated the presence of carotenoids in almost every branch of the Coelenterata; in a number of aloyanarians (first noted by Studer[17] in *Eunicella verrucosa*), in various actinarians, in the madreporarian, *Caryophyllia smithi*, in the ceriantharian *Cerianthus lloydi*, in the scyphozoan *Lucernaria quadricornis*, and in the hydroid gymoblasts *Tubularia larynx* in *Tubularia indivisa* (previously noted in the group by MacMunn[18]) and by Teissier[19] in *Clava squamata*. Regarding the Calyptoblasts the position is less clear; Lönnberg could not detect carotenoids in clean *Antennularia antennina* and emphasised the necessity of removing diatoms from colonies before examination. Similarly, Abeloos and Teissier[20] did not find carotenoids in *Sertularella*, *Aglaophenia*, and *Lafoea* spp.; their occurrence has, however, been reported in *Antennularia ramosa* and *A. antennina*.[21]

Perhaps one of the most interesting problems of carotenoid biochemistry in coelenterates is the relationship between the varied colours which varieties of the same species can assume and their carotenoid disposition.

The pioneer in this field was Schultze[13] who showed that in *Hydra* species colours were a reflection of the nutritional state of the animals.

This only applied when colours were not due to the presence of symbionts. Well nourished animals which are red or black lose all their colour when they are starved. Whether all the colour is due to carotenoids is not clear; it seems unlikely for *H. circumcinta* when fed ostracods or red copepods became orange-red owing to the formation of semi-crystalline granules of carotenoids; when fed *Daphnia* spp. (which apparently contained no carotenoids) they became reddish brown but accumulated no carotenoids. According to Teissier[21] *Clava squamata* eggs contains a grey chromoprotein which liberates a carotenoid during development.

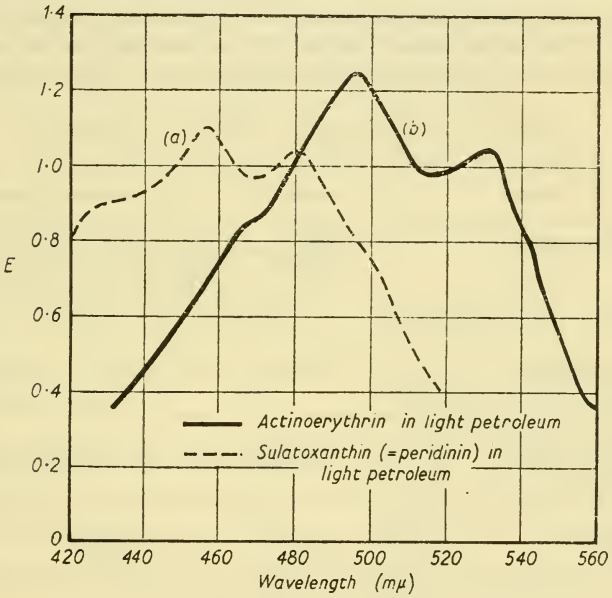

Fig. 22.—Actinioerythrin (*from Fabre, R., and Lederer, E. (1934)* Bull. Soc. Chim Biol., **16**, 105), and Sulcatoxanthin (*from Strain, H. H., Manning, W. M., and Hardin, G. (1944)* Biol. Bull. Woods Hole, **86**, 169).

More recent work has been centred on the three varieties of *Actinia equina*, the red, brown, and green forms. An orange carotenoid was detected in the red and brown variants and a red one in the red animals.[22] Subsequent investigations of the red animals by Lederer[23] and by Fabre and Lederer[24] revealed, apart from α- and β-*carotenes*, a red carotenoid ester *actinioerythrin* combined with a protein; about 1·5 mg. (30% yield) of this pigment was obtained from 30 specimens.

This has been confirmed by Karrer and Solmssen[6] and by Heilbron, Jackson and Jones;[25] the latter isolated, by careful hydrolysis of actinoerythrin, the parent acidic carotenoid which they named *violerythrin*; a rather striking feature of this carotenoid, of which the structure is still unknown, is the considerable difference between its absorption spectrum and that of its ester (Table 24) for esterification of carotenoids does not normally alter the spectra of the parent compounds to any appreciable extent. Heilbron *et al.* also isolated a second ester, very probably a *taraxanthin* ester.

In the green variant Fabre and Lederer found the pigment identified by Heilbron as a taraxanthin ester; as noted with actinioerythin, this pigment was bound to a protein, which, in this case, assumes a green colour. The brown variety appears to be intermediate between the green and red, containing both actinioerythrin and a taraxanthin ester.

TABLE 24.—*Characteristic Coelenterate Carotenoids*

Name	m.p.	Absorption Maxima
Actinioerythrin[1]	75°[1] 83–85°[2]	574, 533, 495 mμ. (CS_2)[1] 577–518* mμ. (ethanol) 574, 538, 497 mμ. (CS_2)[2] 511 mμ. (ethanol)
Violerythrin[2]	191–2°	625, 576, 540 mμ. (CS_2)
Sulcatoxanthin[2]	125–130°	516, 482, (450) mμ. (CS_2)
Metridin[3]	195–2°	495 mμ. (CS_2)

REFERENCES TO TABLE 24

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* Apparently a very wide absorption band.

Other coelenterata also contain specific xanthophylls. *Sulcatoxanthin* ($C_{40}H_{52}O_8$) a xanthophyll of unknown composition (*see* Table 24) was isolated from *Anemonia sulcata*[25] and is probably identical with peridinin (*see* p. 134); a very similar, if not identical, carotenoid was detected in *Cribrina xanthogrammica*[26] and *Metridium senile* (*Actinoloba dianthus*).[27] The pigments in the former are due to the presence

of algal symbionts, but as the latter anenome is free from such symbionts, it refutes the suggestion that sulcatoxanthin was obtained from symbiotic colonies.

M. senile contains an ester which on saponification yields a carotenoid somewhat similar to astacin. Fox and Pantin[27] have concluded that this pigment is not astacin and have named it *metridin*. By analogy with the properties of astaxanthin and astacin, it is very probable that the naturally occurring pigment is not metridin itself but a reduced form. This species also contains an xanthophyllic ester (? taraxanthin)[25] and astaxanthin.[27] The coloured variants of *M. senile* have been studied by Fox and Pantin.[27] It will be seen from Table 25 that xanthophylls play an important part in the coloration of these animals and carotenes little or no part. The concentration of xanthophylls varied from 14·96 mg. per 100 g. dry weight for the red variants to 1·76 mg. per 100 g. dry weight for the white variants.

TABLE 25.—*Colour Variants of* Metridium senile*

Colour	Carotenoid Distribution	
	Relative amounts	Distribution
White	very little	*Astaxanthin esters*† and free astaxanthin.
Brown (varying shades)	least	*Astaxanthin esters* or metridin esters, carotene, xanthophylls and xanthophyll esters.
Yellow orange	considerable	*Metridin esters, xanthophyll esters*, carotenes, xanthophylls
Red with Brown	much	*Metridin esters*, or astaxanthin esters.
Red	much	*Metridin esters*, occasionally accompanied by free or esterified astaxanthin, free metridin, xanthophylls and carotenes.

* After Fox, D. L., and Pantin, C. F. A., (1944), *Biol. Rev.*, **19**, 121.
† Pigments italicized predominate.

Heilbron and his colleagues[25] isolated two esters from *Tealia felina*; one was very similar to actinioerythrin and the other gave on hydrolysis an acidic carotenoid very similar to astacin. Similar pigments were encountered in the red variant of the Pacific Coast *Epiactis prolifera*

by Fox and Moe ;[28] traces of carotene were also present. The green variant was not examined.

A pigment closely related to astaxanthin occurs in *Gorgonia* and *Pennalia* spp.[12,18]

Lederer[1] considers the early work of Krukenberg on corals to be inaccurate, but gives no evidence to support this view. Karrer and Solmssen[6] found no carotenoids in *Asteroides calyculans*.

Formation

(i) *Nutritional Factors*

Schultze's[13] early work had suggested that pigmentation of *Hydra* depended on the nutrition of the animal, so it was not unexpected that experiments on *Actinia equina* indicated the alimentary origin of its carotenoids.[22] Animals raised from eggs on a carotenoid-free diet were without carotenoid pigmentation and starved animals placed on the same diet regenerated the pharynx and tentacular cycles containing only traces of carotenoids ; these animals were pigmented as soon as carotenoids were made available in the foodstuffs. Evidence that these animals can alter ingested carotenoids is less clear, but it is extremely interesting to note that starved " reds ", or " greens ", or " browns " always regained their *original* colour when fed the same carotenoid-rich diet (shrimps' eggs).

No full explanation of the formation of different carotenoids is yet available ; Fox and Pantin[9] suggest that it may be due either to selective assimilation of carotenoids or to selective metabolism.

In the case of *Metridium senile* the pigmentation is not so labile, for Fox and Pantin[27] found no colour changes when these animals were exposed for long periods to varying conditions of nutrition. It has already been noted that differently coloured types contain different amounts of carotenoids, so these two facts together suggest a genetical disposition to store a certain amount of carotenoids which is to a great extent independent of the environment. This further implies that *M. senile* either has the ability to synthesize carotenoids *de novo* ; or, more probably, does not utilize stored carotenoids under any conditions.

(ii) *Effect of Light*

Studer[17] and Elmhurst and Sharpe[29] have investigated the effect of light on pigmentation (? carotenoid), the former in *Hydra* species and the latter in *A. equina*, *Anemonia sulcata*, and *Tealia felina*. In all species high light intensity stimulated increased pigmentation. As might be expected from the results of the nutritional studies, *M. senile* is not susceptible to changes of light intensity.[27]

Function

Fox and Pantin[27] in their work on *Metridium* have ruled out any suggestion that carotenoids play any part in adaptive coloration of the animals and conclude that their function is "biochemical." Just what this biochemical function is, is not at the moment apparent.

It is very possible that carotenoids play a rôle in reproduction for the very small amounts of carotenoids present in the pale variants of *Metridium* are concentrated in the gonads. Other observations suggestive of such a function have been recorded:—M'Intosh[30] and Guberlet[31] state that the ovaries and testes of *Aurelia flavidula* (*A. aurita*) are yellow and red respectively. The stalk tissue of *Corymorpha tomoensis*, which according to Okeda[32] has special powers of regeneration, is deep red, but there is yet no evidence that the pigment is a carotenoid. Perhaps the most important pointer is that provided by Schultze[13] who noted a transference of carotenoids in *Hydra circumcinta* during regeneration and gametogenesis. Carotenoids move from the tissues into the maturing egg leaving the parent deficient in carotenoids. No explanation can be given why the process is not essential to all *Hydra*, because, for example, the gonads of *Hydra fusca* (*Pelmatohydra oligactis*) are not red.

The important relationship between structure and general pigmentation in the coelenterata is discussed by Fox and Pantin.[9]

ECHINODERMATA

Asteroidea

In 1881 Merejkowsky[12] reported that 20 species of echinoderms contained "zooérythrine rouge" which is probably identical with astaxanthin. It was however not until 1934 that Karrer and Benz[33] identified astaxanthin (astacin) in *Ophidiaster ophidianus*. In the same year von Euler and Hellström[34] isolated from *Asterias rubens* a blue chromoprotein which yielded a carotenoid which they named *asteric acid* ($C_{40}H_{56}O_6$). Later work leaves no doubt that asteric acid is astaxanthin.[34A] *Echinaster sepositus* contains astaxanthin[6] as does *Crossaster* (*Solaster*) *papposus*, *Solaster endica*, and *Porania pulvillus*.[34] It occurs in the latter as a violet-red chromoprotein, exhibiting an absorption spectrum in water with maxima at 492 and 458 mμ. The dorsal skin of *Crossaster papposus* contains a water soluble carotenoid protein which yields free astaxanthin on denaturation and extraction with acetone. The red variety gives a protein complex which is precipitated at 50 per cent. saturation with ammonium sulphate, and the blue variety a complex precipitated at 33 per cent. saturation.[35]

Fox and Scheer[36] in their detailed study of echinoderm pigments found that in the asteroids, *Astropecten californicus*, *Patiria miniata*, *Pisaster ochraceous*, and *P. giganteus*, that the predominant pigment was astaxanthin, that no esterified xanthophylls were present except perhaps in traces in *P. giganteus*, and that the free xanthophyll fraction was more abundant than the carotene fraction, which was mainly β-carotene. A pigment very similar to zeaxanthin was present in considerable amounts except in *A. californicus* in which *pectenoxanthin* (*see* p. 176) was detected. *P. ochraceous* apparently contained *mytiloxanthin* and *P. giganteus*, *metridin*. Mytiloxanthin is the characteristic pigment of the Californian mussel and in *P. ochraceous* may be derived from this mollusc on which it feeds extensively. A carotenoid-protein complex existed only in *P. giganteus*, the carotenoid involved being metridin. (*See also* Table 24.)

There is an interesting preferential accumulation of carotenoids in certain organs of these asteroids. For example, the carotenoids in the skin of *P. ochraceous* amounted to 49 per cent. of the total, and in the pyloric caeca to 4·7 per cent. The situation is reversed in *P. giganteus*, the corresponding figures being 7·2 per cent. and 22 per cent.; why this should be so is not easily apparent. The concentration of carotenoids in the pyloric caeca is higher than that in the skin in both species, being fifty times higher in *P. ochraceous* and twice in *P. giganteus*.

Lönnberg[37] has also noted carotenoids in the pyloric caeca of *Astropecten irregularis* and *Henricia sanguinolenta*.

Ophiuroidea

Qualitative detection of carotenoids has been made by Lönnberg[37] in a number of ophiuroids.* In the three species studied by Fox and Scheer,[36] *Ophiopteris papillosa*, *O. spiculata*, and *Ophiothrix rudis*, the outstanding feature was the absence of carotenes. The xanthophyllic fraction contained a pigment similar to taraxanthin and a new very unstable pigment which was not characterized. *O. papillosa* also contained *pectenoxanthin*, and in all species the presence of xanthophyll esters was consistently indicated.

Holothuroidea

Lönnberg[37] claims that the red-yellow gonads of *Mesothuria intestinalis* and the blue gonads of *Cucumaria lactea* contain a mixture of carotenoids. Fox and Scheer[36] found small amounts of carotenoids in *C. lactea* (0·029 mg. per 100g.), of which 48 per cent. is carotene. As echinenone (*see* p. 163) is present, this figure for carotene is probably

* *See* Table 27.

an over-estimate, for in the phase separation technique, echinenone is epiphasic and remains with the carotenes.

Manunta has reported the presence of carotenoids in the lungs, mesentery, intestine and gonads of *Holothuria forskali*, *H. tubulosa*, and *H. polii*. The greatest concentration exists in the gonads of the first two species, whilst in *H. polii*, the intestine is the major site of accumulation.

Crinoidea

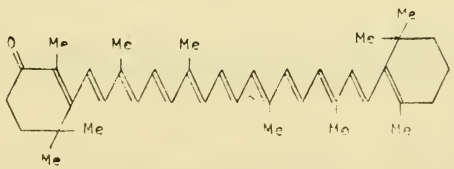
Karrer and Solmssen[6], in finding no detectable amounts of carotenoids in the sea lily *Antedon rosacea*, have confirmed the previous report of Lederer.[1] Lönnberg,[4] however, has noted them in *A. petasus*.

Echinoidea

This class has been investigated rather more fully than have those just discussed.* It was from the gonads of *Paracentrotus* (*Strongylocentrotus*) *lividus* that Lederer[38] obtained the pigment echinenone (Table 26 and Fig. 23). Echinenone also occurs in the gonads of *Echinus esculentus* and *Echinocardium cordatum* and also in the perivisceral fluid of the latter species.[38A]

It was at first thought that echinenone was characteristic of echinoids but recently Goodwin and Taha[38B] have shown that, as suggested by Lederer but rejected by Heilbron and Karrer[7,8], it is probably identical with myxoxanthin isolated from *Oscillatoria rubescens* by Heilbron and Lythgoe[38C] (*see* p. 136) and with aphanin obtained from *Aphanizomenon flos-aquae* by Tischer[38D] (*see* p. 136). It also occurs in the gonads of the gastropods, *Patella vulgata* and *P. depressa*[75] (*see* p. 179) and in the sponge *Hymeniacidon sanguineum*[7,8] (*see* p. 156). It thus appears to be widely distributed in algae and marine invertebrates and the interesting biochemical problem of whether the animals obtain echinenone directly from their algal food or make it by oxidizing a pigment such as β-carotene, remains to be solved.

Goodwin and Taha[38B] consider the structure of echinenone to be in all probability 4-keto-β-carotene :—



Echinenone (probably)

* *See* Table 27.

There is a differential distribution of carotenoids in *Echinocardium cordatum*, for Goodwin and Srisukh[38A] found β-carotene and echinenone to be the major components of the gonadal carotenoids, lutein occurring only in traces ; in the peri-visceral fluid, however, there are equal amounts of echinenone and lutein but no β-carotene. Previously, Lönnberg[4] had not obtained " satisfactory " [sic] results with *P. lividus* but had noted carotenoids in *Psammechinus miliaris*. Apart from α- and β- carotenes, Lederer[38] also isolated a second new carotenoid from *P. lividus*, *pentaxanthin* ($C_{40}H_{56\text{ or }58}O_5$), which appears to contain 3 hydroxyl groups and, although it has an absorption spectrum very similar to that of lutein (xanthophyll) it is much more strongly adsorbed on alumina than is lutein. (*See also* Table 26 and Fig. 23.)

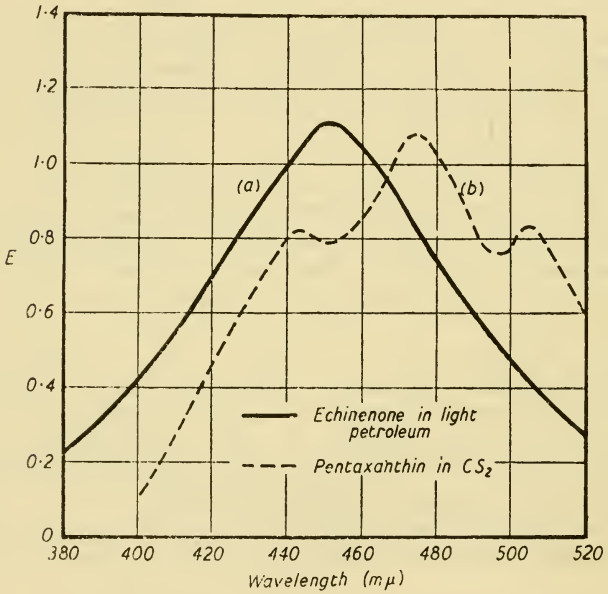


Fig. 23.—The absorption spectra of Echinenone (*from Goodwin, T. W., and Taha, M. M. (1950)* Biochem. J., **47,** 244) and Pentaxanthin in CS_2 (*from Lederer, E. (1938)* Bull. Soc. Chim. Biol., **20,** 611).

Fox and Scheer[36] in their extensive study, to which reference has previously been made, examined the carotenoid distribution in *Dendraster excentricus*, *Stronglyocentrotus franciscanus*, *S. purpuratus*, *Lytechinus*

pictus. At least 60 per cent. of the total carotenoids are carotenes, mostly β-carotene; α-carotene and echinenone were detected in *L. pictus* and zeaxanthin in *S. purpuratus*.

TABLE 26.—*Characteristic Echinoid Carotenoids*

Pigment	*m.p.*	*Absorption spectra maxima (mμ.)* *Carbon disulphide*	*Light petroleum*
Echinenone [1,2]	178–179°	488–494	458–460
Pentaxanthin [1]	209–210°	506, 474, 444	—

REFERENCES TO TABLE 26.

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2. GOODWIN, T. W., and TAHA, M. M. (1950), *Biochem. J.*, **47**, 244

FORMATION AND FUNCTION

Only little is known about the formation of carotenoids but some interesting correlations between the feeding habits of echinoderms and their carotenoid make-up has been revealed by the work of Fox and Scheer.[3 6] The essentially herbivorous echinoids, crinoids and holothuroids, contain on the average five times less carotenoids than do the carnivores, the asteroids and the sphiaroids. The predominating carotenoids of the carnivores are highly oxygenated whilst those of the herbivores are predominantly hydrocarbon. The reasons for these differences are not yet apparent.

There is considerable correlation between sex and carotenoid distribution in the three echinoid species studied by Fox and Scheer.[3 6] Male *Dendraster* contain more carotenoids than do the females, the increase being more marked in the xanthophyll fraction (3 times) than in the carotene fraction (twice). The skin of both sexes contained little and the intestines much pigment but the gonads showed marked sexual differences. The concentration in the ovaries was three times that in the testes, the pigments in both sexes being entirely carotenes. In contrast the testicular tissue of *S. purpuratus* contained five times as much pigment as did the ovarian tissue. This increased pigmentation in the testes was due more to a preferential accumulation of carotenes rather than of xanthophylls. There was also a sexual differentiation in the intestines in this species; the female gut contained three times more xanthophylls and 4/3 more carotene than did the male gut. This is the reverse of the situation in the gonads.

The proportions of the total body carotenoids mobilized in the testes and ovaries of *Strongylocentrotus* and *Lytechinus* are 17 and 25 per cent., and 27 and 77 per cent. repectively. It is most interesting to find that, in spite of the mobilization of carotenoids in testicular tissues, the spermatozoa are colourless.

ANNELIDA

Lönnberg[4,5] has demonstrated the presence of carotenoids in the skin of a number of polychaete worms. (Table 27.)

MacMunn[28] observed a carotenoid, hidden from view beneath the superficial melanin layer of the integument, in the black *Arenicola piscatorum*.

The polychaete *Thoracophelia mucronata* is the only worm for which quantitative data have been recorded.[39] It contains 0·38 mg. per 100g. of carotenes but is completely devoid of xanthophylls. In this respect it differs from most marine invertebrates. Its diet is sand on which is adsorbed marine detritus. The detritus contains both β-carotene and xanthophylls but, as just stated, the worms accumulate only β-carotene. The fate of the ingested xanthophylls is still doubtful, for they are not excreted in the faeces; they may be destroyed in the lumen before they have the opportunity to be absorbed, or they may be absorbed as such and then either completely oxidized or converted into the yellow coloured, blue fluorescent chromolipids which are stored in considerable quantities, but which are not carotenoids.[39A]

PLATYHELMINTHES

Francotte[39B] states that the colours of certain Polycladia (class Turbellaria) are caused by carotenoids obtained from the Ascidia on which they live.

ASCHELMINTHES

Lönnberg[4,15] has noted the presence of carotenoids in a number of Nemerteans (*see* Table 27).

SIPUNCULOIDA

Krukenberg[39A] reported the presence of a carotenoid in the digestive juice of *Siphonostoma diplochaitos* and Lönnberg found carotene in *Phascolosoma elongatum*.

PRIAPULIA

The zephyrean, *Priapulus caudatus*, contains carotenoids.[4,15]

BRACHIPODA

Carotene has been found in *Crania anomala* and *Terebratulina caput serpentis*.

POLYZOA (BRYOZOA)

Early reports indicated the presence of a neutral carotenoid in the epidermis of *Bugula neritina*,[39C] whilst what would appear to be astaxanthin has been found in *Lepralia foliacea* and *Flustra foliacea*.[18]

The only recent work on this phylum is that of Villela,[39D] who could not find carotene in *Bugula neritina* or in *B. flabellata*; it was however, present in *Schizoporella unicornis*, *Steganoporella magnilabris*, and *Trigonospora* sp. Xanthophylls, in the form of esters, occurred only in *Trigonospora*.

ARTHROPODA

Crustacea

Considering the critical position that copepods occupy as a fundamental food supply for the production of more highly organized marine life, one would have expected to find that their carotenoids would have been investigated more thoroughly than is the case.

Recently, however, Kon and his colleagues[39E,F,G] have taken up this problem and have also found the answer to the question " whence does the massive store of vitamin A in fish originate ? " This question is discussed later (*see* p. 173).

Lwoff[40,41] has examined, chiefly histologically, the carotenoid distribution in the copepod *Idya furcata*. He concludes that a carotenoid, laid down in the oocytes as a protein complex, is liberated during development and is fixed in the eye before cellular differentiation of the embryonic intestine. The red and blue pigments of the copepod are respectively free carotenoid and a carotenoid-protein complex; the retina contains both pigments, the protein complex being in an internal retinal layer; the free pigment occurs in the blood, whilst the complex exists in the eggs as a constituent of the vitelline spherules. Lwoff also noted carotenoids in the luminous organs of Euphausidae and in the retinas of *Nebalia* and *Pagurus prideauxii*. It is important to note here that Lwoff[40,41] and his co-workers[42] and Verne,[43,44,45] at almost the same time, were the first to realize that carotenoids existed in marine invertebrates attached to proteins.

As to the identity of carotenoids in copepods, Euler, Hellström and Klussmann[46] reported the presence of small amounts of α- and β-carotene and a great deal of astaxanthin in *Calanus finmarchicus*, and Lederer[1] obtained crystalline astaxanthin (astacin) from *P. prideauxii*. Recently Goodwin and Srisukh[47] have shown that the red pigmentation of *Tigriopus fulvus* is due to the presence of free and esterified astaxanthin; the free pigment also occurs in the eggs.

Lönnberg[4] in his general survey of carotenoids in marine invertebrates noted carotenoids in a schizopod, *Mysis flexuosa*, an isopod, *Idothea baltica*, two amphipods, *Haploops tubicola* and *Neohela monstrosa*; and in the cirripeds, *Balanus balanus* and *Scalpellum scalpellum*. The gonads of the cirripeds *Lepas fascicularis* and *L. anatifera* contain astaxanthin.[48] The amphipods, *Orchestia gammarelus*, and *Gammarus marinus* contain astaxanthin.[61] (*See also* Table 28.)

Wagner[46A] has claimed to have isolated crystalline β-carotene in large amounts from the mixed krill obtained from the stomachs of whales.

The recent work of Kon and his colleagues,[39E,F,G] however, confirms the impression that β-carotene is a very minor component of crustacean carotenoids. They found only traces of this pigment in *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *Pandalus bonnieri*, *Spirontocarus spinus*, *Crangon allmanni* and *C. vulgaris*, whilst astaxanthin occurred in large amounts. As these species are typical of a mixed "krill", this investigation suggests that Wagner[46A] was almost certainly mistaken in identifying his carotenoid as β-carotene. As Moore[46B] points out, however, " . . . in view of Goodwin's observations on astaxanthin and β-carotene in locusts (*see* p. 225), it might be unwise at the moment to discredit completely Wagner's claim ".

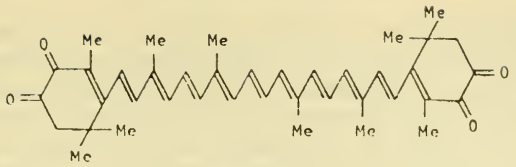
It is on the decapods that most of the work has been carried out. A pigment, presumably astaxanthin, was first obtained from the lobster by Pouchet in 1872. Other early workers (see Lederer[1] for full references) found two pigments:

(1) red, with one absorption band (astaxanthin) variously named crustaceorubin, zooerythrin, tetronerythrin, and vitellorubin.

(2) yellow, with 3 absorption bands, presumably a neutral xanthophyllic carotenoid called vitellolutein.

Lönnberg[4] and Verne[43,44] reported carotenoids in the eyes, blood, carapace and hypodermal chromatophores of a number of decapods. Modern qualitative investigations were however initiated by Kuhn and Lederer,[49] who found that the green chromoprotein (ovoverdin) of the eggs of the spring lobsters (*Homarus vulgaris*) broke down to liberate "*astacin*." It was also found in the hypodermis, ovaries and blood and it now appears to be universally distributed in crustacea (*see* Table 28). Astacin was first obtained crystalline from the eggs of *Maja squinado*[49,50] and is 3 : 4, 3′ : 4′-tetra-keto-β-carotene. (Fig. 24.)

β-carotene also exists in these eggs in small amounts (between 2·5 and 20 per cent. of the astaxanthin present). It is, however, completely absent from lobster eggs.[50A]



Astacin

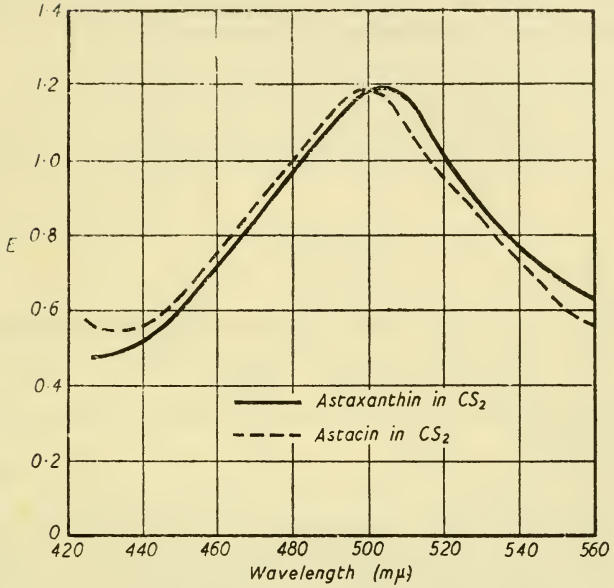


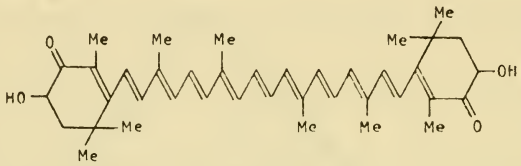
Fig. 24.—The absorption spectra of Astaxanthin and Astacin (*from Goodwin, T. W., and Srisukh, S. (1949)* Biochem. J., **45**, 263).

Kuhn and Lederer found that the pigment from the eggs behaved differently from that of the hypodermis and carapace (blue spots). The former was hypophasic to 90 per cent. methanol and the latter epiphasic. They both gave astacin on saponification. It was assumed that the pigments were two distinct esters of astacin, the first being named " ovoester."

However, in 1938, Kuhn and Sörensen[61] showed that astacin was an oxidative artefact of *astaxanthin*, the naturally occurring pigment. Conditions such as alkaline saponification readily bring about the change. Astaxanthin is 3, 3′-dihydroxy-4 : 4′-diketo-β-carotene and is identical with Kuhn and Lederer's ovoester, the hypodermal ester

is an astaxanthin ester. Recently, Goodwin and Srisukh have re-investigated these pigments and whilst agreeing with Kuhn and his colleagues that the egg pigment is unesterified and hypodermis pigment esterified astaxanthin, they cannot agree that the astaxanthin in the carapace is esterified; they found only the unesterified pigment occurring in combination with protein. Whether these differences are due to the lobsters being obtained from different localities is not known.

Owing to keto-enol tautomerism astaxanthin and astacin exhibit acid properties and will dissolve in dilute aqueous alkali.



Astaxanthin

It is now certain[52] that astacin never occurs naturally and all reports of its presence are due to its formation by oxidation of astaxanthin during the manipulative processes. Because of this, in this book astaxanthin is always reported although the original workers may have described astacin.

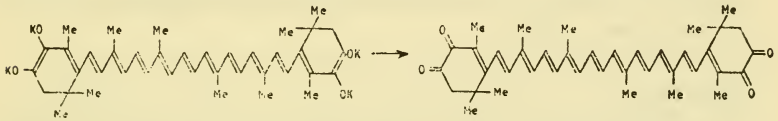
Kuhn, Stene and Sörensen[52] have given a list of all the sources of astaxanthin recorded up to 1939. It is clear from the work of these workers and that of Stern and Salomon[53,54] that ovoverdin and the blue pigment in lobster carapaces are astaxanthin-protein complexes. Ovoverdin is stable between pH 4–8, has an isoelectric point of pH 7, exhibits absorption bands at 640 mμ. and 470 mμ., has one molecule of astaxanthin linked to one molecule of protein,[54] and according to sedimentation data has a molecular weight of 300,000.[55] The complex is reversibly dissociated by short heating to moderate temperatures in the presence of neutral salts.[54]

Astaxanthin was first noted in *Nephrops norvegicus* by Burkhardt, Heilbron, Jackson, Parry and Lovern[55A] and Lederer and Fabre; recently Goodwin and Srisukh[47] have confirmed this and found that whilst the hypodermal pigment is esterified astaxanthin that of the carapace is not.

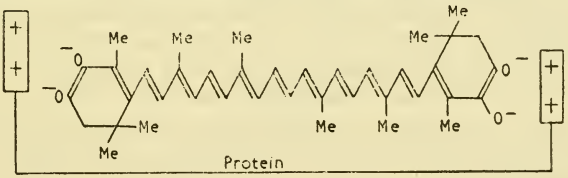
The blue pigment in the ovaries and developing embryo of the goose barnacles *Lepas anatifera* and *L. fascicularis* is also a carotenoprotein, the prosthetic group is astaxanthin and the protein an euglobulin. As the nauplii develop, their colour changes from blue to pink and

hatched nauplii are entirely pink.[48] This is consistent with the liberation by denaturation or some similar process of the carotenoid from its protein complex. Ball's[48] work, however, raises another possibility, that the colour is still due to a chromoprotein but one in which the protein-carotenoid linkage has changed from a salt type (ovoverdin) to some other type, for he has succeeded in producing a reddish-pink chromoprotein from the blue material. A solution of the blue pigment in 25 per cent saturated ammonium sulphate was slowly treated with *N* hydrochloric acid at 0° ; the colour gradually changed from blue to red ; addition of cold disodium hydrogen phosphate solution restored the original colour. If, however, the phosphate was not added but the acid solution brought directly to 50 per cent. saturation with ammonium sulphate the red pigment was precipitated ; it is soluble in water but cannot be reconverted into the blue pigment, the salt linkage having been irreversibly severed.

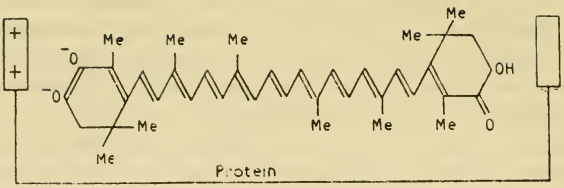
When astaxanthin is treated with potassium butoxide in the absence of air it turns blue owing to the enolization of the hydroxyl groups followed by the formation of a potassium salt.[47,52,56] On admitting air this salt is immediately oxidized to astacin, viz. :



The existence of the blue chromoprotein complex in lobster carapaces is explained in the same way, enolic astaxanthin is bound by ionic forces (salt link) to a protein and is in some way stabilized :



An explanation of green and brown chromoproteins has not yet been put forward. From the simple considerations of chromophoric groups it might be expected that in the brown chromoprotein only one β-ionone residue is enolized, whereas in the green chromoprotein both are enolized. Such a suggestion is probably an over-simplification and the possibility of stabilized resonance structures must be considered.



? Brown Chromoprotein

The difference in physical properties between astaxanthin and astacin are noted below.

	m.p.	*Absorption spectra maximum* Carbon disulphide
Astaxanthin ..	215–216°[46]	502 mμ.[47,51]
Astacin	240–243°[57]	500 mμ.[52]

It must be remembered that astaxanthin, once considered a specific crustacean carotenoid, has recently been detected in plants (*see* p. 135) and insects (*see* p. 221).

FORMATION

It is now generally accepted that synthesis of carotenoids *de novo* by arthropods (or, for that matter, by any animal) does not occur. The little evidence that is available, however, dates back to 1926 and 1927. Lwoff[40,41] showed that the eye pigment (*see* p. 167) of *Idya* was derived from the egg pigment, and by raising this animal on carotenoid-free regimes (marine mussels, washed rabbit red cells, *Escherichia coli*, etc.), he showed that the pigment was for the most part of alimentary origin ; he suspected that the caroteno-proteins were endogenous. According to Fox,[2] Lwoff has now abandoned this view. The crab *Carcinus maenas* stores carotenoids in its hepatopancreas and excretes them in its faeces ; on a carotenoid-free diet the hepatopancreas and the faeces lose their carotenoids.[58,59]

Brown[60] investigated quantitatively the carotenoid variations in the shrimp, *Palaemonetes vulgaris*, when it was maintained on various backgrounds. Those kept on a white background lost most of their pigmentation whilst those on a brown or black background maintained or even increased their carotenoid concentration. Fox[2] has criticized this work and points out that these results cannot be taken as indicating carotenoid synthesis by the shrimp, for many uncontrolled factors

may have been operating. Such factors would include the effect of photo-environment on absorption and retention of carotenoids, egg laying, and temporary sparing of carotenoids by drawing on other foodstuffs. It is now generally assumed that specific carotenoids such as astaxanthin are produced by oxidation of the ingested carotenoids, but which carotenoid is the precursor is unknown (but *see* p. 225).[61]

Function

A sexual function is suggested by the accumulation of carotenoids in the gonads but further than that it is impossible to go. Abeloos and Fischer[58,59] found that in gravid female *Carcinus maenas* the hepatopancreatic stores are transferred to the ovaries via the blood stream. Goodwin[50A] has recently shown that during the development of lobster eggs astaxanthin is not utilized in any way.

The free pigment is, however, liberated from its protein complex (ovoverdin) a week or so before hatching.

IMPORTANCE OF ZOOPLANKTON CAROTENOIDS AS A SOURCE OF PROVITAMIN A FOR FISH

The main sources of foodstuffs for fish which contain large amounts of vitamin A in their liver and intestinal wall are the zooplankton. Copepods make up the " permanent " source of zooplankton whilst the eggs and larvae of fish and invertebrates make up the " transitory " source.[62] Phytoplankton are utilized to a lesser extent.

Copepods live on diatoms which contain small amounts of β-carotene (the chief vitamin A precursor (*see* p. 269)), but whose chief pigment is fucoxanthin. Copepods apparently convert these diatomic carotenoids into their characteristic pigment astaxanthin and store only minute amounts of β-carotene. About the same relative pigment distribution is noted in eggs and larvae. Phytoplankton contain probably a little more β-carotene than do zooplankton.

It had long been realized that the amounts of β-carotene available in zooplankton were insufficient to account for the large amounts of vitamin A accumulated by plankton-feeding fish. It has been suggested that astaxanthin might conceiveably be a vitamin A precursor in fish;[65B] on general grounds this must be considered highly improbable (*see* Chapter 12) even though it has been claimed that astaxanthin isolated from the shrimp, *Aristeomorpha foliacea* (=*Penaeus foliaceus*), is vitamin-A active.[62A] The main objection to this work is that no account was taken of the possible presence in the astaxanthin fraction

of vitamin A itself; furthermore, the technique used to prepare astacin, which was inactive, would also remove any vitamin A originally present.

Assuming then that astaxanthin is inactive, three possibilities remain (i) zooplankton contain pre-formed vitamin A, (ii) fish can synthesize vitamin A *de novo* or (iii) vitamin A precursors, as yet unidentified, are utilized by fish and exist in zooplankton.

All the evidence we possess on all other animal species points away from the last two possibilities. They must, therefore, be considered extremely unlikely to function in fish. This is in spite of the fact that recently Lane[63] has claimed to have separated the vitamin A activity of zooplankton (*Temora turbinata* and *Centropages typians*) from their carotenoid fraction. The vitamin A-active fraction, which had an absorption spectrum with a maximum at 310 mμ., when fed to the fish *Limanda ferruginea*, resulted in the accumulation of vitamin A (λ-max. 325mμ.) in the liver. The conclusion that non-carotenoid material can be utilized as a vitamin A precursor, must be accepted with considerable reservation, pending much more rigorous demonstration of the purity of Lane's active fraction. The presence of considerable amounts of impurities in material containing vitamin A in small amounts could easily displace the absorption maximum to shorter wavelengths.

The first suggestion has always seemed the most likely, but it is only very recently that evidence has been obtained which has transformed it into a certainty. It will be interesting to follow chronologically the investigations leading up to this conclusion.

Drummond and Gunter's[64] pioneer studies showed that zooplankton oils from mixed copepods and *Calanus finmarchicus* exhibited very little vitamin A activity, contained no pre-formed vitamin A and only small amounts of β-carotene; phytoplankton oils from *Chaetoceros* spp. and *Lauderia borealis* exhibited slightly more activity by virtue of their higher β-carotene content, for no preformed vitamin A could be found.

In spite of a careful study completed in 1939 by Gillam, El Ridi and Wimpenny,[65] the problem still remained unresolved. The gross plankton hauls investigated by Gillam *et al.* showed the presence of both vitamin A and carotene. As the most prolific compounds of the phytoplankton fraction, *Rhizosolenia styliformis* and *Biddulphia sinensis* contained no vitamin A, it would not have been unreasonable to assume " by difference " that the vitamin A was located in the zooplankton. Against this is their observation that the preformed vitamin A content was maximal well before the zooplankton population was densest.

Because of this, Gillam and his colleagues rightly refused to consider that their investigation proved the presence of preformed vitamin A in zooplankton and it was not until ten years later that, largely owing to the considerable advances in chromatographic and spectrographic techniques, the problem was solved.

Nielands[65A] found considerable amounts of vitamin A in the eyes and hepatopancreas of the common lobster (*Homarus vulgaris*) and Kon and Thompson,[39E,F] at almost the same time, showed that it was present in a number of species of smaller crustacea, being concentrated in the eyes and the exoskeleton.

It seems, then, that the major portions of vitamin A of plankton-feeding fish comes to these animals preformed. It must not, however, be assumed that fish cannot convert the small amount of β-carotene in their diet into vitamin A. There is some positive evidence concerning this point; Neilands[65A] in 1949 demonstrated this conversion in the Atlantic cod (*Gadus callarias*), whilst as long ago as 1939 Morton and Creed[65B] observed the conversion of β-carotene into vitamin A_2 by fresh water fish (dace and perch).

MOLLUSCS

Lamellibranchs

Although Lönnberg[4] had found that a number of species gave positive tests for carotenoids, the first intensive investigation was carried out by Lederer[66] who isolated a neutral xanthophyllic pigment, *glycymerin*, from the sex glands of the scallop, *Pectunculus glycymeris*. The pigment which is not attached to proteins is distributed on the inside and in the lower superficial layers of the gonads; the upper superficial layer being colourless. Lederer[67] later examined the gonads of *Pecten maximus* and found that the fat-soluble pigments were principally a mixture of non-esterified xanthophylls although a little β-carotene was detected. The main pigment was unique, and termed *pectenoxanthin*. This pigment ($C_{40}H_{56\pm2}O_5$) has also been identified in the gonads of *Pecten jacobaeus*;[6] and a very similar pigment exists in the gonads of *Volsella modiolus*.[46] In this instance a portion of the pigment is attached to the protein. Astaxanthin occurs in *Pleurobranchus* species and in the feet of *Lima excavata*.[61] Preliminary work by the author has revealed the presence of highly oxygenated xanthophylls in *Modiolus modiolus*.[61A]

However, by far the most comprehensive study on Lamellibranchs is that of Scheer[68] on *Mytilus californianus*. This species contains

almost no carotenes, the main pigments being *zeaxanthin* and *mytiloxanthin*. The latter which is apparently a characteristic acidic carotenoid, occurs only in the free state; small amounts of a carotenoid similar to Lederer's glycymerin were also noted.

Both carotenes and xanthophylls were noted in the edible oysters *Gruphea angulata* and *Ostrea edulis*; the visceral mass contains about twenty times as much pigment as the rest of the animals.[68A]

Strain[68B] has recently shown that the rose-pink coloration of the nudibranch mollusc, *Hopkinsia rosacea*, is due to a new carotenoid, *Hopkinsiaxanthin* (Table 27 and Fig. 25). Although insufficient material was available for a complete chemical study, it appears that this pigment contains two hydroxyl groups and one carbonyl group.

TABLE 27.—*Characteristic Carotenoids of Marine Molluscs*

Name	m.p.	Absorption Spectra Maxima
Glycymerin[1]	148–153°	495 mμ. (CS_2)
Pectenoxanthin[2]	182°	454, 488, 518 mμ. (CS_2)
Mytiloxanthin[3]	140–144°	500 mμ. (CS_2)
Hopkinsiaxanthin[4]	—	466, 497 mμ. (Petroleum, b.p. 50–70°)

REFERENCES TO TABLE 27.

1. LEDERER, E. (1933), *C. R. Soc. biol. Paris*, **113**, 1015.
2. LEDERER, E. (1934), *C. R. Soc. biol. Paris*, **116**, 150.
3. SCHEER, B. T. (1940), *J. biol. chem.*, **136**, 275.
4. STRAIN, H. H. (1949), *Biol. Bull. Woods Hole*, **97**, 206

FORMATION AND METABOLISM

Scheer's investigation on *M. californianus* suggests that carotenoids play a positive rôle in mussel economy. There was no marked seasonal variation in any of the carotenoids and prolonged fasting, up to 196 days, resulted in no appreciable change in pigment concentration, although there was a suggestion that mytiloxanthin may be to some extent converted into zeaxanthin. There is, however, a considerable drop in amount of carotenoids present, especially in the gonads, after

fasting and this may be taken to indicate that carotenoids play some part in gametogenesis and/or the gonads act as a reserve store of carotenoids. Contrary to a suggestion of Zechmeister[69] it was found that the pigment concentrations of normal and fasted mussel tissue are independent of lipid concentrations. Mussels, kept in a similar environment to that of the fasted animals, were divided into two groups, one of which was fed a carotenoid-free diet and the other a diet of *Nitzschia closterium*. The animals fed on *N. closterium* increased their concentrations of zeaxanthin and mytiloxanthin, the latter being formed probably

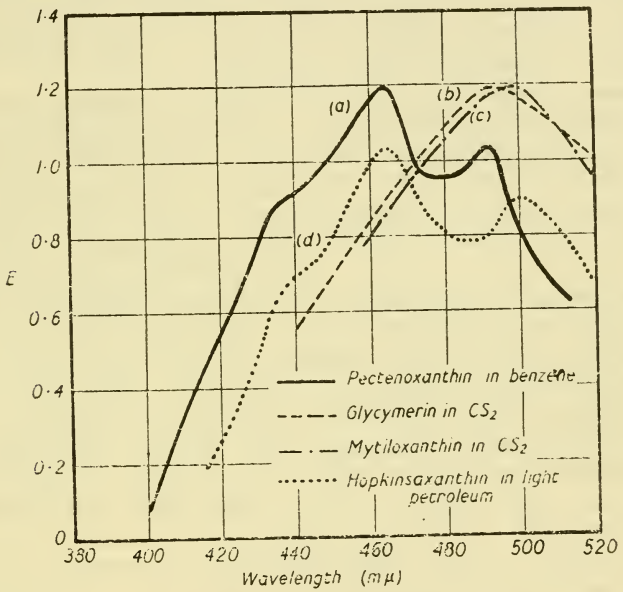


Fig. 25.—The absorption spectra of Pectenoxanthin (*from Lederer, E. (1938)* Bull. Soc. Chim. Biol., **20**, 611), Glycymerin (*from Fabre, R., and Lederer, E. (1934)* Bull. Soc. Chim. Biol., **16**, 105), Mytiloxanthin (*from Scheer, B. T. (1940)* J. biol. Chem., **136**, 275), and Hopkinsiaxanthin (*from Strain, H. H. (1949)* Biol. Bull. Woods. Hole, **97**, 206).

by oxidation of alimentary carotenoids, but which of these were involved could not be determined. The mussels on the carotenoid-free diet lost much more pigment than did those that were merely fasted. This interesting result may be due to the utilization of stored carotenoids by the activities of feeding in a condition of virtual starvation, for body weight data indicated that the carotenoid-free diet was not well absorbed. In the fasted animals no useless energy was expended in " feeding " and the

carotenoids were conserved. Once more a sexual differentiation was noted, for the concentration of xanthophylls in all female tissues was higher than in male tissues. In the case of epiphasic " non-carotene " pigments the highest concentration was found in the male gonads, although the female somatic tissue had a higher concentration than had male somatic tissue. Scheer comments on his observations thus: " the concentration of hypophasic pigments (xanthophylls) can be considered to be a true secondary sex characteristic, while that of the epiphasic pigment is apparently dependent only on the accumulation of pigment in the testes, the composition being relatively uniform in all other tissues." It is difficult to understand why a statistically significant accumulation of epiphasic carotenoids in male gonads cannot equally be considered of sexual significance.

Scheer concluded his exhaustive survey by examining carotenoid metabolism during spawning and his results again suggest the mediation of these pigments in reproduction. In the females the shed ova contained carotenoids which were qualitatively and quantitatively the same as in the female gonads; however, the difference between the carotenoid content of normal and spent females indicates a loss unaccounted for by the shed ova. In the case of the males the spermatozoa contained no carotenoids, but there was a suggestion of loss of body carotenoids in fasted males after spawning; the loss is in the epiphasic and mytiloxanthin fractions and there was a small increase in zeaxanthin almost exactly equal to the loss of mytiloxanthin.

It will have been noted that a characteristic of molluscs is that xanthophylls greatly predominate over carotenes. Although their natural habits predispose to this condition, Scheer[69] carried out an experiment which indicated a definite predilection of *M. californianus* for xanthophylls. He fed the mussels on *Procentrotum micans*, which has a xanthophylls : carotenes ratio of 9 : 1, and the resulting faeces contained xanthophylls and carotenes in the ration of 6 : 1 ; thus, assuming equal stability of the pigments in the intestinal tract, xanthophylls are preferentially absorbed.

CEPHALOPODA

The most outstanding fact concerning carotenoids in this group is their comparative absence.

Lönnberg[70] examined three species, *Sepiola scandica*, *Rossia macrosoma*, and *Eledone cirrosa*. Lutein (xanthophyll) was detected in the eyes of all three species; otherwise, apart from traces of (unspecified) pigments in the mantle and testes, and large amounts in the

liver of *E. cirrosa*, no carotenoids were detected. Specially significant is their absence from the eggs of *R. macrosoma* and *S. scandica*. A xanthophyll and a carotenoid-albumin have been reported in the retinal rods of *E. moschata* but the evidence presented is not compelling.[71] Wagner and Vermeulen[71A] and Leong[71B] state that carotenoids are absent from cuttle-fish.

More recently Fox and Crane[72] have investigated the pigments from two Pacific cephalopods, the two-spotted octopus, *Paroctopus bimaculatus*, and the common squid, *Loligo opalescens*. Carotenoids occurred only in traces in the eyes of the squid, and this had been previously noted by Wald;[73] none or only "suspected" traces occurred in other organs. In the octopus the liver and the ink were the only tissues containing any carotenoids; the liver contained β-carotene and free and esterified xanthophylls both of the neutral and acidic type; the distribution was similar in the ink except that no β-carotene was present. The chief carotenoid was lutein (xanthophyll) and the acidic pigment appeared to be different from both metridin and astaxanthin. Measured as lutein the carotenoid levels in the liver and ink were 3·5 mg. per 100 g. and 0·55–0·70 mg. per 100 g. respectively; these levels dropped during starvation.

The presence of carotenoids in the ink can promote much speculation. As Fox and Crane point out, loss of carotenoids can take place in many ways: sloughing of skin, growth and moult of feathers, discharge of ear wax in cattle, and secretion from the femoral pits in iguanas, but apart from the secretions from internal structures relating to reproduction (eggs in many oviparous vertebrates and invertebrates, and milk from mammary glands), no other such secretion of carotenoids is known save that of the ink.

GASTROPODA

Lönnberg has detected varying amounts of carotenoid, in a number of marine gastropods. The gonads of the limpets, *Patella vulgata* and *P. depressa*, have been studied by Goodwin[74] and Goodwin and Taha.[75]

Five carotenoids were identified: α- and β-carotenes, echinenone, cryptoxanthin, and zeaxanthin. All these occurred in both testes and ovaries and in the same relative proportions, viz.: 1 : 5 : 3 : 3 : 3 respectively. Little variations in both total and relative amounts were found in limpets collected at the same time in various parts of Great Britain. No differences could be found between *P. vulgata* and *P. depressa*.

A small proportion of the ovarian, but none of the testicular carotenoids, was attached to protein. The brownish-green colour of the ovaries was, however, not to any significant extent due to carotenoids, but to a complex formed between a protein and an as yet unidentified greyish-green pigment. This pigment appears somewhat similar to the water-soluble pigment found in the flesh of some fish.

The pink to red colour (according to season) of the testes is due to the carotenoids present, the green pigment being always absent.

After spawning the female gonads are much paler and contain less carotenoids, whereas the male gonads assume a brick red colour and contain, as far as can be ascertained, nearly the same amount of carotenoids as before spawning;[74] from a microscopic examination it appears that the spermatozoa contain only traces, if any, of carotenoids. There is a close analogy here with the mussel, *M. californicus*.

It has recently been stated that young *Aplysia* (*delipans* and *punctata*) contain unidentified carotenoid and chlorophyll derivatives. In dark-adapted neurons, irradiation with light of wavelengths absorbed by the carotenoids increases the reaction time of the neuron; the reverse occurs when light of wavelength corresponding to that absorbed by chlorophyll is used. It is further claimed that irradiation of the carotenoid *in vivo* increases the number of absorption bands in its spectrum. Irradiation of a solution of the pigment *in vitro* reduces the number of bands.[76]

To conclude this chapter, all the available qualitative and quantitative data on carotenoid distribution are recorded in Tables 28 and 29.

TABLE 28.—*Carotenoid Distribution in Marine Invertebrates*

Species	α-Carotene	β-Carotene	γ-Carotene	Lycopene	Torulene	Lutein	Astaxanthin	Zeaxanthin	Myxoxanthin	Pectenoxanthin	Glycymerin	Echinenone	Cryptoxanthin	Hopkinsiaxanthin	Pentaxanthin	Metridin	Taraxanthin	Actinioerythrin	Sulcatoxanthin	Violerythrin	Reference No.
A. ARTHROPODS																					
Ampelisca tenuicornis ..		+				+															1
Anapagurus chiroacanthus							+														2
Astacus fluviatilis ..							+														3, 8
Homarus vulgaris ..							+														4, 5, 6
Balanus cretanus		+				+															1
Calanus finmarchicus ..	+	+					+														7, 25
Calocaris macandreae ..		+				+															1
Cancer pagurus		+					+														8, 9
Carcinus maenas ..		+																			2
Crangon allmani ..		+				+	+														1, 14
Crangon vulgaris ..							+														14, 25
Diaptomus bacillifer ..		+																			10
Ebalia tumefacta		+				+															1
Eupagurus prideauxii ..						+															7
Euphausia superba ..		+					+														25
Galathea intermedia ..		+				+	+														1
Gammarus marinus ..							+														13
Idothea emarginata ..		+				+															1
Idothea neglecta		+					+														1
Leander serratus ..							+														8
Lepas anatifera							+														2
Lepas fasciularis ..							+														2
Maja squinado		+					+														11, 12
Meganyctiphanes norvegica		+					+														14, 15
Munida banffia		+				+	+														1
Mysis flexuosa		+																			1
Nephrops norvegicus ..		+				+	+														1, 6, 8, 44
Orchestia gammarellus ..							+														13
Pagurus bernhardus ..		+				+															2
Pagurus rubescens ..		+																			1
Palaemon fabricii ..		+				+	+														1, 2
Palaemon serratus ..							+														8
Palinurus vulgaris ..							+														8
Pandalus bonnieri ..		+					+														14, 25
Pandalus borealis ..							?														1
Pandalus brevirostris ..							+														1
Pandalus montagui ..		+					+														2
Porcellana longicornis ..		+																			1
Portunus depurator ..		+																			1
Portunus longicornis ..		+				?															1
Portunus persillus ..		+				+															1
Portunus puber							+														8
Spirontocaris spinus ..		+					+														14, 25
Thysanoessa raschii ..		+					+														14, 25
Tigriopus fulvus							+														6
B. MOLLUSCS																					
Acera bullata		+																			1, 23
Anomia ephippium ..		+																			1
Aporrhaispes pelecani ..		+				+															1
Astarte sulcata		+																			1
Buccinum undatum ..		+				+															1
Calliostoma miliare ..		+				+															1
Capulus hungaricus ..		+				+															1
Cardium echinatum ..		+				?															1
Cardium tuberculatum ..						?															1, 7
Chaetoderma nitidulum ..		+																			1

TABLE 28.—*Carotenoid Distribution in Marine Invertebrates*—contd.

Species	α-Carotene	β-Carotene	γ-Carotene	Lycopene	Torulene	Lutein	Astaxanthin	Zeaxanthin	Myriloxanthin	Pectenoxanthin	Glycymerin	Echinenone	Cryptoxanthin	Hopkinsiaxanthin	Pentaxanthin	Metridin	Taraxanthin	Actinioerythrin	Sulcatoxanthin	Violerythrin	Reference No.
Cochleodesma praetenue		+				+															1
Cultellus pellucidus		+				+															1
Cyprina islandica		+				+															1, 8
Dendronotus frondosus						+															1
Dentalium entale						+															1
Dosina exoleta						+															1, 18, 19
Doto coronata		+																			1
Eledone cirrosa						+															24
Gibbula cineraria		+				+															17
Gibbula tumida		+				?															1
Hopkinsia rosacea														+							16
Lacuna divaricata		+				+															17
Leda parvula		+				?															1
Lepidopleurus cancellatus		+																			15
Lima excavata							+														13
Lima loscombei		+				+															1
Littorina littorea		+				+															19
Loligo opalescens		+				+															18
Lucina borealis		?																			1
Modiolaria marmorata		?				?															1
Mya truncata		?				?															1
Mytilus californianus								+	+												20
Mytilus edulis		+				?															19
Nassa incrassata		+																			19
Nassa reticulata		+																			1
Natica nitida		+				?															1
Nucula sulcata		?				+															15
Octopus bimaculatus		+				+															18
Patella depressa		+						+					+	+							22
Patella vulgata		+				?		+					+	+							22
Pecten jacobaeus										+											17
Pecten maximus		+								+											21
Pecten opercularis		+				?															1
Pecten strictus		+																			1
Pectunculus glycimeris											+										7
Philline aperta		?				+															1
Pleurobranchus spp.							?														17
Psammobia ferroensis		+				+															1
Purpurea lapillus		+				+															1
Rissoa spp.		+				+															1
Rossia macrosoma						+															24
Saxicava rugosa		+				+															1
Solen ensis		+				+															1
Sepiola scandica						+															24
Spisula solida		?				?															1
Spisula subtruncata		+				?															1
Syndosmia alba		?																			15
Tapes pullastra		+				+															1
Tellina crassa		+																			1
Thracia convexa		+																			1
Tonicella marmorea		?				+															15
Trivia europaea		+																			1
Trochus zizyphinus		+				?															1
Velutina velutina		+				?															1
Venus fasciata		+																			1
Venus gallina		+																			1
Venus ovata		+																			1
Volsella barbata		+				?															1
Volsella modiolus		+				?															19

TABLE 28.—*Carotenoid Distribution in Marine Invertebrates*—contd.

Species	α-Carotene	β-Carotene	γ-Carotene	Lycopene	Torulene	Lutein	Astaxanthin	Zeaxanthin	Mytiloxanthin	Pectenoxanthin	Glycymerin	Echinenone	Cryptoxanthin	Hopkinsiaxanthin	Pentaxianthin	Metridin	Taraxanthin	Actinioerythrin	Sulcatoxanthin	Violerythrin	Reference No.
C. ECHINODERMS																					
Amphiura chiajei		?				+															1, 19
Asteracanthion glacialis						+															27
Asterias glacialis		+				?															19
Asterias rubens							+														16, 19, 23, 26
Asterina gibbosa						+															27
Astropecten auranticus						+															27
Astropecten californicus		+					+	?		+											33
Astropecten irregularis		+					?														15, 19
Brissopsis lyrifera							+														1
Cribella aculata		+				+															28
Crossaster papposus		+				?															8, 19
Cucumaria elongata		+				?															1
Cucumaria lactea												+									33
Dendraster excentricus		+																			33
Echinaster sepositus							+														17
Echinocardium cordatus		+				+						+									30
Echinus esculentus						?						+									22, 23, 31
Goniaster equestris		+																			28
Henricia sanguinolenta		+				+															8, 19
Hippasteria phrygiana		+				+	?														8
Holothuria brunneas							?														28
Holothuria nigra							?														28
Holothuria polii							?														27
Holothuria tubulosa							?														32
Luida sarsii		+																			8, 19
Lytechinus pictus	+	+										+									23
Mesothuria intestinalis						+															1, 19
Ophidiaster ophidianus							+														29
Ophiocomina nigra		?				+															1, 19
Ophiopteris papillosa																	?				33
Ophiopteris spiculata																	?				33
Ophiotherix fragilis		?					+														1, 19
Ophiothrix rudis																					33
Ophiura texturata		?				+															1, 19
Paracentrotus lividus	+	+										+			+						31
Patiria miniata		+					+														33
Phylloporus lucidus						+															1, 19
Pisaster giganteus		+					+									+					33
Pisaster ochaceous		+					+		+												33
Porania pulvillus		+				?	+														1, 28
Psammechinus miliaris						+															1
Solaster endica						+															
Solaster papposa		+																			28
Spatangus purpureus		+				+															1
Stronglyocentrotus drobachiensis		+				+															1
Stronglyocentrotus franciscanus		+																			33
Stronglycocentrotus purpuratus		+						+													33
D. COELENTERATES																					
Actinia equina	+	+															?	+			8, 34, 35
Alcyonium digitatum		+				+														+	1
Anemonia sulcata																			+		34
Caryophyllia smithi		+					?														1
Cribrina xanthogrammica																		?			59
Epiactis prolifera		+					?											?			36

TABLE 28.—*Carotenoid Distribution in Marine Invertebrates*—contd.

Species	α-Carotene	β-Carotene	γ-Carotene	Lycopene	Torulene	Lutein	Astaxanthin	Zeaxanthin	Mytiloxanthin	Pectenoxanthin	Glycymerin	Echinenone	Cryptoxanthin	Hopkinsiaxanthin	Pentaxanthin	Metridin	Taraxanthin	Actinioerythrin	Sulcatoxanthin	Violerythrin	Reference No.
Gorgonia sp.							?														32, 41
Halcampa duodecirrhata		+				?															1
Lucernaria quadricornis		+				?															1
Metridium dianthus ..		+				?															1
Metridium senile ..		+					+									+	?	?			38
Pennaria spp.							?														32, 41
Protanthea simplex ..		?				+															1
Sagartia undata ..						?															1
Sagartia viduata ..		+				?															1
Tealia felina							?											+			34
Tubularia indivisa ..							?														37
Tubularia larynx ..		+																			1
Urticina felina		+				+															1
E. PORIFERA																					
Axinella crista galli ..	+	+	+				+					+									17, 43
Ficulina ficus	+	+	+	+	+																42
Halichondria albescens							?														37
Halichondria caruncula ..		+																			37
Halichondria incrustans ..		+				+															37
Halichondria panicea ..		+																			15
Halichondria rosea ..		+																			37
Halichondria seriata ..		+				+															37
Halma Ducklandii ..			+								+										27
Hymeniacidon sanguineum	+		+																		40
Leuconia gossei							?														37
Microciona prolifera ..			+																		45
Suberites domuncula ..	+	+	+	+	+		?														17, 37, 42
Suberites ficus		+																			15
Suberites flavus		+			+																41
Suberites massa		+																			1,5 41
Tedania muggiana ..		+																			15
Tethya lycnureum ..		+																			15
F. ANNELIDA, BRACHIPODA, PRIAPULIA, ASCHELMINTHES, POLYZOA																					
Alcyonidium gelatinosum		+				+															15
*Bugula neritina**		+																			27
Chaetopterus variopedatus		+																			28
Cirratulus cirratus ..		+																			44
Cirrutalus tentaculus ..		+																			15
Crania anomala ..		?			+																15
Fascolosoma elongatum ..		+																			15
Flustra foliacea						+															28
Flustra securifrons ..		+			+																15
Glycera goesii		+				+															15
Harmothoe sarsii		+																			15
Laetmonice filicornis ..		+																			15
Leprolia foliacea ..						+															15
Lumbrinereis fragilis ..		+				?															15
Malacobella grossa ..						+															2
Neoamphitrite figulus ..		+				+															15
Nephthys caeca		+																			15
Nephthys ciliata ..		+																			15, 28

* But see Reference 39D at end of Chapter.

TABLE 28.—*Carotenoid Distribution in Marine Invertebrates*—contd.

Species	α-Carotene	β-Carotene	γ-Carotene	*Lycopene*	Torulene	Lutein	Astaxanthin	Zeaxanthin	Myxoxanthin	Pectenoxanthin	Glycymerin	Echinenone	Cryptoxanthin	Hopkinsiaxanthin	Pentaxanthin	Metridin	Tarazanthin	Actinioerythrin	Sulcatoxanthin	Violerythrin	Reference No.
Nereis virens		++																			15, 28
Phascolosoma elongatum		++																			15
Polymnia nebulosa						++															15
Sabella penicillus		?																			15
Siphonostoma diplochaitos						++															27
Stylarioides plumosus		+				++															15
Terebella stroemii		+				?															15
Terebratulina caputserpentis		++																			15, 28
Thelepus cincinnatus		++				+															15
Thoracophelia mucronata		+																			46

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Table 29

Carotenoid Content of some Marine Invertebrates

Species	Amount mg. % of fresh weight		Reference
	Carotenes	Xanthophylls	
Coelenterates			
Metridium senile (red)	0	14·96	1
Metridium senile (white)	0	1·76	1
Molluscs			
Mytilus californianus			
male body	0	2·18	2
female body	0	4·83	
male gonads	0	6·5	
female gonads	0	11·34	
Cephalopods			
Paroctopus bimoculatus			
Ink	0	0·55–0·70	3
Gastropods			
Patella vulgata			
male gonads	120	170	4
female gonads	110	165	
Annelids			
Thoracophelia mucronata	0·38	0	5
Arthropods			
Tigriopus fulvus			
male		5·76 (µg./animal)	6
female (gravid)		6·03 (µg./animal)	
female (egg sacs)		1·58–2·89 (µg./sac)	
Echinodermata			
Astropecten californicus	0·044	0·72	7
Patiria miniata	0·05	0·86	
Pisaster giganteus			
skeleton and skin	0·07	0·90	
pyloric caeca	0·40	1·37	
Pisaster ochraceous			
skeleton and skin	0·23	0·24	
pyloric caeca	0·56	11·45	
Ophiopteris papillosa	0	c. 3·39	
Ophiothrix rudis	0	c. 1·40	
Ophiothrix spiculata	0	c. 1·60	
Dendraster excentricus	0·157	0·049	
Stichopus californicus	0·014	0·015	

TABLE 29.—*Carotenoid Content of Some Marine Invertebrates*—contd.

SPECIES	AMOUNT mg. % of fresh weight		REFERENCE
	Carotenes	Xanthophylls	
ECHINODERMATA—*contd.*			
Lytechinus pictus			
females	0·49	0·35	7
males	0·87	0·43	
female gut	4·48	4·18	
male gut	3·38	1·52	
female gonad	0·86	0·28	
male gonad	0·48	0·53	
female test	0·01	0·01	
male test	0·01	0·01	
Strongylocentrotus franciscanus	0·170	0·025	7
Strongylocentrotus purpuratus			
female gut	2·99	6·04	
male gut	2·41	5·65	
female gonad	2·00	0	
male gonad	0·69	0	

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CHAPTER VI

MARINE VERTEBRATES : AMPHIBIANS : OCEAN BED

PROTOCHORDATA

TUNICATA

The early work of Kruckenberg[1] and Lönnberg[2] had demonstrated the presence of carotenoids in a number of tunicates (*see* Table 30), but only four have been examined in any detail, and this examination has been carried out by Lederer.[3,4]

Halocynthia papillosa (*Cynthia papillosa*), *Microcosmus sulcatus*, *Botryllus schlosseri*, and *Dendrodoa glossularia* all contain considerable amounts of xanthophylls but only very small amounts of α- and β-carotenes. *H. papillosa*, which is dark red, contains both free *astaxanthin* and a unique xanthophyll, *cynthiaxanthin*, in both the tunic and internal organs; astaxanthin predominates. Cynthiaxanthin is of unknown structure but has the following physical properties; m.p. 188–190°; $\lambda\lambda_{max.}$ 517, 483, 451 mμ. (CS_2) and 482 and 452 mμ. (light petroleum); it gives no blue coloration with concentrated hydrochloric acid. Karrer and Solmssen,[5] however, could not detect cynthiaxanthin in their specimens of *H. papillosa*. The rose coloured social tunicate *D. glossularia* contains principally esterified astaxanthin; 15–20 mg. were obtained from 440 g. of animals.

Closely related to *H. papillosa* is the violet *M. sulcatus* which contains a complex mixture of carotenoids, mainly free xanthophylls, which were very difficult to separate. The major xanthophyll was similar to lutein (xanthophyll) but gave a blue colour with hydrochloric acid. The carotenes detected were probably *echinenone* (*see* p. 163) and α-carotene.

The brown-red tunicate *B. schlosseri* contained a mixture from which *pectenoxanthin* (*see* p. 175), capsanthin and capsorubin were isolated. The presence of the latter two carotenoids was probably due to the fact that the animals were obtained from a harbour into which pimento pepper waste had been dumped; Heilbron, Parry and Phipers[6], however, suggest that they might have been formed by oxidation of fucoxanthin contained in the algal foodstuffs.

TABLE 30.—*Carotenoid Distribution in Tunicates*

Species	α-Carotene	β-Carotene	Echinenone	Lutein	Capsanthin	Capsorubin	Cynthiaxanthin	Astaxanthin	Pectenoxanthin	Reference No.
Botryllus schlosseri				+	+	+			+	1, 2
Ciona intestinalis				+						1
Clavellina lepadiformis		+		+						1
Corella parallelogramma		?		+						1
Cynthia papillosa	+	+					+	+		3, 4
Dendrodoa grossularia	+	+						+		2
Microcosmus sulcatus	?		?							2
Molgula occulta		?		?						1
Myxilla mammillaris		?		?						1
Styela rustica		+		?						1

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ENTEROPNEUSTA

The only report is that of Lönnberg[7], in which the presence of a xanthophyllic carotenoid is indicated in *Harrimania kupferi.*

FISH

Some of the brilliant skin colours of marine fish are due to carotenoids which exist in the chromatophores. The carotenoids are almost always entirely xanthophyllic and there is very little variation from species to species. Either astaxanthin, or lutein (xanthophyll), or a taraxanthin-like carotenoid or a mixture are the only pigments generally present. According to Lönnberg[8], fish fall roughly into two groups, those containing lutein (xanthophyll) and those containing the taraxanthin-like carotenoid; he appears to have overlooked the presence of astaxanthin. Even with this small number of alternatives it is very difficult to forecast the carotenoid make-up in any single member of a species. A review of the biochemistry of fish carotenoids has recently appeared.[8A]

TABLE 31.—*Qualitative Carotenoid Distribution in Skin of Marine Fish*

Species	Pigments			References
	Lutein	Astaxanthin	Taraxanthin	
Ammodytes lanceolatus			+	1
Ammodytes tobianus			+	1
Anguilla anguilla	+			2
Aphiga minuta			+	3
Beryx decadactylus		+		4
Bothus maximus			+	3
Bothus rhombus	+		+	3
Callionymus lyra			+	2, 3
Caranx trachurus	+			3
Centrolabrus exoletus	+	+	+	3
Clupea harengus	+		+	3
Copeina guttata			+	16
Cottus bubalis	+		+	2, 3
Crenilabrus melops / *Crenilabrus suillus*	either tar	axanthin or lute	in	3
Cyclopterus lumpus		+		3
Cymatogaster aggregatus			+	4, 5
Fundulus parvipinnis			+	4, 5
Gadus aeglefinus			+	3
Gadus callarias	+		+	3
Gadus merlangus			+	3
Gadus minutus	+		+	2, 3
Gadus pollachius			+	3
Gadus virens			+	3
Gaidropsarus cimbrius	+			2
Gaidropsarus mustela	+			2
Gasterosteus aculeatus			+	3
Gillichthys mirabilis			+	5
Girella nigricans			+	5
Gobius niger	+			2
Hypsypops rubicunda			+	5, 6
Labrus bergsmyltrus	+			3
Labrus melops	+			5
Labrus ossifagus	+			3
Neurophis aequoreus	+			2
Neurophis ophidon			+	3
Pholis gunellus	+		+	3
Pleuronectes flesus	+			2
Pleuronectes kitt	+		+	2, 3
Pleuronectes limanda	+		+	2, 3
Raja batis	+			3
Raja clavata	+			3
Raniceps raninus	+			3
Salmo gairdneri		+		7
Salmo salar		+		8, 9
Scomber scombrus	+		+	2
Scophthalmus norvegicus			+	3
Scorpaena scrofa		(?) +		19
Sebastes marinus		+		4, 11
Siphonostoma typhle	+		+	3
Syngnatus acus	+		+	3
Trachinus draco			+	3
Trigla gurnardus			+	3
Zoarces viviparus	+		+	3

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Skin

Examples of fish containing esterified astaxanthin in the skin are *Onchorhynchus nerka*,* [9] *Salmo gairdneri*, [9] *Cyclopterus lumpus*, [10] the marine dorado (*Beryx decadactylus*), and the rock cod (*Sebastes marinus*). [11] In the case of *Beryx decadactylus* the term "skin" includes gills and mouth mucus.

The taraxanthin-like xanthophyll (esterified) was noted in the fish examined by Sumner and Fox [14-15] and Fox, [15] *viz.*: the Pacific killifish (*Fundulus parvipinnis*), the greenfish (*Girella nigricans*), the long-jawed goby (*Gillichthys mirabilis*), the marine goldfish (*Hypsipops rubicunda*) and the surf perch (*Cymatogaster aggregatus*). Recently Fox and his associates [15A] have reinvestigated the "taraxanthin" of *Hypsypops rubicunda* in detail. They resolved this fraction into a number of components, none of which is identical with taraxanthin. They rather more closely resemble dinoxanthin (*see* p. 135), although one has properties similar to lutein epoxide (*see* p. 17). An esterified neutral xanthophyll pigment (probably taraxanthin) was found by Goodwin [16] in the sand eels *Ammodytes tobianus* and *A. lanceolatus*. Lönnberg [7, 8] has noted the taraxanthin-like pigment and lutein in a considerable number of species. Carotenoids have also been reported in the dorsal skin of ells. [17] The known distribution of carotenoids in fish skin is recorded in Table 31.

Eyes

In the large number of species examined by Lönnberg, [18, 19] the eyes of only two were devoid of carotenoids: *Gadus esmarkii*, and *Squalus acanthias*. *Raja clavata* was first thought to be in this class, but was later found to contain traces. Wald [20] noted the esterified taraxanthin-like pigment in the combined pigment-epithelia and choroid layers of eyes of the sea robin (*Prionotus carlinus*), the black bass (*Centropristes striatus*) and the sarp (*Stenotomus chrysops*). *Beryx decadactylus* contains considerable amounts of astaxanthin in the iris and sclera, [11] but none in the retina.

Flesh

Carotenoids are not widely distributed in the flesh of fish, but astaxanthin has been found in the flesh of *Salmo salar*, [21, 22] *Coregonus albula*, [22A] *Lophius piscatorus* [23] and *Onchorhynchus nerka*. [9]

Liver

The qualitative distribution in the livers of fish is recorded in Table 32. The only special comment which need be made here is that in

* Anadromous fish are treated in this chapter for convenience.

the angler fish (*L. piscatorus*) the liver astaxanthin is attached to a protein.[23,24] The liver of *Beryx decadactylus*, as well as the heart, is devoid of carotenoids.[11]

TABLE 32.—*Carotenoid Distribution in Liver of Marine Fish*

Species	Pigments				References
	β-Carotene	Lutein	Astaxanthin	Taraxanthin-like pigment	
Cyclopterus lumpus ..			+		1, 2
Lophius piscatorius ..	(? also lutein epoxide)		+	+	1, 4, 5, 6
Orthagoriscus mola ..	(? also α-carotene)		+		4
Regalescus glesne ..			+		5
Salmo salar		+			3

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TABLE 33.—*Carotenoid Distribution in Ovaries and Ova of Marine Fish*

Species	Pigments				References
	β-Carotene	Lutein	Astaxanthin	Taraxanthin-like pigment	
Ammodytes tobianus	+			+	1
Coregonus albula ..			+		2
Eliginus navaga ..	+ (+ 3 unidentified xanthophylls)				3
Gadus callarias ..	+	+			4
Gadus morrhua ..	+		+		5
Hippoglossus hippoglossus ..	+	+	+		4
Lota vulgaris ..	+	+			6
Salmo salar			+		7, 8
Solea vulgaris ..	+				4

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Reproductive Organs

Unesterified xanthophylls occur in the ova and ovaries of many fish (Table 33). Emphasis should be laid on the fact that β-carotene which has rarely, if ever, been observed in other fish organs, is a universal constituent of egg carotenoids, although it is always only a very minor component. The complete absence of carotenoids from the milt of the marine fish (*Ammodytes tobianus*[16] and *Clupea harengus*[25]) contrasts strongly with their relative abundance in the eggs.

Quantitative Studies

Little is known of the quantitative distribution of carotenoids in fish ; the figures collected in Table 34, are all that are available.

Table 34

Carotenoid Content of some Marine Fish

Species	Amount mg./100g. wet wt.		Ref.
	Carotenes	Xanthophylls	
Ammodytes tobianus			
Whole fish	trace	0·36	1
Whole fish	trace	0·99	
Spermatoza	0	0	
Ova	0·03	0·80	
Cymatogaster aggregatus ..	0	c. 0·27	2
Fundulus parvipinnis	0	0·17–1·2	3, 4
Gillichthys mirabilis	0	0·41–·062	3
Girella nigricans		c. 1·4	5
Hypsipops rubicunda	0	80–500*	6

* According to age

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Formation and Metabolism

Although little work has been carried out on the formation of piscine carotenoids, it seems reasonably certain that they are of alimentary origin ; they are not, however, always stored unaltered. The work of Sumner and Fox[13] on carotenoid formation in the Pacific killifish,

Fundulus parvipinnis is outstanding; aquaria-kept *Fundulus* were divided into three groups fed respectively, a carotene diet (beach worm *Thoracophelia mucronata*), a xanthophyll diet (garibaldi, *Hypsypops rubicunda*) and a carotenoid-free diet (flesh of Californian halibut, *Paralichthys californicus*). On the first two diets the *Fundulus* carotenoid, entirely taraxanthin-like, increased in both concentration and absolute amounts although the increase was much greater on the xanthophyll diet, whilst in the third group there was a drop in concentration but no change in total amount. This experiment makes it clear that *Fundulus* cannot synthesize carotenoids *de novo* but that they do have the ability to oxidize carotenes to xanthophylls.

In the case of *Hypsypops rubicunda*, however, a diet containing only β-carotene did not allow the animals to maintain their normal xanthophyll content, although some β-carotene was stored in the skin. This latter observation is of special interest because it is the first time that it has been possible experimentally to produce storage of β-carotene in the skin of fish.[15A].

Observations on surf perch (*Cymatogaster aggregatus*) also show that carotenoids are not metabolized in the same way in all fish.[26] When fed the red shrimp (*Hippolyte californiensis*) which contains β-carotene and neutral (? taraxanthin) and acidic (? astaxanthin) xanthophylls, *Cymatogaster* only utilizes the neutral xanthophyll; the other two pigments are excreted quantitatively. After hydrolysis in the lumen the taraxanthin was esterified, presumably as it passed across the gut wall, and transported to the skin, where, in the sexually inactive fish, it remained in constant amount. Excess taraxanthin was stored unesterified in the rectal segment of the gut; it rapidly disappeared from this site when food was withheld. The dietary astaxanthin esters although hydrolysed in the gut were not absorbed. The function of the xanthophylls stored in the rectal segment is somewhat obscure but the following facts were established:

(*a*) the segment does not absorb carotenoids directly from the lumen but is provided with them from the blood stream;

(*b*) it neither excretes xanthophylls into the rectal lumen nor oxidizes them *in situ*;

(*c*) it is not a temporary storehouse for replenishing skin carotenoids.

The concentration of xanthophylls in the skin of *Hypsypops rubicunda* increases with age (*see* Table 34). This is in agreement with the colour changes observed in the developing fish, *i.e.*, from the dull orange of the half-grown specimens to the brilliant orange adults.[15A]

Some fish appear to make little or no use of their dietary carotenoids

for Glover[27] found no carotenoids in the halibut (although traces may be present in the ova)[28] in spite of its main food being a fish, *Sebastes marinus*, rich in astaxanthin.[4]

Glover, Morton and Rosen[29] have followed the metabolism of astaxanthin in salmon (*Salmo salar*) eggs. For the six weeks between the fertilization of the eggs and hatching no change took place in the amount of astaxanthin. Two months after the hatching the content had decreased by 12 per cent. ; whether this drop is significant or not it is difficult to say. At hatching 92 per cent. of the total astaxanthin present was in the yolk sac. As the embryos develop there is a constant transfer of pigment from the sac to the embryo, so that a two month embryo contains 80 per cent. of the total pigment. Not only is there a transfer but the astaxanthin is esterified as it is laid down in the embryo. The same type of change occurs in entirely fresh water fish (*see* p. 207).

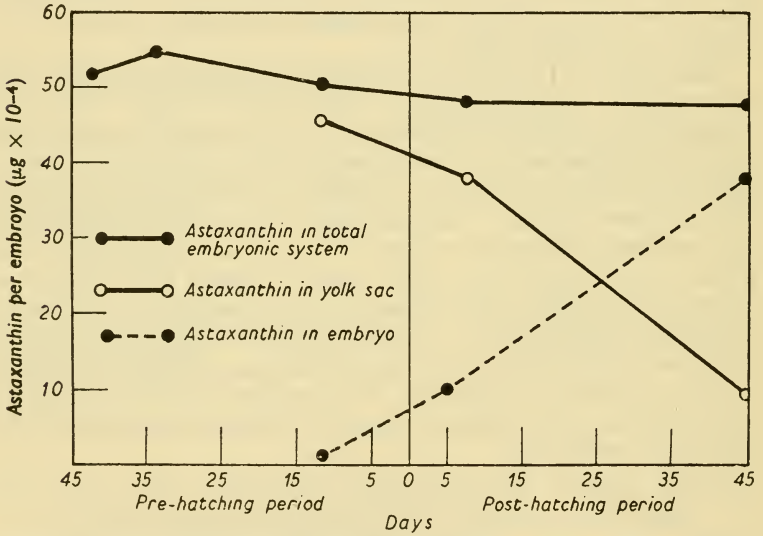

Fig. 26.—Showing the Astaxanthin distribution in the developing salmon embryo. (*After Glover, M., Morton, R. A., and Rosen, G. D. (1949) Biochem, J.*, **50**, 425).

Function

(*a*) *In Photoresponses.* The work of Sumner and Fox[12-14] indicates that carotenoids do play a part in photoresponses in some fish. *Fundulus parvipinnis* and *Gillichthys mirabilis* maintain their carotenoid content in different optical environments although the colours of the fish

alter. Alteration of colour is due to contraction and expansion of chromatophores without any quantitative variation in the carotenoid content of these cells. *Girella nigricans* on the other hand, which loses xanthophylls in captivity, tends to lose them more quickly on a white background than on coloured backgrounds. Much more is known of carotenoid distribution and function in the chromatophores of fresh water than of marine fish ; this is discussed on p. 206.

Owing to the wide distribution of carotenoids in fish tissues, Wald[20] does not consider their presence in some retinae of overwhelming significance, although there is a possibility that they increase visual acuity by reducing chromatic aberration and glare.[30]

(*b*) *In Reproduction.* As in all other animals so far discussed the differential mobilization and distribution in fish gonads is suggestive of a specific function, but no such function has as yet been definitely established.

Both sexes of *Cyclopterus lumpus*[10] mobilize astaxanthin from the liver to the skin and flesh during the summer spawn, and female *Fundulus parvipinnis*[13] transfer free xanthophylls to the ripening eggs whilst the males increase the xanthophylls in their skin. Similar appearances of free xanthophylls in ova have been noted in *Ammodytes tobianus*[16] and *Salmo salar.*[29]

As with the problem of phototropic responses, the problem of carotenoids in reproduction has been much more fully investigated in fresh water than in marine fish. A full discussion of the problem is, therefore, postponed until Chapter VII.

MAMMALS

Of the two main groups of whales, the *Odontoceti* (toothed whales), which subsist on " Krill " (small crustacea), would be more likely to contain carotenoids than the *Mystacoceti* (whalebone whales) which subsist on larger prey such as seals and smaller whales.

Drummond and MacWalter,[31] however, demonstrated that faeces of a Krill-eating whale were very rich in astaxanthin. This suggested that the pigment is probably not absorbed to any appreciable degree and this probability is emphasized by the fact that Burkhart, Heilbron, Parry and Lovern[23] only very occasionally encountered astaxanthin in whale body oils ; further Barua and Morton[32] in a wide investigation have never encountered whale liver oils containing astaxanthin, but Schmidt-Neilson *et al.*[10] do report observing red oils from blue-whale livers. Burkhart *et al.*,[23] however, consider the pigmentation of whale oils to indicate a pathological condition.

According to Wagner and Vermeulen[33] the flesh, liver and milk of blue and fin-backed whales contain no carotene and Morton and Rosen[33A] could find no carotenoids in whale ovaries.

As might be expected, the liver oil from the killer whale (*Grampus griseus*) contains no carotenoids.[34]

Porpoise livers contain variable amounts of carotenes, but none was ever found in embryos.[35]

AMPHIBIA

There is only one report of the presence of carotenoids in a marine amphibian ; Lwoff[36] reported carotenoids in the retina of an unidentified amphipod collected in a cove at St. Martin (Manche).

CAROTENOIDS OF THE OCEAN BED

It is not possible to leave a discussion of the carotenoids of the sea without considering the important work of Fox and his collaborators[37-39] on the carotenoids of the ocean floor. The carotenoids which they examined in ocean mud are, biochemically speaking, of great age. Their stability is due to the prevailing conditions : low temperatures, absence of O_2, and absence of light. The most striking fact which emerges on examination of these carotenoids is that the relative proportions of carotenes and xanthophylls are the inverse of those generally found in marine flora and fauna ; whilst xanthophylls predominate in living material carotenes predominate in the mud. The amount of carotenes in marine living material varies (apart from in some echinoderms) between 0 and 35 per cent. of the total carotenoids present ; in mud values fall between 35 and 83 per cent. β-Carotene is the predominating mud carotene but smaller amounts of carotenes characteristic of fungi, bacteria, ascidians, and sponges also occur in small amounts. Mud xanthophylls are mainly those originally occurring in algae, especially diatoms, but compounds very similar to antheraxanthin (*see* p. 50) and petaloxanthin (*see* p. 47) have been detected. Astaxanthin rarely appears in mud.

Fox, Updegraff and Novelli,[39] suggest three possible reasons for the preferential storage of carotenes in ocean mud :

(*a*) preferential oxidation of xanthophylls ;

(*b*) selective assimilation followed by oxidative destruction of xanthophylls by many marine animals, especially ilytrophic (bottom feeding) animals ; and

(*c*) anaerobic carotene synthesis and/or reduction of xanthophylls by chromogenic micro-organisms.

Some approximate but very suggestive calculations have been made by Fox *et al.*[39] Taking mussel faeces as typical of marine detritus, they contain 12 per cent. of lipids, 68 mg./100 g. of xanthophylls, and 12 mg./100 g. of carotenes;[40] corresponding mean values for bottom sediments are 2 per cent. of lipids, 2 mg./100 g. of xanthophylls, and 8 mg./100 g. of carotenes. The process of early fossilization thus represents a loss of 83 per cent. of lipids, 97 per cent. of xanthophylls, but only 33·3 per cent. of carotenes. Basing the figures on the pigment content of the lipids, the comparatively great stability of the carotenes is even more evident; the concentration of xanthophylls in lipids decreases by 83 per cent. whilst that of the carotenes is increased by 400 per cent.

No phytofluene occurs in extracts of bottom sediments, but some unidentified material exhibiting a blue fluorescence in ultra-violet light and an absorption spectrum around 260 mμ. have been noted.[41] Recently, Petracek, Fox and Zechmeister[42] have shown that carotenes also predominate over xanthophylls in inter-tidal ocean mud (from Mission Bay, California) and that blue fluorescent materials are present but that phytofluene is absent.

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CHAPTER VII

FRESH WATER ANIMALS : DEPOSITS : AMPHIBIA

INVERTEBRATES

Information on the carotenoid distribution in the lower forms of fresh water life is meagre. Some fresh water crustacea are very similar to marine crustacea in containing astaxanthin, *e.g.*, *Gammarus pulex*,[1] *Daphnia longostra* and *Cyclops* species.[2] Crystalline astacin (the oxidative artefact of the naturally-occurring astaxanthin) has been obtained from the crayfish (*Potamobious astacus*). Others, however, do not contain this carotenoid, for *Daphnia magna* contains only hydrocarbon carotenoids[3] and *Asellus aquaticus* only β-carotene and cryptoxanthin.[4] In mixed samples of zooplankton containing 85 per cent. of *D. longostra* and the rest *Cyclops*, astaxanthin is by far the predominating pigment, the amounts of astaxanthin, other xanthophylls, and carotenes being respectively 6·52 mg., 1·38 mg. and 0·12 mg. per g. dry weight.[2]

The caverniculus amphipods *Niphargus* (*Sygodytes*) spp. contain no carotenes, but their eggs are reported to be pink.[2A]

Helix pomatia, according to the very old work of Kruckenberg and of McMunn, possesses a hepatic carotenoid[5], probably a mixture of alimentary origin, as does another unspecified snail.[6] Seybold and Egle[7] state that, compared with its food, the edible snail excretes faeces containing relatively more carotenes than xanthophylls. Their claim that this indicates preferential destruction of the xanthophylls by the gastric secretion of the snail, must be accepted only with reservation.

Cain[9] has recently provided histochemical evidence that carotenoids accumulate in the interna of the Golgi apparatus of the neurones of *Helix aspersa*, *Planorbis corneus*, and *Limnaea stagnalis*. The accumulation is greatest in the two last-named species.

Recently Comfort[8] has examined the highly-coloured egg mass of the South American gastropod *Pila canaliculata*.* The pigmentation is due to a carotenoid protein complex very similar to those commonly found in marine crustacean eggs. On denaturation of the complex a mixture of a number of carotenoids, mainly hypophasic in a 90 per cent.

* In his paper, Comfort describes his material as *P. glauca* but in a private communication to the author he states that it is more probably *P. canaliculata*.

ethanol petroleum ether partition, was obtained. This is in contrast to the typical crustacean complex which generally contains only astaxanthin. The pigment complex from embryonic *P. canalicuata* fixed in formol shows a single absorption band at 550 mμ. The livers of both newly born larvae and adults contain carotenes. It is interesting to note that only *Pila* which lay eggs out of water produce pigmented eggs, eggs which are buried are unpigmented. The former varieties occur in the New World and the latter in India. It would be interesting to know if the " unpigmented " forms were completely devoid of carotenoids.

VERTEBRATES (FISH)

The difference between the carotenoids of marine and fresh water fish is slight. Fresh water species are known which contain lutein (xanthophyll), astaxanthin, and the " taraxanthin-like " carotenoid either singly or together. A possible difference is the more widespread distribution of lutein (xanthophyll) in fresh water fish, but the " sample " of species so far examined is not sufficiently large to say whether this is definitely so.

TABLE 35

*Carotenoid Distribution in the Brown Trout (Salmo Trutta)**

MATERIAL	AMOUNT μg./g. fresh tissue				
	Carotenes(*a*)	Xanthophylls not astaxanthin (*b*)		Astaxanthin	
		Free	esterified	Free	esterified
Skin and fins (whole) ..	0	0	87	0	81
Skin, excluding red spots	0	0	120(*a*)	0	73
Skin, red spots only ..	0	0	306(*b*)	0	1608
Liver	7·5	8·5	3·5	0	0
Ovary	3 (0·13)†	19 (0·43)†	0	152 (0·17)†	0
Muscle	trace	3·5	0	32·5	0
Eyes (posterior hemisphere)	0	0	0·2	0	0

* From Steven, D. M. (1948), *J. exp. Biol.*, **25**, 369.
† Values for *S. irideus* eggs.[12A]

(*a*) Mostly β-carotene. (*b*) Mostly lutein (xanthophyll).

Lederer,[10] in 1935, was the first to demonstrate the presence of astaxanthin in a fresh water fish; the skin of the common gold fish *Carassius auratus* contains the esterified pigment as do the fins of *Esox lucius* and skin of *Lota lota*. Somewhat earlier Euler and Virgin[11] had found xanthophylls in the liver of the pike, *Esox lucius*, and the roach, *Leuciscus rutilus*. Some specimens of the fresh water perch, *Percha fluviatilis*, contain astaxanthin esters whilst others contain neutral carotenoids. Steven,[2,12] has shown that the skin of the wild trout *Salmo trutta* contains esters of both astaxanthin and lutein (xanthophyll), the muscles esterified astaxanthin and free luetin (xanthophyll), the liver only carotene and neutral xanthophylls.

Rainbow trout (*Salmo irideus*) ova contain the same carotenoids as those found in the eggs of wild trout. Quantatively, however, the two species differ considerably.

The eggs of *Salmo fario* probably contain astaxanthin.[12A]

Goodwin[13] has examined three species of fresh water fish, the char, *Salvelinus* spp., the Ceylon *Bartus nigrofasciatus* and the Argentine *Copeina guttata*; the first two contain astaxanthin esters and the third probably lutein esters as the predominant pigments; Steven[12] has also noted astaxanthin in the char. Astaxanthin was not present in the gonads of *Eliginus navaga*[13A] which do however contain large amounts of neutral xanthophylls and traces of carotenes. According to Lönnberg[14] the spermatozoa of *Esox lucius* contain carotenoids. No astaxanthin is present in the following tropical fish: *Platypoecilus maculatus*, *Xiphophorus helleri*, *Oryzias latipes*, *Macropodus opercularis*, *Colisia lalia*, *C. fasciata* and *Betta splendens*. However, they all contain lutein (xanthophyll) and the first two and the last three also contain zeaxanthin and violaxanthin respectively.[15]

Steven has examined lampreys and found that ammocoete larvae of *Lampetra planeri* contain only xanthophylls with lutein the major component; similarly, spawning adults of *L. fluviatilis* contain only xanthophylls. A difference is noted, however, in that 50 per cent. of the pigments in the larvae are esterified, whilst all those of the spawning adults are unesterified. The pigment accumulates in the non-expandible lipophores in the dermal and subdermal layers of the skin; there are only negligible amounts in the liver and other tissues.

Quantitative Data

Very few quantitative investigations have been carried out on fresh water fish, the most important being those of Steven. His data on wild trout (*S. trutta*) are summarised in Table 35.

The results of Hartmann *et al.* on *S. irideus* ova are included in this table, and it will be seen that considerable inter-species differences exist.

TABLE 36—*Qualitative Carotenoid Distribution in Fresh Water Fish*

Species	Pigments			References
	Lutein	Astaxanthin	Taraxanthin	
Bartus nigrofasciatus ..		+		1
Betta splendens	+ (? also violaxanthin)			2
Carassius auratus ..		+		3
Colisia fasiata	+ (? also violaxanthin)			2
Colisia lalia	+ (? also violaxanthin)			
Copein guttata	+			1
Esox lucius	(? also lutein 5 : -epoxide)		+	3
Lampetra fluviatilis ..	+			4
Lampetra planeri	+			
Macropodus opercularis ..	+ (? also zeaxanthin)			2
Oryzias latipes	+ (? also zeaxanthin)			
Percha fluviatilis		+	+	13
Platypoecilus maculatus	+ (? also zeaxanthin)			3
Salmo trutta	+	+		5, 6, 7
Salvelinus spp.		+		1, 4
Xiphophorus helleri ..	+ (? also zeaxanthin)			2

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FORMATION AND METABOLISM

(i) *Invertebrates*

The storage of carotenoids by fresh water amphipods may well depend on the presence of light, for Beatty [2A] showed that whilst ephigean amphipods store carotenoids, hypogean do not, although they were not short of carotenoid-containing food.

Beatty quotes two old reports which support his observations: *Gammarus puteanus* and G. fluviatilis lose their colour when kept in the dark for six months, whilst *Niphargus puteanis* becomes greenish when transferred to light, although *N. plateaui* does not; the dull transparent white of *Gammarus dueberi* becomes flecked with red spots when exposed to light.

(ii) *Fish*

Although it is probably true that the extreme variations in the pigmentation of trout are due to genetic factors, it is also true that the lipochrome pigments are of alimentary origin.

Davis[16] in 1930 claimed that hatchery trout, fed dried salmon eggs showed the same coloration as wild trout of the same age. Mann[17] confirmed this using as food goldfish, carrots, and mixed *Daphnia* spp. The results of McCay and Tunison's[18] experiments were, however, not so clear cut. More recently, Steven [2,12] has confirmed that trout carotenoids are of alimentary origin and has provided a much more quantitative picture of the situation than had previously been available.

Steven found that trout kept in captivity maintained their natural pigmentation when their diet consisted of natural food supplemented by live *Entomostraca* (chiefly *Simocephalus* and *Daphnia* spp.) and *Corethra* larvae. When placed on a diet of chopped meat and earthworms the fish lost their red and yellow pigmentation and their carotenoids were reduced almost to zero; these trout when transferred to a diet of salmon eggs rapidly assumed their original coloration and after 35 days their carotenoid content was back to its original level.

TABLE 37—*The Effect of Diet on the Carotenoid Content of the Brown Trout*

From Steven, D. M., (1949) J. exp. Biol. **26**, 295.

Group	Duration on experimental diet	Carotenoid content of skin and fins (μg./g. tissue)	
		Lutein	Astaxanthin
1. Wild trout	—	120	75
2. Aquarium-reared on natural food and carotenoid-rich supplement (*see* p. 70)	—	90	80
3. Aquarium-reared on natural food and carotenoid-free supplement	9 months	20	0
4. Group (3) transferred to supplement of salmon ova	35 days	80	65
5. Group (3) transferred to supplement of β-carotene	46 days	15	0
6. Group (3) transferred to supplement of lutein	24 days	30	0
7. Group (3) transferred to supplement of astacin	38 days	20	0

Steven attempted to produce carotenoid pigmentation by feeding earthworms previously injected with either β-carotene, lutein (xanthophyll), or astacin dissolved in arachis oil. No increased pigmentation was observed in any of the diets. The failure was attributed to failure of the fish to deal adequately with the comparatively large amounts of oil in the diet although Lovern[19A] states that fish absorb fat well.

As realized by Steven, there is no reason why fish should absorb astacin under any conditions. As was pointed out in Chapter V(*see* p. 170) this is an artefact produced by oxidation of an astaxanthin and under natural conditions fish would never be presented with it. Steven's suggestion that failure to absorb lutein might be due to a similar reason cannot be easily upheld; with careful handling in extracting this pigment from plant materials the only possible change it is likely to undergo is *cis-trans* isomerization, and it is well known that *cis* isomers of xanthophylls which are vitamin A precursors (*e.g.*, cryptoxanthin) are absorbed by rats and still possess some vitamin activity.[19]

There is one report that the (? carotenoid) pigmentation of *Esox lucius* is also of alimentary origin.[20]

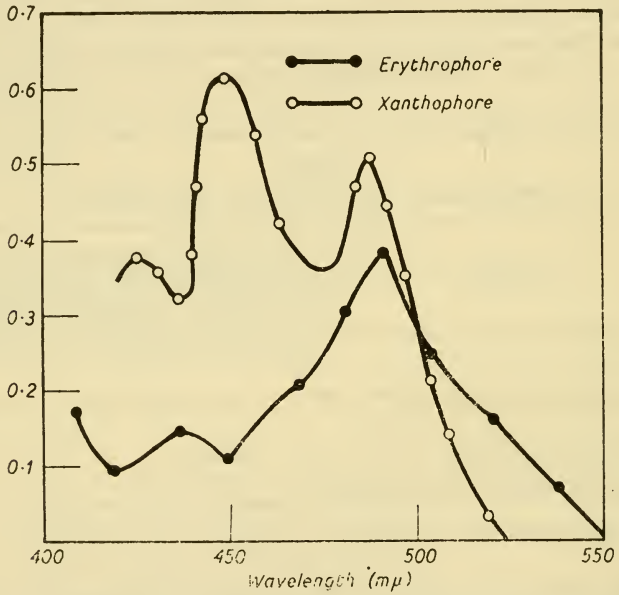

Fig. 27.—The absorption spectra of single Chromatophores of the trout (*Salmo trutta*).
(*From* Steven, D. M. (*1948*) J. Exp. Biol., **25**, 369.)

Function

(i) *In Phototropic Responses*

Goodrich, Hill, and Arrick[15] examining a number of fresh water tropical fish showed that the xanthophylls, lutein, zeaxanthin, and taraxanthin were all concentrated in the xanthophores. The erythrophore pigment was not a carotenoid but a pterin-like pigment which

was named erythropterin. *Platypoecilus* and *Xiphophorus* varieties carrying the gene which controls red pigmentation, possess a hybrid chromatophore which Goodrich *et al.* term a xantho-erythrophore ; in this cell are concentrated both the carotenoids and erythropterin.

Steven[2,12] has also obtained evidence that carotenoids are connected with the chromatophore system by his work on trout and lampreys, but found that xanthophores contain only lutein whilst the red pigment of the erythophores is not erythropterin but astaxanthin (*see* Table 36). The cause of this difference is erythrophore pigmentation in different species is an interesting problem for further investigation.

It remained, however, for Steven to provide the quantitative evidence which quite clearly demonstrated that in trout lutein and astaxanthin are associated with the xanthophores and erythrophores respectively. He showed that the red spots of the skin contained about twenty times as much astaxanthin as did the regions which did not contain any yellow spots. With the aid of a micro-spectrophotometer he measured the absorption spectra of single chromatophores and found that the xanthophores and erythrophores exhibited spectra corresponding quite closely with lutein and astaxanthin respectively. It was estimated that a single xanthophore contained 11–28 10^{-6} μg. of lutein and a single erythrophore 200–340 10^{-6} μg. of astaxanthin.

Further evidence of the association of carotenoids with the chromatophores of *S. trutta* came from dietary studies. After nine months on a carotenoid-free diet, yearling fish exhibited pale-yellow xanthophores, generally distributed over the skin and fins, and a very few orange-pink erythrophores mainly situated at the tip of the adipose fins ; the erythrophores which generally accumulate along the lateral line had disappeared. These observations agree with the quantitative studies (Table 37) which demonstrated the presence of small amounts of lutein and no astaxanthin in these fish.

Steven's studies on the metabolism of carotenoids during the reproductive cycle of trout also indicate the close relationship between these pigments and chromatophores. Muscle carotenoids, but not skin (chromatophore) carotenoids are mobilized into the developing ovaries. During larval development lutein and astaxathin are not utilized, but are quantitatively transferred in constant ratio from yolk to embryo ; during this process they are esterified and accumulate in the skin and fins. This rate of transference follows much more closely the increase in length of the posterior end of the developing embryo than the overall growth, measured either as length or weight. As the majority of the carotenoid-containing chromatophores develop in the skin of the posterior end of the body, especially in the tail and

adipose fins, this correlation indicates that the rate of transfer of pigments is related to the development of the cells which receive them.

Making use of an ingenious technique, Steven managed to remove about 90 per cent. of the yolk carotenoids from the newly hatched trout larvae. Fish treated in this way developed normally on a carotenoid-free diet of the oligochaete worm *Enchytraeus*, with the exception that they were very pale in colour and completely lacking in both xanthophores and erythrophores.

It seems then that a major reason for the mobilization of carotenoids into the ova is to ensure that the newly-hatched larvae are adequately equipped with chromatophores. The most interesting question which remains to be answered is why, in salmon as well as trout, are the xanthophyll esters saponified during their transference to the eggs ? The presence of free xanthophylls in the eggs suggests ,by analogy with vitamin A, that the pigments are " metabolically "* as well as " physically " functional, because the functional form of vitamin A is probably the free vitamin (but *see* p. 271), whilst the esters are the storage form. This possibility will be considered in the next section.

Steven[40] has recently observed that lampreys (*Lampetra palneri* and *L. fluviatilis*) accumulate lutein in the non-expandible lipophores in the dermal and subdermal layers of the skin (*see also* p. 203), but that they play no part in coloration.

In Reproduction

In spite of the proof by Steven that carotenoids are not needed for the normal embryonic development of brown trout, the work of Hartmann *et al.*[12A] on rainbow trout indicates that they may be metabolically functional and of importance in the fertilization process rather than in embryonic development. They report that astaxanthin acts as a fertilization hormone, gamone I (G_I). Two gamones, G_I and G_{II} occur in trout eggs and the ovarial fluid surrounding them. Two natural antagonists A_I and A_{II} occur in spermatozoa. Astaxanthin, β-carotene and lutein all have some positive effect on motility of spermatozoa, whilst astaxanthin and β-carotene, but not lutein, have a positive chemotactic action on the spermatozoa ; β-carotene is, however, much less active than astaxanthin. Astaxanthin is, however, the only pigment which effectively antagonizes the naturally-occurring androgamone I ; it is thus concluded that astaxanthin is a true fertilization hormone.

It must be emphasized that in this investigation no proof was

* A distinction must be drawn between a possible " metabolic " function and a " physical " function, such as participation in photo-responses which could be carried out by the esterified pigments.

provided that astaxanthin is the naturally-cocurring hormone, In fact, no demonstration of its presence in the ovarial fluid, which was used as the natural source of G_I, was presented. It was reported that the fluid was pale yellow; the presence of astaxanthin in the fluid in the concentrations found active in this experiment should have imparted a much stronger colour to it.

It will be very interesting to see if other workers will confirm these findings, but it appears already that preliminary work has failed to do so.[213]

A logical difficulty arises with regard to this work of Hartmann *et al.* When the earlier reports from the same laboratory on the gamones present in sea urchin eggs are considered, it is found that echinochrome (2-ethyl-3, 5, 6, 7, 8,—pentahydroxy-1, 4-naphthoquinone) in the form of a protein complex exhibited all the properties attributed to astaxanthin in the trout. This is in spite of the fact that sea urchin eggs are very rich in carotenoids (*see* p. 163) but do not always contain echinochromes. Carotenoids were, however, not tested for gamone activity in sea urchins.

The whole problem of the function of carotenoids in reproduction is obviously in a very interesting state and the next few years should considerably clarify the position. The present position has recently been reviewed.[411]

In Respiration

It has recently been stated that amongst the carp family, carotenoids accumulate mostly in the eggs of those species living under conditions of poor oxygen supply. This has been taken to mean that carotenoids function as a supplement to the embryonic circulation and furthermore, that the pigmentation of fish is an indication of the amount of oxygen available to them.[20A]

DEPOSITS

In 1932 Trask[211] encountered carotenoids in an algal deposit from a lake in North Florida in water less than 30 cm. deep.

Baudisch and von Euler[222] examined two types of peat-like deposits ("gyttja") located near Stockholm. The red and green algal gyttja contained only carotene whilst the "littoralgyttja" (detritus gyttja) contained only xanthophylls. Both deposits are formed from materials rich in both xanthophylls and carotenes and the suggestion has been made that in the littoral peat, which is rich in $CaCO_3$, "natural" chromatography has been in operation, xanthophylls but not carotenes being adsorbed on the $CaCO_3$; an explanation for the accumulation of carotenes in algal deposits has not been offered.

Samples taken of Russian marsh sapropels[23] were richer in carotenoids than were samples taken from Muscovite lakes.[24] The superficial layers of the lake muds carried the highest concentration of carotenoids, which amounted, on the average, to 1·67 mg./g. and 2·62 mg./g. of carotenes and xanthophylls respectively.[24] Karrer and Koenig[25] found that rhodoviolascin was by far the predominating pigment in a red mud obtained from Kenya. Van Niel[42] considers that this is due to the fact that the material was a natural mass-culture of the purple sulphur-containing bacteria.

Beattie[26] examined the detritus in caves in North Italy and found that carotenoids are associated with underground rivers, for large quantities were found in the mud of a pool fed by the river whilst none was found in mud in a drip pool unconnected with the river. Airborne detritus from the mouth of the cave and similar material from Chislehurst contained little or no pigment. As in the case of Baudisch and von Euler's[22] "littoralgyttja," xanthophylls predominate in the river-borne detritus.

Amphibia

As early as 1882 Kühne[27] found evidence for the presence of carotenoids in the skin of the following species of frogs and toads: *Hyla arborea*, *Rana esculenta*, *Bufo viridis*, *B. calamita*, and *B. vulgaris*. This was confirmed qualitatively for *R. esculenta* by Kruckenberg[6] and *R. esculenta* and *R. temporaria* by Lönnberg.[28] Dietel[29] found carotene in the liver and ovaries of *R. temporaria*, and van Eekelen[30] carotene and xanthophyllic esters in the skins of both *R. esculenta* and *R. temporaria*. Manunta,[31] studying *R. esculenta* and *H. arborea*, found large amounts of carotene and small amounts of free xanthophylls in the former and equal amounts of carotenes and esterified xanthophylls in the latter.

It was Rand,[32] however, who first demonstrated that the frog was a veritable living storehouse of carotenoids for they occur in skin, liver, kidney, lungs, ovaries, ova, oviducts, testes, and fat bodies of both summer and winter frogs. This was later confirmed by Brunner and Stein[33] by Zechmeister and Tuzson,[34] and Morton and Rosen.[35] Zechmeister and Tuzson identified α- and β-carotene, lutein (xanthophyll), and zeaxanthin and Morton and Rosen, whilst confirming this work, also found evidence for the presence of mono- and di-xanthophyllic esters. Lutein (xanthophyll) has also been detected in the frogs retinae[36] where it may exist as a protein complex.[36A,37] Lutein (xanthophyll) is also present in the pigment epithelium of the eyes of the bullfrog *Rana catesbiana*[36] and the edible frog *R. esculenta*.[36B]

Bartz and Schmidt[37] detected carotene, lutein and zeaxanthin in the brachial and sciatic nerves of bull frogs, and Ackerman[36A] carotenoids in the phagocytes in the intestinal walls of frogs.

Proteus anguineus,[26] a blind and nearly colourless salamander inhabits dark caves in Yugoslavia and North Italy; the liver contains small amounts of carotene and the body unesterified xanthophylls.

The carotenoids which are widely distributed in the Great newts (*Triton cristata* and *T. carnifex*) have not yet been unequivocally identified; both xanthophylls and carotenes exist and of the xanthophylls, the two major components are probably cryptoxanthin and taraxanthin. The xanthophylls are completely esterified in the skin but only partly so in the liver. Love[38] has made a study of the quantitative distribution of carotenoids in *Triton carnifex*. (Table 38).

Axolotls (*Ambystoma triginum*) kept in captivity did not absorb to any appreciable extent β-carotene dissolved in arachis oil.[38]

TABLE 38.—*The quantitative distribution of carotenoids in the organs of the great newt* (Triton carnifex)

Organ	Amount of carotenoids μg./g. (fresh wt.)	
	Carotenes	Xanthophylls
Liver	59·0	34·5
Fat body ..	17·6	36·8
Ovary	6·3	7·4
Testes	3·8	6·1
Spleen	1·1	1·4
Gut	1·1	1·1
Remaining tissues	2·6	7·6

From Love, R. M. (1951), Ph.D. Thesis University of Liverpool.

METABOLISM

The most important contribution to the further understanding of carotene metabolism in frogs is that of Morton and Rosen.[35] By using much more rigid experimental techniques than any of those previously employed and by following the changes occurring during complete annual cycles, they obtained results which command considerable attention.

There were little, if any, seasonal variations in the carotenoids of kidneys, skin, muscle, tongue, lungs, pancreas or eyes. The skin, liver, and muscle contain considerable amounts in both sexes, but females differ from males in mobilizing relatively enormous quantities of carotenoids into the ovaries and mature eggs. The expulsion of the ova represents a considerable loss of carotenoids to the female.

The levels in male and female livers remained constant during hibernation ; on spawning there was a decrease which continued until December. Gonads showed a marked seasonal variation which was independent of dietary changes ; and the concentration in both testes and ovaries dropped precipitately at spawning. During spawning there was a very marked depletion of the carotenoids of female fat bodies which indicates their significance in ovarian nutrition. Once more there is a hint that carotenoids function in reproduction, and this is enhanced by the fact that gonadal xanthophylls are unesterified.

TABLE 39

Typical Values for the Carotenoid Content of various Frog Organs

ORGANS	Carotene (μg. per g.)	Xanthophylls (μg. per g.)
Fat body	0·158	1·40
Testes	0·32 –0·59	0·40– 0·77
Liver	1·25 –3·4	7·2 –13·0
Stomach	0·35 –0·52	0·90– 1·24
Skin	2·19 –7·25	24·4 –43·6
Leg muscle	0·69 –1·04	5·0 –10·6

From Morton, R. A., and Rosen, G. D. (1949), *Biochem. J.*, **45**, 213.

Ackerman,[36A] who followed the seasonal variations in the liver of *R. esculenta*, obtained results somewhat at variance with those of Morton and Rosen. He found a fall in the liver carotenoids during hibernation and a very sharp rise in the spring.

Morton and Rosen noted a drop in the carotenoid content of both male and female fat bodies during hibernation ; this indicates that carotenoid metabolism continued during this period. Marked fluctuations were recorded in the carotenes : xanthophylls ratio during deve-

lopment, this may indicate selective utilization, but the general significance of the variations is not yet apparent.

An important point emphasized by Morton and Rosen is that one must recognize a distinction between carotenoids carried with fat in lipid transfer and those mobilized in accordance with a cycle of specific utilization. The practical recognition of these two phases of carotenoid metabolism is not always a simple matter.

It is claimed by Rokhlina[39] that carotene antagonizes the thyrogenic stimulation of axolotl metamorphosis.

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CHAPTER VIII

INSECTS, ARACHNIDS AND REPTILES

INSECTS

Although a considerable amount of work has been carried out on the carotenoid biochemistry of insects, a great deal still remains to be done, especially on problems of metabolism and function.

Coleoptera

Zopf[1] in 1893 discovered a carotenoid in the elytras, body wall and eggs of *Lina populi* and *L. tremulae* and named it " linacarotene " ; it also occurs in the yellow juice which excited animals excrete. Zopf also found that it was present in the same tissues of the lady birds, *Coccinella septempunctata* and *C. quinquepunctata*. In 1933 Wall[2] reported the presence of carotene in *C. novempunctata*, but it was Lederer[3] who in 1934 carried out the first intensive chemical investigation on the Coleoptera ; he used *Coccinella septempunctata* and found that the elytral linacarotene was, in fact, a mixture of α- and β-carotenes and lycopene. No free xanthophylls were present but very small amounts of xanthophyll esters were found in the rest of the body.

According to the early investigations of Palmer and Knight[4] the potato (Colorado) beetle *Leptinotarsa decemlineata* contains carotene but no xanthophylls ; much more recently Manunta[5] has claimed that the main carotenoid of this beetle is similar to that extracted from the fat of the flamigo (*Phoenicopterus roseus* (*see* p. 260) and named phoenicoxanthin (but *see* p. 260). It is obvious that this beetle deserves further investigation. Palmer and Knight found an extraordinarily high concentration of carotene 13,600 μg. per 100 ml. in the haemolymph of *L. decemlineata* compared with that found in mammalian (*e.g.*, human) plasma (*see* p. 236), similar high values are found in locust haemolymph (*see* p. 219).

The flour beetle, *Tenebrio molitor*, can exist on diets devoid of carotenoids and does not manufacture any of these pigments *de novo*.[6,7]

Lepidoptera

In 1885 Poulton[8] stated that the haemolymph larvae and chrysalides of lepidoptera, contained carotenoids which originated in the insects' food ; the pigment in the haemolymph being attached to the protein.

Meyer[9] stated that carotenes were present in the haemolymph of nine families of lepidoptera. In particular, only β-carotene existed in the haeolymph and fat body of *Caradrina quadripunctata*, in the intestine and pupae of *Pieris brassicae* and *Vanessa urticae* and in the excretion of newly moulted imagines. Manunta,[10] however, claimed that both carotenes and xanthophylls occurred in the haemolymph and hypodermis of *P. brassicae*, the relative amount of xanthophylls being greater in the hypodermis. Later the same investigator indicated that these carotenoids were *α-carotene* and *taraxanthin*.[11]

Gerould[12] found that the colour of a blue mutant of the normally green *Colias philadice* was due to the destruction of alimentary " xanthophyll " by the intestinal epithelium, thus unmasking the blue-green of " chlorophyll-a " in the haemolymph. The eggs of these insects are also devoid of carotenoids.

It appears that the clothes moth (*Tineola biseliella*) does not need a dietary source of carotenoids, neither does it manufacture them on a carotenoid-free diet.[7] The skin of the larvae of *Sphinx ligustri* contains lutein attached to a protein.[13]

The haemolymph of male *Xanthia flavago* is colourless, while that of the female is greenish-yellow, owing to the presence of carotenoids.

Metabolism

The remainder of the work to be described in this section has been carried out on the economically important silk worm, *Bombyx mori*.

According to Geyer[14] the sex difference in *B. mori* (males are colourless and females bright yellow), is due to the absence of carotenoids from the male. The eggs contain carotenes and xanthophylls with the xanthophylls in excess[14] and the cocoons carotene and lutein[15] (xanthophyll) with free lutein the predominant pigment[14]; esterified lutein and violaxanthin are also present. The pigments of the haemolymph are similar to those of the eggs. Manunta[15] showed there was a differential distribution of carotenoids in the four layers of the cocoon coat. As one moves from the outside inwards the carotene content drops steadily, whilst the lutein content increases until it reaches a maximum in layer III; in layer IV it is slightly less.

The factors producing yellow (carotenoid-containing) silk and white silk have been studied.[16] Ude[17] claimed that there are two genes which control pigmentation of silk; *C* determines that the blood contains carotenoids and *Y* that these are passed on to the serigenous glands. This view implies the possibility of the occurrence of insects with yellow haemolymph and white silk (absence of *Y*); such have been found, both by Ude himself and by Gerould.[12]

Manunta[15] has shown that the carotenoid concentration in the serigenous glands increases with maturity; this is accompanied by a corresponding drop in haemolymph concentration. The amount of pigments concentrated in the glands is not dependent on the maximum levels attained in the haemolymph but on the efficiency with which the carotenoids are transferred to the glands. This efficiency is primarily controlled by the permeability of the gland to carotenoids, although the rate of movement of pigment from the intestine to the haemolymph is also of importance. According to Manunta[18] *Philosamia ricini* (eria silk moth) larvae absorb only β-carotene and violaxanthin from the complex mixture of carotenoids which occur in the leaves which they eat; furthermore, they seem to concentrate the β-carotene in the skin and the violaxanthin in the intestinal mucosae. On maturation, carotene makes its way *via* the haemolymph to the intestinal mucosae of the pupae.

HEMIPTERA

Two of the most interesting reports of investigations into the carotenoids of this group concern the predaceous *Perillus bioculatus*, and the parasitic *Apanteles flaviconchae*. The former obtains its β-carotene* by sucking the haemolymph of the larvae and adult potato (Colorado) beetle, *Leptinotarsa decemlineata*, which obtains its carotene in the first place from potato leaves.[4] *A. flaviconchae* normally feeds on the green larvae of *Colias philadice* from which it obtains xanthophylls for excretion in the yellow silk with which it spins its cocoons. When fed on the blue-green mutant of *C. philadice* from which xanthophylls are absent (*see* p. 215), the resulting cocoons are quite colourless.[12] β-Carotene appears to be the only carotenoid present in *Apis gossypii*, and it is interesting to note that there is as much β-carotene in the green as in the yellow forms.[2]

Lederer[19] has re-examined *Pyrrhocoris apetrus* which since 1894 has been known to contain carotenoids[20]; somewhat surprisingly the carotene present was identified as lycopene; β-carotene was not present.

Recently Okay[21] has claimed that the green wing pigments of *Nezara viridula* and *N. viridula* var. *torquata* are two component systems. One component is yellow—a carotenoid-protein complex and the other is blue. No free carotenoids could be detected in the wings; the identity of the blue pigment is obscure.

Knight[21A] found that in hemiptera the carotene content of the hypodermis was increased in insects reared at low temperatures and

* It is not certain that this pigment is β-carotene (*see* p. 214).

decreased at high temperatures; it will be noted later that Goodwin could find no effect of variation of rearing temperature on the carotene accumulation in the locusts.

Hymenoptera

Only two short reports exist concerning the tissue carotenoids of the hymenoptera. Von Euler, Hellström, and Klussmann[22] state that the eggs of certain ants contain carotene and Manunta[11] observed that the parasitic *Microgaster congleromatus* contains the same carotenoids as does its host *P. brassicae*, viz., α-carotene and taraxanthin (but *see* p. 215).

Honey, beeswax,[23,24] and bee glue (propolis)[22] all contain carotenoids, presumably directly derived from pollen (*see* p. 52). Scheutte and Bott's[23] early work suggested that only β-carotene was present in honey but Tischer[25] has found in addition lutein (xanthophyll) esters; in any case the constituent pigments very probably vary with the source of the pollen. It is interesting to note that amber honey is not acceptable to the American market, which likes its honey colourless and thus ignores the potential vitamin A activity in the coloured product.

Orthoptera

In 1907 Podiapolsky[26] stated that *Locusta* (*Tettigonia*) *viridissima* contained xanthophylls but it was not until 1933 that Przibram and Lederer[27] undertook the first exhaustive investigation into a member of this family, although Pannier and Verrier[28] in 1929 found large amounts of carotenoids in some red *Phyllium siccifolium* produced by raising them on the oil of green oak leaves.

Przibram and Lederer[27] considered that the green pigmentation of the walking-stick insect, *Dixippus morosus*, was produced by the combination of three components; a mixture of α- and β-carotene, "chlorophyll," and a blue water-soluble pigment. It seems, at the present stage of knowledge, very doubtful if "chlorophyll" actually exists in the integument of any insect, and the recent work of Junge[13] has shown that the pigment of *D. morosus* is due to a combination of the carotene as a protein complex and a blue ("bile") pigment, probably mesobiliverdin; the resulting green complex, called by Junge "insectoverdin," is probably widely distributed in insects.[21,29] The pigment in the red coloured femoral swellings of *D. morosus* is almost entirely α-carotene.[27] Przibram and Lederer[27] also found carotenes but no xanthophylls at every stage of metamorphosis in *Phyllium pulchrifolium*. They further stated that the green Mantis *Sphodromantis bioculata*

contains carotenoids even when reared from colourless eggs on a carotenoid-free regime ; the brown variant, on the other hand, never contained carotenoids under any circumstances.

Lederer[19] later examined the bright red wings of *Oedipoda miniata* and found the carotenoid fraction to consist of a mixture of β-carotene and an unidentified hydroxy-carotenoid. This pigment has a spectrum very similar to those exhibited by capsanthin and capsorubin but differs from these pigments in giving a strong blue coloration with HCl. The red spots on the femora of *O. miniata* contain the same mixture of carotenoids as do the wings. The blue wings of *O. coerulescens*, on the other hand, contain only traces of carotenoids. Okay[21] has verified Lederer's results and has put forward evidence that the carotenoid(s) exist in the wings as water soluble carotenoid-protein complexes. The orthopteroerythrin, obtained by Okay[30] from *O. minata*, and which he first considered to be a bile pigment, is now also believed to be a carotenoid-protein complex. Other orthoptera having similar chromoproteins with apparently the same carotenoid as the lipid constituent are *Acrotylus insubricus*, *Calophenus italicus*, *O. schochii* and *O. aurea*. Lately Okay[31] has indicated the presence of carotenoproteins in the carnivorous *Mantis religosa*, and in the phytophagic *Acrida turrita*, *Phaneroptera quadripunctata* and *Isophya kraussi*. It is interesting to note that the acridid *A. turrita* contained the greatest amount of the complex. It appears that the carotenoid in the wings of Orthoptera is astaxanthin, the free pigment as well as its protein complex occurring in the red wings, whilst the blue and yellow wings contain only the free pigment.[32] A lutein (xanthophyll) protein complex occurs in the skin of *Locusta* (*Tettigonia*) *viridissima*, *Tettigonia cantans*, and *Meconema varium* as part of the insectoverdin complex.[13]

Recent American work[33,34] on *Melanoplus bivitattus* indicates that its main carotenoid pigment is β-carotene, although a small amount of xanthophylls may be present. β-Carotene is located mainly in the body cavity and is most concentrated in the gonads. The average amount of β-carotene present in males and females is 43·6 μg. and 39·6 μg. per gram (body weight), respectively ; the concentration in the gonads can reach 266 μg./g. Although β-carotene accumulates in the body cavities of both light and dark phases of the grasshopper, it is deposited directly below the cuticle only in the light phase.

The first report of carotenoids in locusts is that recorded by Lederer[35] of unpublished work by Volkonsky and himself. This work indicated that the red integumental pigment of young Desert Locusts, *Schistocerca gregaria*, was not a carotenoid but that the yellow pigment of mature insects was. Chauvin[36] reported an unidentified rose-coloured

carotenoid in immature insects and a mixture of α- and β-carotenes in mature insects.

Recently Goodwin and Srisukh[37,38] have investigated both *Schistocerca gregaria* and the African Migratory Locust, *Locusta migratoria migratoriodes* R and F., and have identified the rose-coloured carotenoid as astaxanthin and the yellow pigment as β-carotene. β-Carotene exists principally in the fatty tissues, haemolymph and gonads, and astaxanthin in the integument only. In mature males some β-carotene finds its way into the cuticle (*see* p. 222).

As the haemolymph of both *Locusta* and *Schistocerca* contains only β-carotene 600 and 3000 μg. per 100 ml. respectively, but no astaxanthin, and as there is no astaxanthin in the locusts' food, it can only be assumed that astaxanthin can be synthesized in the integument from β-carotene ; for as previously stated there exists no confirmed case of carotenoid synthesis *de novo* by animals.

There is good evidence that in the wings of *Schistocerca* and *Locusta* as well as of the red locust (*Nomadacris septemfasciata*) astaxanthin occurs as a protein complex.[38]

In some regions of Africa, locusts are an important dietary constituent and as well as other nutrients can provide a fair amount of provitamin A (β-carotene). Goodwin[39] has arrived at a mean figure of 10–15 μg./g. (fresh wt.) for laboratory-reared insects. This value (which is somewhat lower than the values quoted by Brodskis and Rungs)[40] can easily be doubled in females containing fully developed eggs. It is appropriate to notice here that Brodskis[41] and Brodskis and Rungs[40] claim that vitamin A *per se* exists in locusts ; Goodwin and Srisukh,[38] however, could not find vitamin A in either *Locusta* or *Schistocerca*, although they had available much more sensitive apparatus than had Brodskis and Rungs. Two further points which make the absence of vitamin A most probable are (*a*) its absence from all other insects examined, and (*b*) the fact that some insects, at least, can exist on diets completely devoid of either carotenoids or vitamin A,[6,12] amongst which is the orthopteran *Blatella germanica*.[42,43] If, however, the similarity between insects and crustacea in producing astaxanthin can be extended, it is possible that some insects may produce vitamin A, because it has recently been shown to be present in a number of crustacea.[44,45]

Chauvin[46] has demonstrated the presence of carotenoids in the oenocytes of *Schistocerca*, and the orange-red pigment found by Roonwal[47] at the base of the ovarioles is probably β-carotene. Goodwin and Srisukh[38] have shown that β-carotene is the only carotenoid in the newly-hatched eggs of both *Locusta* and *Schistocerca* ; the β-carotene

content amounts to about 100 μg./egg. Unesterified astaxanthin occurs in locusts' eyes.[38]

The genetic control of carotenoids metabolism has been noted in a number of insects, *e.g.*, *Bombyx mori*, *Sphodromantis bioculata*, and *Colias philodice*, and Goodwin[39] has recently observed what is possibly a manifestation of this in *Locusta migratoria*. Four very dark blue immature adult female *Locusta*, progeny of normal gregarious parents, were examined. The body fat of these specimens was completely white and contained no carotenoids, whereas the integument contained a much higher concentration of astaxanthin than normal. This pigment was probably attached to a protein to form a complex which gave the integument its deep blue colour, for the amounts of the other (non-carotenoid) pigments presents were not different from normal.

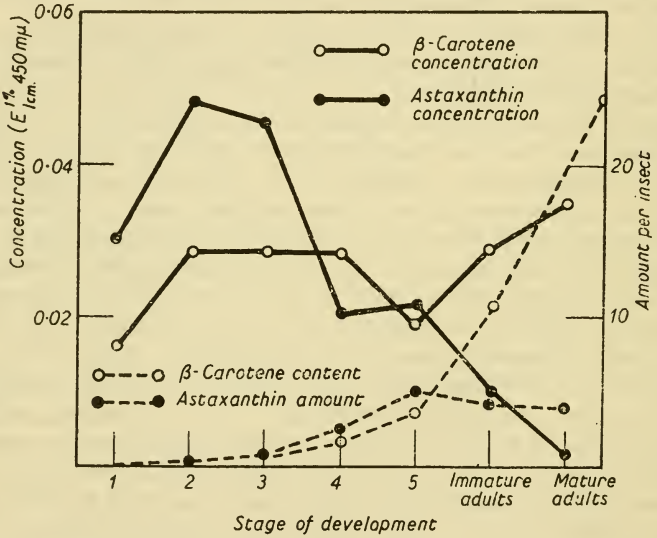

Fig. 28.—The carotenoid distribution in developing locust (*from Goodwin, T. W (1949) Biochem. J.*, **45**, 472).

Goodwin[34] has traced the changes which are identical in both gregarious and solitary phases of both species, in carotenoid distribution during the development of the insects. Figure 28, which can be considered typical, indicate how the metabolism of the two carotenoids differs. After the third stage the concentration of astaxanthin begins to decrease until in mature adults it is extremely small. The carotene concentration on the other hand increases as soon as the locust begins

to eat, remains steady through the various hopper stages and increases considerably during adult life (Fig. 28). When the amounts of the pigments per insect are considered, the carotene content continues to increase throughout life, whilst that of astaxanthin becomes stationary after the fifth hopper stage. The reason for these variations have not yet been ascertained.

During incubation of locust eggs the β-carotene disappears and astaxanthin is formed (Fig. 29). This is further evidence that β-carotene is the precursor of astaxanthin in locusts (*see also* p. 219).

Variation of breeding temperature of locusts, which has a considerable effect on the production of other pigments, has little effect on the accumulation of carotenoids.[48]

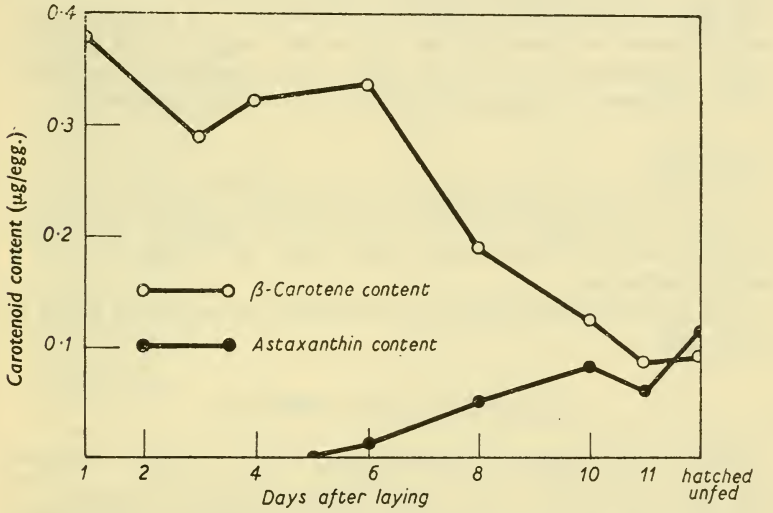

Fig. 29.—Carotenoid metabolism in the developing locust egg (*from Goodwin, T. W. (1949)* Biochem. J., **45,** 472).

Carotenoids and phase transformation in locusts. When locusts swarm, *i.e.*, change from the solitary to the gregarious phase, they undergo marked colour changes; these have been recently described in detail,[49,50] suffice it to say here that the most marked change is the loss of the green pigment, characteristic of the solitary phase, and its replacement by a much darker coloration.

Although the gross carotenoid metabolism is not different in the two phases, these pigments play an important part in the coloration of the green solitaries, whilst they play no part in the gregarious

coloration except in mature males and, in the case of *Schistocerca*, to a limited extent in hoppers. The green pigment occurring in the integument of solitary locusts is a typical insectoverdin as described by Junge.[13] It consists of a yellow and a blue component, the yellow component is a caroteno-protein complex (containing both astaxanthin and β-carotene) whilst the blue component is a chromoprotein containing mesobiliverdin as its prosthetic group.[29] The green haemolymph of solitary locusts (gregarious haemolymph is golden) is also due to an insectoverdin but this differs from that of the integument in that astaxanthin is absent from its yellow component.[29]

In gregarious locusts, although astaxanthin and β-carotene are still present, mesobiliverdin is absent, and the carotenoids are generally masked by either melanin or insectorubin.[49] Only in male adults do they play a major part in gregarious coloration; by migrating from the subcutaneous tissue into the cuticle they confer on the insects a yellow appearance. Similarly, the yellow coloured areas of the abdomen of the *Schistocerca* hoppers is produced by cuticular β-carotene.

Neuroptera

The only report concerning insects of this group is that by Okay.[21,31] The green spring pigment of *Chrysopa peila* consists of two components one blue and the other yellow; the yellow is considered to be a caroteno-albumin.

The qualitative distribution of carotenoids in insects is given in Table 40.

FORMATION IN INSECTS

Most of the work on carotenoid metabolism in insects indicates that they accumulate their carotenoids in one of three ways:—(1) indiscriminate storage of dietary carotenoids, (2) the preferential storage of one or two dietary carotenoids, and (3) the alteration of absorbed dietary carotenoids before storage.

A number of examples of the first two processes have been cited in the preceding sections, although it should be noted that opinion is not always unanimous as to which process occurs to some species (*e.g.*, *Pieris brassicae*). Locusts are an interesting example because processes (2) and (3) occur together; β-carotene and astaxanthin are stored in the various organs but plant xanthophylls never appear. The precursor of astaxanthin is unknown but is quite likely β-carotene, for in the developing egg, the appearance of astaxanthin parallels the disappearance of β-carotene. Further evidence that β-carotene is the precursor

of astaxanthin comes from the observation, already quoted on p. 219, that astaxanthin occurs in the integument, but never in the haemolymph, where only β-carotene is found.

In the animals so far considered the production of new carotenoids has always been an oxidative process, and locusts are no exception to

TABLE 40.—*Qualitative Carotenoid Distribution in Insects*

Species	α-Carotene	β-Carotene	Lycopene	Lutein	Taraxanthin	Phoenicoxanthin	Astaxanthin	Violaxanthin	References
Apis gossypii		+							15
Bombyx mori		+		+					1, 2, 3, 4, 16, 17
Caradrina quadripunctata		+							14
Clythra quadripunctata		+							6
Coccinella novempunctata		+							11
Coccinella septempunctata	+	+	+						5
Coleoptera coccinella	+	+	+						5
Dixippus morosus	+	+							19
Leptinotarsa decemlineata		+				?			11, 7, 18
Locusta migratoria		+					+		18
Locusta (Tettigonia) viridissima					+				15
Meconema varium					+				15
Melanoplus bivittatus		+							21
Microgaster congleromatus	+				+				8
Nomadacris septemfasiata							+		18
Oedipoda miniata		+							5
Perillus bioculatus		+							7
Phyllium pulchrifolium	+	+							19
Phylosamia ricina		+						+	23
Pieris brassicae	+	+		+	+				8, 10, 14
Pyrrhocoris apterus			+						5, 9
Schistocerca gregaria	?	+					+		12, 18, 20, 22
Sphinx ligustei					+				15
Tettigonia cantans					+				15
Vanessa urticae		+							14

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this. Attention should, however, be drawn to the ladybirds, *Coccinella* spp., which accumulate lycopene, although it is extremely unlikely that this carotene occurs in their food. A study of the mechanism of formation of lycopene in these insects should be extremely revealing.

The storage of an individual carotenoid does not imply that others are not absorbed; they may be absorbed and oxidatively destroyed whilst traversing the intestinal tract. Goodwin[39] attempted to settle this point in locusts by examining the diet (grass) and faeces of locusts for carotenoids. However, it was found that as so much undigested grass was excreted in the faeces, it was impossible to decide whether carotene was preferentially absorbed or not. The same objection undoubtedly applies to accepting the suggestion that the lowered xanthophylls: carotenes ratio in *B. mori* faeces compared with that of its diet, implies preferential destruction of xanthophylls in the lumen.[51]

It seems certain that insect carotenoids are of alimentary origin for on a carotenoid-free diet neither *Tineola biseliella*,[12] *Tenebrio molitor*,[7] nor *Blatella germanica*[43] produces carotene, and Chauvin[36] has produced some evidence suggesting that *Schistocerca* carotenoids are reduced on a low carotenoid diet. The work of Przibram and Lederer[27] on the green Mantis *Sphodromantis bioculata*, should be repeated for, if it be true, it is the only known example of animals producing carotenoids *de novo*. It will be recalled that Przibram and Lederer claimed that the green varieties, bred for colourless eggs and maintained on a carotenoid-free diet, always contained carotenoids.

FUNCTION

No specific function has been assigned to insect carotenoids. The accumulation of astaxanthin in the eyes of *Schistocerca* and *Locusta* suggests that it may play a part in photoreception, for in other lower animals from which vitamin A is absent astaxanthin undoubtedly functions in this way.

There is a sexual differentiation in carotenoid disposition in some insects; the bright yellow of female *B. mori* compared with the whiteness of the male, is due to carotenoid deposition, but the difference between the colour of male and female haemolymph in *Pieris brassicae* is, however, not due to different carotenoid concentration but to different amounts of protein "oxidation products"[8] No sex-differences have been noted in the total amounts of carotenoids present in *Locusta* and *Schistocerca*, but in mature males some β-carotene migrates from the fatty tissues into the cuticle and this is mainly the cause of the yellow colour which the mature males assume[50] (*see also* p. 221).

A possible function in reproduction is suggested by the presence of carotenoids in insect eggs; carotenes and xanthophylls in *B. mori*[14,15] and β-carotene alone in *Melanoplus bivitattus*,[33,34] *Locusta migratoria* and *Schistocerca gregaria*.[38] Goodwin[39] has shown that β-carotene is metabolized during development of eggs of *Locusta* and *Schistocerca*; at about the 6–7th day of incubation β-carotene begins to disappear and astaxanthin to appear (*see* Fig. 29). This production, in the embryo, of astaxanthin may be to ensure that the newly-hatched hopper is well equipped for vision, for Wald[52] believes that photoreception is the main raison d'être of astaxanthin in invertebrates. If, however, a sexual function is eventually assigned to insect carotenoids it cannot be universal for, as has previously been stated, a number of insects can develop normally without the aid of carotenoids.

ARACHNIDS

Heim[53] found a pigment, probably a carotenoid, in a mite (*Thrombidium*) and according to Manunta[53A] this is astaxanthin.

Within the last few months, Beament[53B] has found that the eggs of the apple tree mite (*Metatranychus ulmi*) contains two carotenoids, the minor component, about 10 per cent. of the total, is α-carotene, whilst the other remains, at the moment, unidentified. Beament has also shown that the rate of development after hatching is directly related to the colour of the egg; whether this has anything to do with the carotenoid content remains to be seen. The summer eggs of *M. ulmi* are normally orange, whilst the diapausing and winter eggs are bright red and contain about three times as much pigment as the summer eggs.

Hueck[53C] has noted that blue light has a profound effect on the hatching of the apple tree mite; if this is confirmed, it may well be that the carotenoids are the sensitizing pigments for this photo-action.

REPTILES

Although not closely connected with the animals discussed in the previous section of this chapter, it will be convenient to consider here the little that is known about carotenoids in reptiles.

Kruckenberg and McMunn, during their pioneer experiments on animal pigments, came to the conclusion that the fat-soluble pigments of a number of snakes and alligators were not carotenoids (lipochromes).[35] On the other hand Kruckenberg[35] did obtain evidence of the presence of a hydroxy carotenoid in a number of chameleons, *viz.*, *Lacerta muralis*, *L. agilis*, *Chamaleon vulgaris* and

Bombinator igneus; this pigment Kruckenberg named *lacertofulvin* and in 1917 Schmidt[54] found it in crystalline form in the chromatocytes of, *inter alia*, *L. vivipara*. More recently Manunta[15] investigated the carotenoid distribution in an African chameleon (*L. viridis*). The skin contained predominantly xanthophyll esters, together with small amounts of unesterified xanthophylls and traces of carotenes; the liver contained free xanthophylls, carotenes and esterified xanthophylls in the ratio 5 : 3 : 2, and the eggs almost wholly free xanthophylls. The xanthophyll fraction from the skin and the eggs could be divided into two components, one of which was very probably lutein (xanthophyll); the eyes contained only lutein. Lovenich, von Studnitz and Wigger[55] consider that lacertofulvin which occurs together with β-carotene and lutein in the skin of *L. sicula*[56] is identical with their *chlorophane* extracted from chicken retinas (but *see* p. 260).

Many years ago (1885) Halliburton[57] demonstrated the presence of lipochromes in the serum and body fat of turtles, but no further work was reported until 1938 when Lederer[19] investigated the Japanese turtle *Chrysemys scripta elegans* with what can only be considered unexpected results. The red spots on the skin near the eye yielded γ-carotene, the yellow dorsal carapace α-carotene, and the gut a mixture of α-carotene and lutein (xanthophyll). Not sufficient material was available to identify these pigments unequivocally. The skin of *Chrysemys* terrapins contains a pigment similar to lutein (xanthophyll) but it was adsorbed more strongly on alumina than is lutein and exhibited an absorption spectrum with maxima at 450, 475 and 505 mμ. (CS_2).[58] The retinas of *Clemmys insculpta* probably contain astaxanthin.[59] The monitor *Varanus comodensis* stores carotenoids in its liver to about the same extent as do humans (*see* p. 231), but only about 10–20 per cent. of the total is carotene.[60]

Fox[61] reports that the spiny tailed iguana (*Ctenosaura acanthura*) excretes a xanthophyllic (taraxanthin) ester in the "hard, waxy, corn-grain shaped, yellow-brown kernels of the femoral pores."

Villela and Prado[62] report the presence of xanthophylls but no carotenes in the blood of the Brazilian snakes *Bothrops jararaca* and *Eudryas bifossatus*; on the other hand the plasmas of the rattle-snake, *Crotalus terrificus* and the "boipeva," *Xenodon merremii*, were devoid of carotenoids.

The liver of the tortoise, *Testudo graeca* contains considerable amounts of carotenoids.[63]

Function

Carotenoids probably play a part in the colour changes which

chameleons and lizards can produce, for at the end of the last century Kruckenberg[35] realized that " lipochromes " existed in the chromatophores which were under nervous control ; von Geldern[64] has also discussed this topic.

Attention is drawn to the presence of a *free* (unesterified) xanthophyllic carotenoid in the eggs of at least one species of chameleon.[15]

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CHAPTER IX

MAMMALIAN CAROTENOIDS

All mammalian carotenoids are of alimentary origin and there is no instance of any mammal manufacturing a "specific" carotenoid either *de novo* or from ingested carotenoids. Interest, then, centres mainly on how mammals metabolize the carotenoids which they eat. They have a general ability to convert certain carotenoids into vitamin A, but this aspect deserves a chapter to itself (Chapter XI.) and will not be considered further here. Apart from this common feature of carotenoid metabolism, mammals can be divided into three groups according to whether they accumulate in the fatty tissues of their body :

(*a*) carotenes and xanthophylls (carotenoids) ;

(*b*) primarily carotenes.

(*c*) no carotenoids.

Apart from a genetic variant of the rabbit (*see* p. 248), no mammal has yet been observed which preferentially stores xanthophylls.

These groups will be considered separately and emphasis will be laid on the carotenoid distribution in certain special structures (*e.g.*, adrenals, retina), for it is in the apparently similar distribution which one finds in these special structures in animals of all the groups, that a function of carotenoid *per se* in mammals may eventually be recognized.

It has just been stated that the problem of the conversion of a carotenoid with vitamin A activity into the vitamin will be discussed later, but at the moment it should be borne in mind that these carotenoids are converted to vitamin A in the intestinal wall [1, 2, 3, 4, 5] and not in the liver as had been assumed until recently ; this necessitates a reorientation when considering the older literature and also affords a simple explanation of a number of facts which were difficult to reconcile with the old liver-conversion theory.

CAROTENOID ACCUMULATORS

(i) Humans

The only mammal which absorbs its carotenoids unselectively and which has been extensively studied is man. This statement may require slight modification in the light of the unconfirmed claim of Karrer and

Krause-Voith[6] that ingested epoxides do not appear in the blood of humans (*see* p. 250).

The presence of both carotene and xanthophylls in human blood plasma (but not in the red cells) and milk was first demonstrated by Palmer and Eckles[7] in 1914, although the presence of lipochromes was first noted in 1869 by Thudichum.[8] According to Palmer[9] blood carotenes and xanthophylls are attached to proteins; more recent work[10,11] has confirmed this and indicated that the pigments are probably attached to an albumin*. That such a carrier-complex is involved is implicit in the results of the work of Chalmers, Goodwin and Morton[12] who found that whilst carotene dissolved in organic solvents is destroyed by ionizing radiations with an ionic yield of almost unity, in plasma the ionic yield drops to 0·01; Goldblith and Proctor[13] have recently confirmed these observations in organic solvents.

It has not been unequivocally decided whether xanthophylls are free or esterified in human plasma; Palmer[9] considered them to be free but more recent work by Süllman and Vischer[14] and by Pratt and Stern[15] indicates that they are esterified.

The suggestion of Sullman and Vischer that some xanthophylls are converted into carotenes as they cross the gut wall is in all probability incorrect.

The number of reports confirming Palmer and Eckles'[7] original observation on the presence of carotenoids in blood is legion and it is now considered that blood carotenoids are merely a reflection of the carotenoid intake in the diet:[16-18] this similarity is carried over into the body fat[19-21] and, in general, milk;[21-27] on the average about 25 per cent. of the total carotenoids of human milk is made up of α- and β-carotenes,[27-32] and of this fraction 35–40 per cent. is α-carotene.[32] With and his collaborators consider that lycopene is not transferred to the milk[28-30] but the recent work of Kon and Mawson[27] makes this conclusion improbable; further more, lycopene ic present in human fat[33] and blood.[34] The values obtained for the carotenoid distribution in human milk in war-time Britain are given in Table 41. Kon and Mawson found that although considerable variations occurred from mother to mother the values obtained during a single complete lactation were very constant. It should be noted that although the milk carotenoids generally reflect the blood carotenoids, as stated above, Kon and Mawson's studies revealed some small but important variations. The unknown pigment referred to in Table 41, separates on chromatography with vitamin A from which it could not be separated; it had an absorption spectrum with maxima in hexane at 450 and 476 mμ.

*β-Globulin has recently been reported as the carrier.[9a]

TABLE 41.—*The Relative Proportions of Carotenoids in human Milk**

Pigment	*% of total carotenoids*	*Range of values*
α- and β-carotene	23	9–43
Lycopene	9	2–19
Unknown pigment	21	12–24
Lutein	47	11–71

*From Kon and Mawson (1950) *Med. Res. Council Sp. Rept. Series No.* 269.

Other organs which store carotenoids in approximately the same ratio as that in which they occur in the diet are the adrenals,[10,35] seminal vesicles,[36] the placenta,[35,37] heart tissue,[38] the liver,[37,39] pancreas,[40] nerves[41] and bone marrow.[42] On the other hand corpora lutea contain almost exclusively β-carotene with only traces of xanthophylls,[9,43] whilst the pigment of the macular region of the retina may be simply lutein.[42] Carotenoids accumulate in the fatty depots throughout life and this accounts for the fact that adult fat is yellow and infant fat almost white.[21]

Zechmeister and Tuszon isolated carotene and lycopene in crystalline form from human fat. They also noted the presence of an unidentified carotenoid with an absorption spectrum exhibiting maxima in CS_2 at 456 and 428 mμ. From modern knowledge, this may be either aurochrome, ζ-carotene or auroxanthin

When, for any reason, body fat is mobilized, carotenoids remain in the depots and this is the reason, according to Thompson[21] why fat from emaciated humans is much yellower than that from well nourished humans. Thompson's work also suggests that fat from the abdomen and chest is richer in carotenoids than is that from thighs and arms, and that there is also a sex difference in amounts deposited. That this latter difference is significant is doubtful although Poulssen[45] had previously claimed that female fat had twice the vitamin A activity (? carotenoid content) of male fat. Thompson further noted that fat from atherosclerotic aortae contained much greater concentration of carotenoids than did the corresponding fat depots and that the concentration in suprarenal fat was ten times greater than in body fat. About the same time Aschoff[46] reported that fat from adrenals contained twenty and that from atheromatous fat ten times as much as did subcutaneous fat.

No carotenoids have been detected in human sweat[46], spermatozoa[47] or cerebro-spinal fluid.[48] Drigalski[47] found no carotenoids in bile but Willstaedt and Lindquist[39] claimed to have isolated two new carotenoids from bile (and, incidentally, from plasma) which they consider to be metabolic products of β-carotene.

Carotenoid Metabolism in Humans

(i) *Metabolism of Carotenoids during reproduction and lactation.* It is now well established that foetal blood contains a much lower concentration of carotenoids than does the corresponding maternal blood, and that the placenta acts as partial barrier to the transfer of carotenoids to the foetus.[49-60] The relative amounts of carotenes compared with xanthophylls is also lowered. Typical values for the total carotenoids in maternal venous cord (placental) and arterial cord (foetal) blood are 96·9 and 90 μg. per 100 ml. plasma respectively.[53] Lewis and Bodansky and their collaborators[56,57] and Neuweiler[53] agree that there is no difference between arterial and venous cord blood for they do not confirm Clausen and McCoord's[52] claim that owing to the utilization of carotene by the foetus, venous cord blood contains more than does arterial cord blood. Lund and Kimble[56] found a direct correlation between concentration of foetal and maternal plasma carotenoids but not between their vitamin A levels. This led them to believe that the placenta is permeable to carotenoids but not to vitamin A, the foetal vitamin A being produced *in situ* from carotenoids. This is a possibility in humans, in whose blood β-carotene normally occurs, but it cannot be applicable to those mammals whose blood is devoid of carotene but whose foetal livers contain vitamin A. Examples of such animals are pigs and goats, and in these species Thomas, Loosli and Williams,[61] have demonstrated transference of vitamin A across the placenta.

It is now well established that there is a fall in carotenoid concentration of blood at or just after parturition in cattle (*see* p. 240); it has been reported only once[62] in humans, but there is no reason to believe that it is not a normal occurrence.

The ability of the newly born infant to absorb xanthophylls must be slight for compared with birth values, the blood xanthophylls only begin to increase, when the child is two years old whilst the carotene levels begin to increase immediately after birth.

A number of observers have noted that the carotenoid concentration decreases rapidly as the colostrum changes into mature milk[22,53,63-66] and this has been confirmed by intensive investigations carried out by Lescher *et al.*[25] in America, by Kon and Mawson[27] in England and

by Chanda *et al.*[32] in Scotland. Lescher *et al.*[25] in their wide survey of the vitamin content of human breast milk found that the colostral carotenoid levels diminished rapidly from the first day of lactation to the 5th or 6th day when they were stabilized at levels which were maintained for as long as lactation lasted (up to 300 days in some of the cases investigated). They found that the average carotenoid concentration of 1st day colostrum was 241 μg./100 ml. and the mean maintenance level about 25 μg./100 ml. Kon and Mawson,[27] stating their results with respect to fat, recorded concentrations of 2mg./100g. at the 3rd–4th day of lactation; this quickly fell to a maintenance level of 0·36 mg./100 g. (*See* Tables 42 and 44 for further values.)

In the same way as cows milk, human milk shows seasonal variations according to the availability of green food.[32,67]

Milks rich in fat have a high concentration of carotenoids, whilst those poor in fat, although the total amount present was low also, have a high carotenoid concentration. The concentration in the milk of β-carotene is always lower than in the blood, whilst that of the other carotenoids was higher. The lower β-carotene values may be due to (*a*) its conversion into vitamin A in the mammary gland or (*b*) the mammary gland acting as a partial barrier. The transfer of breast milk to infants must be efficient for there are reports of sucklings with carotenaemia.[68,69]

(ii) Pathological Aspects

Excessive carotenoid intake can lead to intense yellow pigmentation of the skin owing to deposition of carotenoids in the hypodermis. This pseudo-icterus or xanthemia is always accompanied by carotenaemia and, according to van den Bergh, Hymans and Snapper,[70] a carotenuria; however, recent exhaustive investigations by Lawrie, Moore and Rajagopol[71] indicate that the appearance of carotene in the urine is highly improbable under any conditions. The condition of carotenaemia is apparently without ill-effect and disappears on removing carotenoids from the diet,[72,73] similarly the withdrawal of carotenoids from the diets of normal humans quickly reduces the blood levels to zero. There is one very interesting report of a case in which carotenaemia was due to the failure of the patient to convert carotene into vitamin A in the intestine. The carotene which thus escaped transformation passed into the blood in such quantities that the carotene : xanthophyll ratio was reversed.[75]

The pigmentation of the skin in diabetics, " xanthosis diabetica," is also due to subcutaneous accumulation of carotenoids. This is generally accompanied by a carotenaemia and has led to the conclusion that the

conversion of carotene into vitamin A is impaired in diabetics.[76-81] Recent extensive studies have shown that this assumption is not warranted.[82-85] The high carotene blood levels that are often found in diabetics are, in all probability, the result of high carotene intake associated with a typical diabetic diet; when a large enough "sample" of diabetics was examined the scatter in the carotene plasma levels was normal.[84] A recent interesting report which still requires confirmation is that which states that although the plasma carotene levels of diabetics respond normally to the presence or absence of carotene in the diet, the response of those patients which are insulin sensitive is much greater.[86]

Conditions associated with hyperlipaemia are often also characterized by carotenaemia.[87] Although carotenoid plasma levels in sick humans are often not very different from normal,[88,89] they are

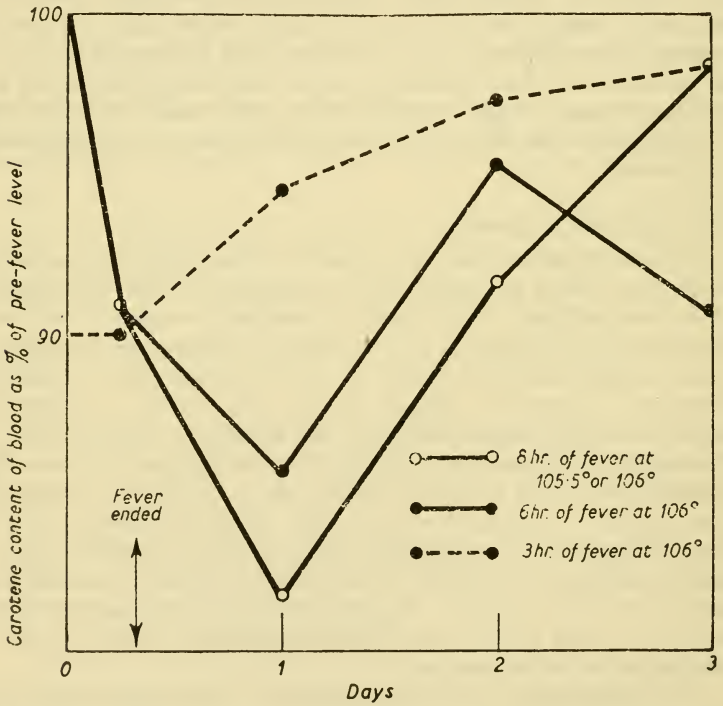

Fig. 30.—Illustrating the effect of fever on the carotene content of human blood. (*After Aron, H. C. S., Craig, R. M., Farmer, C. J., Kendell, H. W., and Schwenlein, G. X. (1945)* Proc. Soc. Exp. Biol. Med., **61**, 271).

generally lowered in scarlet fever,[52] in artificial hyperthermia,[52,89] (*see* Fig. 30), glomerular nephritis,[52] coeliac disease,[90] and in the acute stages of jaundice.[52] In scarlet fever, reduction may be due to reduced carotenoid intake, and in jaundice to impaired absorption (*see* p. 250). Lowered plasma carotene levels are recorded in the case of pernicious anaemia, but this seems of little importance in the aetiology of the disease.[91]

According to Heymann,[92] absorption of carotene in children is decreased in pneumonia, sepsis and gripe. Not all the effect could be attributed to the accompanying fever, because fevers induced by smallpox vaccines and an unspecified hyperpyrexic drug did not bring about decreased absorption. Josephs[93] has confirmed the drop in plasma carotenoids in pneumonia in infants. Thiele and Guzinski[94] claimed that adrenalin injections produce slight increases in the plasma carotenoid levels of humans suffering from a variety of diseases. A close scrutiny of their data compels one to the view that no such increases have been unequivocally demonstrated. Liver levels in health and disease have been reported.[94A]

The relationship between the thyroid gland and carotene metabolism is now attracting much attention and the biochemical aspects will be discussed more fully in Chapter XI. Here will be mentioned only the chief clinical findings.

Clausen and McCoord,[50] Anderson and Soley,[95] and Soskin and Mirsky[96] reported carotenaemia associated with hyperthyroidism, but in their cases of hyperthyroidism, Popper and Steigmann[97] found that the plasma carotene levels fell within the normal range. Escamilla[98] and Mandelbaum, Candel and Millman[99] report a carotenaemia associated with myxoedema which was improved by thyroid therapy; from the evidence provided it is clear that in neither study was the criterion used for carotenaemia sufficiently rigid. Recent work by Cohen,[100] however, quite clearly shows that carotenaemia is very often associated with myxoedema. Durupt[101] takes a case of carotenaemia associated with a decreased B.M.R. (–25) to indicate that the thyroid controls the conversion of carotene into vitamin A; however, it is difficult to see how he reconciles this conclusion with the high plasma vitamin A values also recorded in his patient.

Contradictory reports concerning carotenoid levels in aged humans exist; Wagner[102] claims that plasma carotenoid levels are increased whilst Rafsky ,Newman and Jolliffe[103] note decreased levels in old people. However, Kirk and Chieffi[104] and Yiengst and Shock[105] in wider surveys found no statistically significant differences between old and young subjects. This has recently been confirmed.[331]

An interesting report, which requires confirmation, is that high altitudes reduce the carotene plasma levels; a flight of 2 hr. at 500 metres caused a fall of 14 per cent. and a 1½ hour flight at 4,000 metres a 30 per cent. fall.[105] The drop in the carotenoid levels of the plasma and milk of cows which occurs after massive doses of vitamin A (*see* p. 243) does not occur in humans.[106] The plasma carotene levels of humans with various pathological conditions are recorded in Table 42.

TABLE 42

Carotenoid Content of Blood of Normal and Pathological Human Cases

CONDITION	AMOUNT μg./100 ml.		REFERENCE
	Mean value	Range	
Normal	166–187	50–340	1
		32–290	2
		108–141	4
	87–95	28–282	5
	199–227	102–420	6
	183	96–327	7
	128	18–347	8
	122	50–241	9
	142–146	64–260	13, 14
	80		21
	115		17
	230		23
Normal aged 16–39	190–180	10–380	12
,, 40–59	120	30–270	12
,, 60–69	120–190	20–210	12
,, 70–79	100–110	20–260	12
over 80	120–100	20–240	12
aged 40–90	113	—	24
Pregnant	47		3
	106		5
	127–146	16·6–235	11
			19
1st 5 months	119·5		20
last 3 months	145·9		20
Eczema	30		3
Laennec's cirrhosis (decompensated)	72	17–166	13
,, ,, (compensated)	88	8–247	13
Miscellaneous diseases	121	17–498	13
Infant blood	8		3
(umbilical)	16–21	0–13	11, 19
Hyperthyroidism	182		24
Folliculosis	116	45·7(S.D.)	15
Premature infants	30·6	7·5–41·7	16
Full term infants	25·2	14·4–46·2	16
Children	92		5
	136	60–258	10
	101	16.5 S.D.)	15
Sprue	8–21		17
	48		18
Rheumatic fever (children)	117	33–319	10
Various common skin diseases	216		23
Darier's disease	193		
Pityriasis rubra pilaris	186		
Ichthyosis	167		

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Carotenoid Assimilators other than Man

A study of the data of Jensen and With,[107] who examined the liver carotenoids of a large number of mammals, indicates that only the fox, *Canis vulpes*, and the fitchet, *Mustela putorius*, resemble humans in storing both carotenes and xanthophylls without any sign of preference. However, there are some indications that the badger (*Meles taxus*) and the roedeer (*Capreolus capreolus*) are in the same category.

MAMMALS PRIMARILY CAROTENE ASSIMILATORS

Cattle

The preferential accumulation of carotene in the tissues of cows was first noted in the pioneer work of Palmer and Eckles.[71] The carotenes constitute 92–95 per cent. of the total carotenoids present[108,109] although small amounts of xanthophylls such as lutein (xanthophyll) and cryptoxanthin do, however, occur.[109-113] β-Carotene is, naturally, the chief component of the carotene fraction but α-carotene[110,114,115] and lycopene[116] are present in varying small amounts.

Plasma

The carotenoid content of cows' plasma shows a marked seasonal variation which has been firmly established by a very large number of workers; in other words the plasma (and milk and butter) levels

reflect quantitatively but not qualitatively[116,117] the dietary intake of carotenoids. Sharp increases in plasma values are obtained just after going out to grass during the spring flush[116,118] although maximum levels are generally reached later in the summer.[119] Even in the case of cows which are grazing all the year round, the seasonal variation corresponding to periods of active growth and dormancy of the forages, is still marked.[120] (*see* Fig. 31)

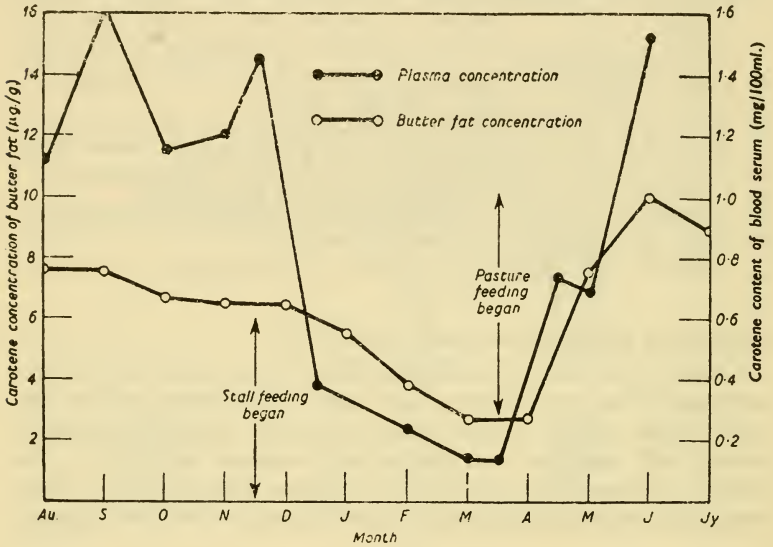

Fig. 31.—Illustrating the seasonal variations in the carotene content of butter fat and blood plasma of Ayrshire Cows (*from Lord, J. W. 1945,* Biochem. J., **39**, 372.)

The carotenoid level of cow plasma can be up to five times greater than bull plasma,[110,120] This may be due to the greater metabolic turnover in females,[121] to the greater efficiency of bulls in converting carotene into vitamin A (*see* p. 275), or to different management of bulls which are often out at grass only for very short periods. Which is the true reason has not yet been finally decided.

As well as seasonal variations, there are marked variations in plasma levels[122] in different breeds of cattle (Table 43) and these are reflected in the colostrum,[123] milk[122,124] and butter.[117,118,124-129]

Tarassuk and Regan[124] claimed to have established a direct relation between carotene levels in plasma in the corresponding milk fats; the numerical values expressed as mg. per 100 ml. plasma and mg. per

100 g. of fat should be the same. The carotene content of the colostra and mature milk of different mammalian species and different breeds of cows are recorded in Tables 43 and 44.

TABLE 43

Variations in Plasma and Milk Carotenoids in Different Breeds of Dairy Cattle[1]

BREED	CAROTENOIDS	
	Milk Fat μg/g.	Plasma μg/100ml.
Guernseys	5·8	487
Holsteins	3·4	385
Ayrshires	4·1	340

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TABLE 44

Carotene Content of Colostra

SPECIES	AMOUNT μg./100 ml.	REFERENCE
Cows — Jerseys	347	1
Holsteins	100	1
	59–136	2
"Beef"	124	1
Hariana & Sahiwal	33·6–153·9	6
Ayrshire	244	7
Humans	51	3
	25–34	4
	150–153	5
Swine*	24	1
Goat	12–46	8

(* but *see also* p. 248)

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Typical carotenoid plasma levels for new-born cattle of any breed are 22-50 μg./100 ml.[130,131] but breed differences become obvious within eight days of birth.[132]

Colostrum and Milk

As in the case of humans the colostrum of cows is very rich in carotene.[109,110,121,133-135] The pre-nursing value of colostrum is between 5–10 times higher than that of seventh-day milk when calculated on the whole fluid, and three times higher when measured on the fat basis.[129,133] Dann[136] had previously reported greater differences between colostrum and late milk, and claimed that heifers produced colostrum richer in (vitamin A) than did cows. Recently it has been shown that the carotene content of the first successive 2 lb. samples of colostrum increases considerably,[137] that interruption of milking increases the concentration of carotene in the milk but does not alter the 3-day yield,[138] that vitamin E therapy has no effect on

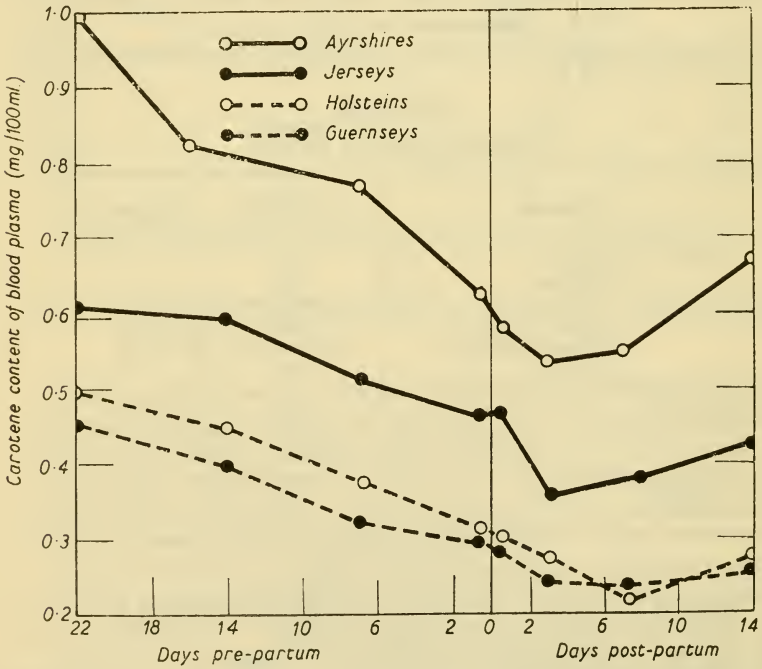

Fig. 32.—Illustrating the drop in plasma carotene levels of cows at parturition. (*From Sutton, T. S., Kaeser, H. E., and Soldner, P. A. (1945)* J. Dairy Sci., **28**, 933.)

the carotene content of colostrum,[139] and that the carotene content of colostrum from one half of the udder milked before parturition was the same as that from the other half milked just after parturition.[139A]

A drop occurs in the carotene plasma levels at parturition[109,140,141] (*see* Fig. 32) and thus there is no possible positive correlation between plasma levels and colostral levels.[28] Spielman, Thomas, Loosli, Whiting, Norton and Turk[129] have, however, noted a correlation between the carotene plasma values measured 18 days before parturition, and colostral levels ; this indicates that the storage of carotenoids in the mammary gland is proportional to the blood levels operating during gestation. The importance of colostrum as a vehicle for carotene (and vitamin A) in feeding of new born cows has been well illustrated by the work of Spielman, Loosli, Thomas, and Turk[142] and of Wise, Caldwell, Parrish, Atkeson, and Hughes.[143] Spielman *et al.* showed that newly born dairy calves maintained on a basal skim milk (low carotene) diet, would not absorb carotene concentrates of various types (crystalline carotene in oil, lucerne meal, etc.), but scoured badly. Only after control of scours during the first week of life by the daily administration of sulphathalidine was the carotene reasonably well absorbed. Wise *et al.* have shown that on good quality hay the blood carotene levels of calves do not increase until the animals are at least six weeks old. It has further been noted that prepartum milking of the dam considerably lowers the carotene levels of the calves compared with those of calves whose dams were not so treated.[144]

Some further points concerning carotenes in cows' milk should be mentioned before considering further the drop in plasma carotenoids in the parturient cow. Cows' milk contains about half the concentration of carotenoids found in human milk,[145] but it should be emphasized that from the point of view of vitamin A activity the qualitative inter-species differences result in the cows' milk being more potent as a source of pro-vitamin A. The active carotene fraction represents about 85 per cent. of total bovine milk carotenoids[109,111] but only 25 per cent. of total human milk carotenoids.

Berl and Peterson[146] have studied the carotene distribution during butter making ; 10–14 per cent. remains in the skim milk, 89–94 per cent. is transferred to the butter and only 0·8–2·0 per cent. remains in the buttermilk. Kon, Mawson and Thompson[147] examined the carotene content of the fat obtained from the different fractions produced during butter making and found that the carotene concentration in separated milk fat and separated whey fat was many times greater than in the fat of other fractions ; *i.e.*, concentrations were highest in the fractions containing the smallest fat globules. Cholesterol, but not

vitamin A has the same distribution. It is suggested that, as carotene accumulates in fat droplets which have the greatest surface area per unit weight, it is concerned in some way with the globular membrane.

TABLE 45—*Carotene Content of Mature Milks*

SPECIES	AMOUNT μg./100 ml.		REFERENCE
	Winter (stall)	Summer (pasture)	
Cow—mixed	3·8–14	17–32	1
	5(min.)	25(max.)	2
	5	35	3
	5	30	4
	3	24	5
	14·07	20·4	13
	18[a]		16
Jersey	5·9	105	6
	20·5	121	7
	27 51[a]	121	8
Guernsey ..	34·5	205	7
	35		8
	104[a]		
	23[a]		16
Holstein	19·5	180	7
	31		8
Ayreshires ..	14·4		7
Hariana & Sahiwal	10·8	43·7	14
Brown Swiss ..	12		7
	31[a]		
Sahiwal	6–50[a]		9
Buffalo	none		15
Human	*mean*		
	16–60		10
	0–430		11
	24–25		12
	2–17		14
	15		17

(*a*) mean of summer and winter values

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Cheese

Cheese undergoes the same seasonal variations in carotenoids as does milk.[148] About 85 per cent. of the milk carotenoids are transferred to the cheese[149,150] and these carotenoids persist through ripening. Little difference was noted between carotene levels in Cheddar and Cheshire cheeses, mean values 2·0 μg. and 10μg./g. of cheese fat for winter and summer cheeses respectively;[151] a typical value when calculated on the whole cheese is 1·8 μg./g.[148]

The sharp fall in plasma carotenoid levels at parturition[109,140,141,152,152A] is stabilized for about three weeks post-partum when the levels are about 46 per cent. of the pre-partum levels; thereafter the levels begin to increase (Fig. 32). The physiological factors controlling this drop are not yet completely understood; the claim of Sutton, Kaeser and Soldner[147] that the sharp decline coincides with a rapidly filling udder, *i.e.*, with the drawing off of colostrum cannot be the only factor operating, because a similar fall occurred when a mammectomised cow gave birth to a premature calf.[153] Goodwin and Wilson[109] consider that the changes have no significance for carotenoids *per se*, but merely indicate a general variation in the concentration of blood constituents at parturition, for similar changes have been noted with plasma lipids,[154] Ca and P,[154,155] vitamin E,[156] and cholesterol.[332]

It is interesting to note that the drop does not occur in cows which develop milk fever.[152] In induced mastitis, however, the milk carotenoid levels are increased but the total amount secreted per day is always below average because of the lowered milk yield; on recovery the values returned to normal.[157] Calves which were born with no plasma carotenoids had 4·5–17·7 μg./100 ml. according to diet within 3–4 days of birth. At about 7 days this had dropped to a steady level.[139A]

Spielman *et al.*[142] found that foetal livers and blood contain carotenoids and that the liver levels but not plasma levels were proportional to the maternal intake of carotenoids. This is contrary to the work of Lund and Kimble[56] on humans which demonstrated a direct correlation between maternal and foetal plasma carotenoids. As has been previously stated a normal plasma carotenoid level for a full-term foetus is 22 μg./100 ml.[130]

Factors other than dietary and reproductive can play some part in controlling plasma carotenoid levels in cattle. For example, cows with acetonaemia have high blood carotene accompanied by low vitamin A levels. Massive doses of vitamin A cure the acetonaemia and restore both the carotenoid and vitamin A levels to normal.[158-160] A similar reaction to vitamin A therapy can be produced in normal cattle; massive doses of vitamin A reduce the plasma and milk

carotenoid levels in healthy beasts,[161-163] but have no effect on the carotenoid content of the early mammary secretions.[139] There is apparently a factor in raw soya bean oil which has the same effect as vitamin A, for Squibb, Cannon and Allen[164] found that supplements of this oil decreased the carotene levels of both the blood and milk of cattle. The carotene levels are also depressed when the diet contains 30 per cent. of ground soya beans.[164A]

Feeding of tocopherols tend to neutralize the depressant action of vitamin A.[165] Sulphonamide therapy has no effect on the carotene plasma levels of cows[166] and neither have changes in the ambient temperature of the animals.[167]

The relationship between carotene metabolism and the thyroid gland is discussed in Chapter XI ; information on the effect of thyroglobulin and iodinated casein, which are used to increase milk yield, on the carotene level in blood and milk,[168,168A] is somewhat contradictory.

Other Organs

Carotenoids, preferentially β-carotene, accumulate in a number of other body organs in cattle, viz. body fat,[45,169,170] ovaries,[171,172] testes,[26,173] adrenals,[169,174-176] corpus luteum,[8,172,177-179] corpus rubrum,[180] thymus,[176] liver,[174,181] retina,[182-184] the pigment epithelium and iris,[184] pituitary,[185,186] bile,[187,189] muscle,[190] kidney[169,174,176] and placenta.[7,50] The spleen appears to contain no carotenoids.[10,181] The reason for the accumulation of carotenes rather than xanthophylls in all these organs as well as in the blood and milk is probably due to the failure to absorb xanthophylls from the intestinal tract, for these greatly preponderate over carotenes in cows' faeces ;[191] even so the faeces of cows feeding on green pasture contain over 100 μg./g. of carotene.[192]

The carotene deposited in the body fat of cattle can accumulate with age to a considerable extent, as witnessed by the colour of the fat of a particularly tough week-end joint. Zechmeister and Tuzson[170] isolated 2 mg. of crystalline carotene per 2 kg. of cow fat. The claim that cow fat contains considerably more carotene than does bull fat[45] has not yet been confirmed, but it is possible for there may be analogous sexual differentiation in plasma carotenoid levels,[133,141] although, as mentioned previously (*see* p. 238), this may be partly due to different dietary habits.

The amount of carotene present in the corpus luteum and corpus rubrum is considerable and can reach 6 and 120 mg./100 g. for these two organs respectively. No other mammalian tissue contains such a

high concentration of carotene; in fact, "carotene" was first obtained crystalline from corpora lutea.[177] Ovaries contain as much as five times more carotene than do testes,[118] this again may reflect dietary habits.

The liver carotene levels vary with the seasonal intake of carotenoids as do the plasma and milk levels.[193]

According to Studnitz, Neumann and Loevenich[183] and Bielig and Busch[184] cattle retinas not only contain β-carotene but also astaxanthin and a pigment which has not been characterized satisfactorily but which might be lactertofulvin (*see* p. 226); Brunner, Baroni and Kleiman, however, could only detect β-carotene,[181] probably in colloidal solution.[169] The iris contains β-carotene only.[184]

The carotene in cattle muscle is not bound to protein and its concentration in muscle is 25 μg. per 100 g. of tissue, which is a lesser concentration than that in the blood plasma.[190] That carotene may possibly be an excretory product in cattle is suggested by the fact that it can be extracted from the yellow patches of olfactory tissue in the upper region of the nasal cavity,[194] and from ear wax.[9]

Other Mammals

Other mammalian species which preferentially accumulate carotene in their tissues, although to a very much lesser extent than cattle, are the horse,[195,196] sheep,[197-198] Indian buffalo,[199] deer,[198] antelope,[198] carabao,[190,120] guinea pig,[198] and hedgehog.[201]

(*a*) *Horses*

Palmer[195] noted the preferential accumulation of carotenes in horse plasma and Zechmeister and Tuzson[196] confirmed this in the depot fat. In 1935 Zechmeister and Tuzson[202] fed a horse a large amount of green fodder (carotenes and xanthophylls) and collected both the jugular and portal blood at slaughter; both samples contained only carotene. Palmer and Eckles[7] considered that the accumulation of carotenes was due to destruction of the xanthophylls as they traversed the gut wall; this may be true (for it appears to be so in rats (*see* p. 248) but selective absorption probably plays a part because Zechmeister and Tuzson[196] found the total carotenoids of horse dung to contain a higher proportion of xanthophylls than did those of the fodder. The carotene values of mares' plasma follow the usual seasonal variations.[203]

(*b*) *Guinea Pigs*

Guinea pigs' milk contains small amounts of carotenoids.[204] Mitolo[205] claims that the carotene content of the liver of scorbutic

guinea-pigs is less than that of those fed a diet adequate in vitamin C. This report should, at the moment, be treated with some caution for the same investigator has recorded the presence of carotene in rat liver,[206] an observation which is at variance with most other published reports (*see* p. 247).

(*c*) *Sheep*

Peirce's extended studies[197] on sheep in Australia reveal that the carotene plasma level on dry pasture is about 2 μg./100 ml.; the level responds to a diet of green fodder and can reach a maximum of about 18 μg./100 ml.; but this is much less than the maximum which can be reached in cows (of the order of 500–1,000 μg./100 ml.).

Paulson, Hilmoe and Moxon[198] working in South Dakota confirmed these low figures, the levels which they obtained varied from 0–17 μg./100 ml.; however, they claimed that those levels bore no relation to carotene intake. Palmer,[9] Goodwin and Gregory,[1] and Pope, Phillips and Bohstedt[207] found no carotene in the plasma of sheep which they examined. Sheep's livers contain a trace of carotene, about 1–3 μg./g., which remains constant even on a diet which completely depletes the liver of vitamin A.[197] Ewes' colostrum contains only a little (about 10 μg. per litre) and, unlike other colostra, this value does not fall soon after partition but is maintained even in the late milk.[208] The efficiency of absorption of carotene in sheep is of the same order as that recorded for other mammals.[209]

(*d*) *Buffaloes*

Buffalo butter fat contains about 1/10 the amount of carotene present in cows' butter fat,[199] and in some specimens it is completely absent

(*e*) *Carabao*

Although it has colourless body fat carabao flesh contains about 0·2 μg./g. of carotene which is greater than the corresponding plasma levels; this is the opposite to the situation obtaining in cattle. The fact that the concentration of carotenes in carabao flesh is only 1/10 that of cattle flesh is used in the Phillipines to differentiate between the two in suspected cases of fraud.[190,200]

(*f*) *Hedgehogs*

Only 5 per cent. of the total carotenoids in the liver and plasma of hedgehogs are xanthophylls.[201] The normal carotene values for plasma and liver levels (90 μg./100 ml. and 6 μg./g. respectively) vary during the season and are highest during the period of activity; they fall during hibernation to 32 μg./100 ml., and 3·4 μg./g. respectively.

Feeding carotene to hedgehogs maintained on a carotenoid-free diet increases the blood and liver levels of both carotene and vitamin A, but in spite of this only 3 per cent. of the dose can be accounted for. It has not yet been decided whether this is due to inefficient conversion of carotene into vitamin A or to inefficient absorption of the carotene.

(*g*) *Asses*

According to Manunta[213] the corpus luteum of an ass contained only β-carotene.

(*h*) *Dogs*

There is one report of carotenaemia in a dog[210] although only traces of carotene, if any, are present in dog blood[211] and liver[212] and bitches' milk does not normally contain carotene.[213]

(*i*) *Elephants*

Goodwin[213A] has found small amounts of a single carotenoid in the fat of an elephant; surprisingly this was not β-carotene but was more strongly adsorbed on alumina than this pigment, and had absorption maxima at 445 and 425 mμ in light petroleum. It closely resembles the unidentified carotenoid detected in human fat (*see* p. 231). It will be interesting to see if this observation is confirmed when further experimental material becomes available. No carotenoids were present in the liver lipids of the elephant.

Mammals which Accumulate no Carotenoids

Although they have attracted little attention, mammals which do not accumulate carotenoids in their body tissue are much more common than are those of the other groups. A survey of a large number of mammalian livers by Jensen and With[107] indicated that the majority contain, either only traces or no carotenoids.

The failure to accumulate carotenes is now considered to be due to the efficient conversion of absorbed carotene into vitamin A in the gut wall (*see* Chapter XI). The fate of ingested xanthophylls in this group is not so clear (*see* p. 248).

Amongst the most important animals in this group are goats,[1,173,214,215] swine,[217-220] rats,[221-224] rabbits,[1,225] hares,[226] and guinea-pigs.[227] Sheep have been dealt with under "carotene accumulators" but can really be considered borderline cases, for even under the most favourable nutritional conditions their plasma contains only traces of carotenoids and massive doses of carotene does not appreciably increase this amount[1] (*see* p. 246). Foxes might be placed in the same category as sheep, for they have very low plasma levels and store no carotenoids in the liver.[228]

Goodwin and Gregory[1] never detected carotene in either the systemic blood or the portal blood of goats, after feeding them massive doses of carotene in various forms. They also failed to find it in the thoracic lymph of goats; however, the lymph of goats recently removed from green pasture does contain a fat soluble yellow pigment which is not carotenoid; it may be a chlorophyll degradation product. Goodwin and Gregory also failed to detect carotenoids in the butter, liver, ovaries and adrenals of goats. Goats' colostrum may contain small amounts of carotene,[208] and traces have been recently found in the liver of some but not all goats.[333]

High doses of carotene have also failed to produce accumulation in either blood or body fat of rats,[222,223] pigs,[126,217,229] or rabbits.[1] β-Carotene, however, may be present in pigs' retinas,[124] but is absent from other organs.[230] Beadle, Wilder and Kraybill[231] found that the yellow fat occasionally encountered in pigs does not contain carotenoids; the colour was due to large amounts of linoleic acid in the fat which arose from the feeding of excessive amounts of flax seeds.

Pease[232] and Willimott[233] encountered rabbits with a recessive factor which allowed xanthophylls but not carotenes to accumulate in the subcutaneous fat. Willimott explained the phenomenon by postulating the absence of a liver enzyme capable of oxidising xanthophylls. In the light of present day knowledge this cannot be accepted, for in normal rabbits Goodwin and Gregory[1] have never found xanthophylls in the portal or systemic blood in transport to the liver, nor in the liver itself.

As was previously stated, carotene does not appear in the plasma of these animals owing to the efficiency of the mechanism converting it into vitamin A. Very little is known of the fate of ingested xanthophylls, but in rats Goodwin[234] has obtained evidence that some at least are absorbed, for after feeding free lutein (xanthophyll) he recovered small amounts of mono- and diesterified lutein (xanthophyll) from the intestinal wall; no lutein (xanthophyll) was detected in any other organ, and the fate of this absorbed pigment could not be traced. It is interesting to note that Prelog and his collaborators consider that the ionone derivatives which they have isolated from pregnant mares' urine, may have been produced from the degradation of alimentary xanthophylls.[235]

The milk of the following species contain no more than minute traces of carotenoids: goats,[208] rats,[208] Indian[236] and Egyptian[237] buffalo, and, by implication, elephants.[238] Ewes' and sows' colostrum contain no carotenes.[207,231]

Function of Carotenoids in Mammals

The potential vitamin A activity of certain carotenoids is by far the most important function which can be ascribed to this class of pigments in mammals. It is especially important in herbivorous animals for they never obtain any preformed vitamin A in their diet. This is discussed in Chapter XI.

The preferential accumulation of carotenoids in ovaries, corpora lutea, corpora rubra, and adrenals is a good *a priori* reason for suggesting that the pigments *per se* take part in the metabolism of these organs. This cannot be a general mammalian function, however, because Goodwin and Gregory[1] could find no carotenoids in the ovaries or adrenals of goats. Reports of similar investigations on other animals which have no carotenoids in their blood, liver, or depot fat are awaited with interest. The fall in the plasma carotenoid levels around parturition which has been observed in cows does not appear to be of specific importance because similar changes have been observed in other blood constituents (*see* p. 243).

Reports have been published in which it is claimed that carotene has a specific effect *per se*; none of these has yet been confirmed. It is claimed, *inter alia*, that carotene possesses antihistamine activity,[239] sensitizes the action of the gonadotrophic hormone,[240] potentiates the action of insulin and adrenaline,[241] takes part in production of volatile fatty acids in liver fat,[242] depresses arginase activity under aerobic conditions,[243] inhibits pepsin,[244] cathepsin and trypsin,[245] and increases the rate of glycolysis in muscle,[246] blood,[246A] and liver.[246A-C] The investigations on blood and liver were carried out on guinea pigs; as these animals do not contain carotenoids in their liver or blood (*see* p. 247), the action of carotene cannot be considered to have any *in vivo* significance.

Recently it has been suggested that carotenoids are linked with the cytochrome system in transferring oxygen to the macular regions of the human retina which has no blood supply.[247] Denton and Pirenne[248] and Hartridge[249] deny this. Hartridge considers that the presence of a macular pigment in humans is not yet unequivocally proved, whilst Denton and Pirenne, assuming that it does occur, consider that its main function would be to improve foveal acuity. Barnicot[250] inserted very small crystals of vitamin A acetate into small pieces of parietal bone cut from ten days old mice and then grafted these pieces of bone into the cerebral hemispheres of litter mates. The presence of the vitamin A led, within 14 days, to well-marked resorption accompanied by numerous osteoclasts and leading

eventually to perforation of the bone. β-carotene examined in a similar way was without effect.

ABSORPTION OF CAROTENOIDS

In general, carotenoids are very badly absorbed by mammals and a considerable proportion of any ingested carotenoids is excreted in the faeces. This is not the case with vitamin A which is readily absorbed.[18,252,253]

(*a*) *Humans*

In humans the experience of a group of workers in England confirmed Clausen's original statement[254] that human faeces contain carotenoid in more or less the same ratio as that of the ingested food, but Wald, Carroll and Sciarra[252] found that only 8 per cent. of ingested xanthophylls but 65 per cent. of ingested carotenes was excreted.

Absorption from vegetable foodstuffs is generally poor but is even worse if the foodstuffs are uncooked or not finely divided.[18,252-279] Although reported figures are somewhat variable it is well proved that absorption is facilitated by the presence of lipids[18,255,257,259,267,275] especially lecithin,[280] even when there are adequate amounts of tocopherols in the diet.[281,282] There are also indications that the efficiency of absorption depends on the type of fat in the diet,[259,263] although Virtanen[274] thinks that this is not so. Very recently, however, Aldersberg and his colleagues[283] have shown conclusively that butter is a better vehicle than cotton-seed oil and Deuel and his group that carotene has a greater biological activity when fed incorporated into margarine than into limpid cotton-seed oil.[284]

It is claimed that carotene adsorbed on to a protein coagulate, obtained by heating leaf juice, is 50 per cent. better absorbed than is carotene in carrots;[285] it should be noted, however, that a water-soluble carotene protein complex is present in carrots (*see* p. 54). Absorption is reduced in fevers,[92] jaundice,[52] and coeliac disease.[90] It is probably poor absorption in the last-named condition which accounts for the subnormal plasma levels observed (*see* Table 42) for their increase following treatment parallels the improved clinical condition.

From a clinical point of view it is important to note that administration of mineral oils reduces the absorption of carotenoids,[286-290] the pigments being easily soluble in the oils which are not absorbed. Mahle and Patton,[292] however, have recently made the important observation that hydrophilic mucilloids, which can replace mineral

oils as a purge, have no effect on carotene absorption. Similarly, it is important to note that alumina gel which may be administered over protracted periods in the treatment of peptic ulcer, has no effect on carotene absorption.[292] Absorption is improved by dispersing agents.[306]

(*b*) *Other Mammals*

When the absorption of carotenoids in animals other than humans is considered, the same general situation is apparent except that in animals which preferentially store carotenes these are probably preferentially absorbed ; in the case of rats some xanthophylls are, however, absorbed and then presumably suffer oxidative destruction.[234] Although, as in humans, fat facilitates absorption, it is not essential in rats and absorption has been achieved on a diet containing only 0·04 per cent. of fat.[294] Even under optimum conditions and using doses of only 1–2 μg. per day, rats excrete 10–15 per cent. of the dose.[295,296] Cama and Goodwin[297] have recently shown that in the rat, the activity of the thyroid gland conditions the efficiency of the absorption of carotene; hypothyroidism reduces the absorption and *vice versa*. This has recently been confirmed in cows, sheep[298] and goats.[168A] Fraps[299] has recorded somewhat indefinite results on the effect of bulk on the absorption of carotene in rats and is has been stated that small rats utilize β-carotene better than large ones.[300] These and other considerations raise considerable problems when the biological assay of vitamin A preparations using crystalline β-carotene as the International Standard is considered. A discussion of this is outside the scope of this chapter and readers are referred to a critical discussion of the problem by Morton.[301] It has recently been agreed; internationally, that crystalline vitamin A, and not β-carotene, shall be used as the International Standard for vitamin A assays although crystalline β-carotene is retained as the standard for assaying pro-vitamins A.[302]

Experiments on choledochocolostomized animals[303] and on isolated intestinal loops of normal animals[304] indicate that bile is essential for the absorption of carotene but not of vitamin A. This accounts for the poor carotene absorption in jaundice.[52] Dispersing agents increase the rate of absorption of carotene in cows.[305]

Even the presence of 0·08 per cent. of mineral oil in the diet of cows (just sufficient to prevent dustiness in salt mixtures and lucerne leaf meal) has a pronounced deleterious effect on carotene utilization.[291]

β-Carotene when homogenized in milk is well absorbed by cows when a nipple feeder is used for administration ; it is less well absorbed when given by stomach tube.[284A]

Destruction in Lumen

Little work has been carried out on the mode of action of destruction of carotene in the intestinal tract, but Hove[307] found that clear stomach extracts in the presence of methyl-linoleate oxidize carotene very much in the same way as does soya bean lipoxidase (Chapter III); extracts of small intestine, however, are much less active.

Much more work has been carried out on the practical aspects of protecting carotenoids in the intestinal tract. Moore's[308] original observation that α-tocopherol (vitamin E) when administered with vitamin A or carotene to rats results in a greater storage of vitamin A in the liver owing to improved stability of the vitamin A and carotene in the gut, has been repeatedly confirmed using varying experimental conditions.[309-315] Recently, however, using the plasma carotenoid levels as criterion, it has been stated that a diet low in vitamin E does not impair the utilization of carotene by cattle.[316]

Hickman and his colleagues[309-312] have also found that substances other than the tocopherols protect carotene and vitamin A against intestinal destruction. Such stabilizers, which are termed co-vitamins, are laurylhydroquinone, ascorbic acid, and palmityl ascorbic acid. One result of such stabilization is that on a diet rich in covitamins a marked increase occurs in the percentage of ingested carotene which is excreted in the faeces.[313] This indicates that the absorption may not be affected by covitamins. Although intestinal stabilization is probably the greatest single locus of action of these covitamins, the work of Davis and Moore[317] suggests that a similar action may also be exerted in the blood stream and tissues. It should be noted that recently Johnson and Baumann[318] found that large amounts of tocopherol administered to rats with rather high doses of carotene reduced the amount of vitamin A stored in the liver; when tocopherol was administered eight hours after the carotene no effect was noted; they could not find any change in carotene excretion during tocopherol administration.

It is difficult to decide whether the action of these covitamins is in inhibiting a lipoxidase-type of enzyme noted by Hove[307] or in protecting the pigment against chemical oxidation, for α-tocopherol inhibits both soya bean lipoxidase[319] and the atmospheric oxidation of carotene solutions.[320,321]

It has recently been stated that, in the presence of adequate amounts of tocopherols, lutein (xanthophyll) tends to interfere with the utilization of carotene in rats.[322] Confirmation of this has been reported,[323,324] but Sherman,[325] has found that the presence of lutein actually protects carotene from intestinal destruction. Johnson and Baumann[326] also failed to observe the interfering action of lutein.

A resolution of these differences may be arrived at by following up Vavich and Kemmerer's[327] various experiments on chicks[328] They found a differential effect ; the amount of vitamin A laid down in the liver of vitamin A-deficient chicks (about 14 days old) fed 65 μg./day of β-carotene was not affected by daily supplements of 300–600 μg, of xanthophylls. When the β-carotene supplement was raised to 130 μg./day, then the xanthophylls had a marked deleterious effect. This persisted even when the β-carotenes was administered in three doses at three hourly intervals. In a recent large-scale biological assay, Callison, Hallaan, Martin, and Orent-Keiles[329] found that in rats lutein had no demonstrable effect on the utilization of β-carotene for growth.

In the experiments in which lutein was found to impair carotene absorption, it was found that vitamin A absorption was equally affected. This observation rules out the possibility of xanthophylls inhibiting carotene utilization by competing with it for the hypothetical intestinal enzyme carotenase.[324]

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CHAPTER X

AVIAN CAROTENOIDS

OCCURRENCE

Birds resemble mammals in having the property of converting carotene into vitamin A which is stored in the liver and the eggs. They differ from mammals in preferentially storing xanthophylls in the liver, eggs, body fat, skin, feathers, shanks, etc. In this they resemble marine animals with which they also share the common ability of altering absorbed carotenoids and storing these altered products in some special structure such as feathers; especially interesting in this connection is the appearance in this phylum of astaxanthin.

Eggs

The first avian pigment to be investigated was that of the yolk of the domestic hen's egg. Willstätter and Escher[1] in 1912, considered that it was a homogeneous pigment which was an isomer of "leaf xanthophyll;" they named it *lutein*. This statement was left undisputed for eighteen years until Karrer and Helfenstein[2] showed that the yolk pigment, although having the same m.p. as leaf xanthophyll (lutein), differed from it in optical rotation. Kuhn, Winterstein, and Lederer[3] then showed that the yolk pigment of hens on common rations was a mixture consisting of 70 per cent. of lutein (xanthophyll) the remainder being principally zeaxanthin. The xanthophylls are stored in the egg mostly in the free form, only about 8 per cent. being esterified.[4] Carotene is stored only to a small extent, constituting on the average about 2–10 per cent. of the total carotenoids present.[5-7] It is not known whether the observations that hens' eggs contain less carotenoids than ducks' eggs, which in their turn contain less than gulls' eggs,[8-10] are of physiological importance. In general, it can be said that hens carry to their yolks at least part of any carotenoid fed to them. Apart from lutein (xanthophyll), zeaxanthin, and carotene, this has been proved in the cases of cryptoxanthin,[11-13] capsanthin,[13,14] lycopene,[15,16] neoxanthin, flavoxanthin, and isolutein;[18] violaxanthin is apparently an exception.[10] It should be noted here that a discrepancy exists between the claims of Karrer and Krause-Voith[19]

and those of Strain.[18] The former claim that epoxides are not transferred to the yolk, whilst, as just stated, Strain found that violaxanthin (a 5 : 6 - diepoxide) was not transferred but that flavoxanthin (a 5 : 8-monoepoxide) was. Although it has not been directly proved, hens can apparently accumulate astaxanthin, for those feeding on crab and lobster shells produce very dark-red yolks, which are not acceptable in the market.[20] With regard to the yolk pigments of other birds, lutein (xanthophyll) is the main pigment in the case of the canary (*Serinus canaria canaria*) but it is absent from the eggs of black-headed gull (*Larus ridibundus*) and the stork (*Ciconia ciconia*), its place being taken by astaxanthin.[16,17] *S. canaria* does not store in its eggs dietary lycopene, carotene, or violaxanthin.[16]

Skin, Fat, Liver, Eyes, etc.

Palmer's pioneer work in the early 1920's proved that, in hens, dietary xanthophylls but not carotenes occur in the blood plasma, fat, and skin, especially of the shanks and claws.[21] The main pigment is lutein (xanthophyll)[22] which, in these cases is esterified ;[16] recent work has confirmed this.[23,24] Flamingo fat contains a pigment very similar to astaxanthin ; it has been named *phoenicotterin* but from the data provided it is premature to consider it different from astaxanthin.[25] Xanthophylls are stored in the liver of hens and turkeys,[26] and in the skin, fat, face and bills of 15 species of wild birds[27] (*see* Table 47). Brockmann and Völker[16] found astaxanthin in the red wattles of pheasants but apart from the retina (*see* next section) Wald and Zussmann[20] could find no astaxanthin in any organ of the hen. Lönnberg[28] found xanthophylls in the eyes of 27 species of wild birds and Hollander and Owen[29] noted them in the irides of numerous species of domestic hen but not in pigeons ; in fact, the iridial carotenoids are so labile that alteration of diet can alter the eye colour of hens.[30]

The carotenoids of the hen's retina have been examined in some detail ; it was in 1877 that Capranica[31] described three types of oil droplets in hens' retinas ; these were characterized by different lipochromes, one greenish, one yellow, and one red, named chlorophane, xanthophane and rodophane respectively. In 1937-38 Wald and Zussmann[20] fully investigated these globules and found that the pigments present were carotenoids ; the greenish component is similar to the bacterial carotenoid *sarcinene* (*see* p. 119), the yellow componentis a mixture of *lutein* (xanthophyll) and *zeaxanthin* in the same ratio as they occur in the egg, and the red component is *astaxanthin*. It should be noted that von Studintz and his colleagues[32] consider that the

green component is identical with lacertofulvin (*see* p. 226). Recently Wald[33] has detected a new carotenoid, *galloxanthin*, in chicken retinas. This pigment, which has not yet been obtained crystalline, has absorption maxima, at wavelengths, which at the time of this investigation, were lower than those normally encountered in the carotenoid series (Table 45). Karrer's work on carotenoid epoxides has since been published (*see* p. 15), and it is possible that galloxanthin belongs to this group of pigments. It is strongly adsorbed on calcium carbonate and gives a band at 785–795 mμ. with $SbCl_3$; the position of galloxanthin in relation to the three types of oil droplets has not yet been defined.

Feathers

The contribution which carotenoids make to the pigmentation of the plumage of birds is considerable, and feather carotenoids can be considered analogous to the carotenoids stored in the external structures of sea creatures. In both cases the carotenoids are xanthophyllic, produced by the animals from the alimentary carotenoids, and as often as not are characteristic of the species.

Palmer[21] gives a full description of the earlier work on plumage lipochromes which was originated by Krukenberg. This has also been briefly summarized by Karrer and Jucker.[34] Lönnberg[35] has reported the presence of carotenoids in the feathers of a large number of species of birds but more searching chemical investigations have been undertaken by Brockmann and Völker,[16] Test,[36] Kritzler[37,38] and Völker.[39-42] Brockmann and Völker[16] discovered that canary feathers contained a pigment which was very similar to both violaxanthin and taraxanthin but distinct from either and they named it *canaryxanthophyll*. It was characterized by its absorption spectrum, by its failure to give a blue coloration with ethereal HCl, and by its greater adsorbability than lutein (xanthophyll). They found that the canary was the only bird whose feathers contained only canaryxanthophyll. The other widely distributed feather carotenoid is lutein (xanthophyll) and Brockmann and Völker found that they could divide birds into three groups according to whether their feathers contained (*a*) mainly lutein (xanthophyll) and a little canaryxanthophyll, (*b*) a little lutein (xanthophyll), or (*c*) considerable canaryxanthophyll. Two other less common pigments were also noted; *picofulvin*, in the green feathers of *Picus canus* and *P. viridis*, and a red pigment in *Pyromelana franciscana*.

The work of Test[36] on the feathers of the yellow woodpecker (*Colaptes auratus*) indicated the presence of three pigments, (i) a taraxanthin-like pigment, (ii) an unidentified red neutral carotenoid

occurring both as an ester and in the free state, and (iii), somewhat surprisingly, α-carotene. The red carotenoid was the minor constituent but in the closely related scarlet *C. cafer* it was the major feather constituent. The feathers of a suspected *auratus cafer* hybrid contained an intermediate amount of the red substance. The difference between the feather coloration of the two species, *C. auratus* and *C. cafer*, is thus merely quantitative. Kritzler[37,38] also found three carotenoids (R_1, R_2, and lutein (xanthophyll)) in the display plumage of the African bishop birds, *Euplectes franciscanus*, *E. orix*, and *E. nigroventis* and the head plumes of the woodpecker, *Melanerpes erythrocephalus*. The three pigments in order of decreasing adsorption on alumina were "R_1," lutein (xanthophyll), and "R_2." *M. erythrocephalus* contains more of R_1 than do the bishop birds. R_1 may be identical with canary-xanthophyll. Völker[39-41] reports the presence of unidentified carotenoids in the yellow and red feathers of a number of parrot species, but the yellow pigment of *Melopsittacus undulatus* is apparently not a carotenoid. More recently, Völker[42] has reported the presence of

TABLE 46.—*Characteristic Avian Carotenoids*

Name	Absorption Spectra Maxima
Canary xanthophyll[1]	472, 443, 418 mμ. (ethanol)
Picofulvin[1]	450, 424 mμ. (ethanol)
R_1 (from bishop birds)[2]	460, 485 mμ. (benzene) 450, 475, 505 mμ. (CS_2)
Red pigment from *Pyromelana franciscana*[1]	512, 432 mμ. (ethanol)
R_2 (from bishop bird)[2]	480 mμ. (benzene) 450, 495 mμ. (CS_2)
Galloxanthin[3]	421, 400, 378 mμ. (ethanol) 422, 401, 380 mμ. (hexane) 427, 407, 387 mμ. ($CHCl_3$) 446, 424 mμ. (CS_2)

None of these pigments has been isolated crystalline.

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astaxanthin in the red feathers of a S.W. African shrike, *Laniarius atrococcineus*.

There is only one report of preferential storage of carotenes in birds. Some years ago Rosenheim and Webster [4 3] claimed that the fulmar petrel (*Fulmarus glacialis*) stores in the proventriculus an amber coloured oil

TABLE 47.—*Qualitative Carotenoid Distribution in Birds*

Species	α-Carotene	β-Carotene	Lutein	Astaxanthin	Taraxanthin	Violaxanthin	Picofulvin	Sarcinene	Galloxanthin	Canaryxanthophyll	Taraxanthin	Reference No.
Acanthis flammea				+								1
Ampelis garrulus				?								1
Anas platyrhyncha domestica		+	+									1
Anser domesticus		+	+	+								2, 3
Aprosmictus metanurus				?								4
Astur gentilis		+	+									1
Cardulis spinus			+									5
Cardulis cardulis			+									5
Chloris chloris			+									5
Chloronerpes yucatensis					?	?	+					5
Ciconia ciconia				+								5
Colaptes auratus	+										?	7
Colaptes cafer	+										?	7
Dryobates major							+					5
Emberiza citrinella			+									1, 5
Emberiza icterica			+									5
Euplectes franciscanus			+									8, 9
Euplectes nigroventis			+									8, 9
Euplectes oryx			+									8, 9
Hypoxanthus rivolis						+						5
Gallus spp.			+	+				+	+			2
Lamarius atrococcineus				+								10
Larus ridibundus				+								5
Melanerpes erythrocephalus			+									8, 9
Motacilla cinerea			+									5
Oriolus oriolus			+									5
Oriolus xanthomus			+									1
Parus caerulens			+									1
Parus major			+									1
Phasianus colchicus				+								1, 5, 6
Phylloscopus sibilatrix			+									5
Picus canus							+					5
Picus viridis							+					5
Pyrrhula pyrrhula			+									1, 5
Serinus canaria										+		5

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containing only carotenes. As Fox[44] rightly points out, this accumulation must represent a secretion of carotenes manufactured either *de novo* or from alimentary carotenoids. Further, it is difficult to see how the oil fails to trap some alimentary xanthophylls. A reinvestigation of this oil in the light of modern developments in carotenoid chemistry is urgently required. Desselberger[45] has described a histological study of the processes involved in the deposition of carotenoids in feathers.

The qualitative distribution of carotenoids in birds is given in Table 47.

Metabolism

Very little advance in our understanding of carotenoid metabolism in birds (apart from that of the vitamin A-active carotenoids), especially hens, has taken place since the general picture was provided by the classical researches of Palmer and Kempster,[46] although a considerable amount of detail has been filled in.

Xanthophylls only are laid down in the skin and shanks as esters and these are mobilized in the free state into the eggs during the laying season. This transfer occurs even if during the laying period the hens were maintained on a carotenoid-free diet, having had, until laying began, access to a carotene-rich diet.[47] Reports differ concerning what happens when laying hens are maintained on a xanthophyll-free diet; Bohren and his colleagues[47] found that the xanthophylls slowly disappeared from the yolks of the eggs, whilst Grimbleby and Black[47A] found a high pigment content in the first three eggs laid after transference to the xanthophyll-free diet, followed thereafter by a rapid decline owing to exhaustion of the body stores of xanthophylls. On the other hand the laying down of xanthophylls in eggs is a rapid process[48] for hens maintained on a carotenoid-free diet will transfer xanthophylls to the yolk within 48 hours of being transferred to a carotenoid-rich diet.[13] About five eggs must be laid before maximum colour is obtained.[49]

Titus, Fritz, and Kauffman[49] found no difference in pigmentation of yolks of cross breeds and white Leghorn pullets on the same diet.

The change in relative concentrations of carotenes and xanthophylls in the egg and liver compared with the food is very striking. In normal green food this ratio is about 1 : 3 and in eggs and liver, although it depends on the diet, it can reach 1 : 30.[50] This is mainly due to the fact that carotene (and cryptoxanthin) are efficiently converted into vitamin A[24,50,51] presumably in the gut wall,[52,53] and not because they are poorly absorbed from the lumen. It should be noted, however, that the material in which the carotenoids are fed, affects the efficiency

of absorption,[54,55] for it has recently been shown that the carotene in lucerne is better utilized than carotene dissolved in arachis oil.[54]

The biological activities of the vitamin A precursors are very much the same in hens as in rats.[53,55-60] A claim that cryptoxanthin is more active in chicks than in rats[61,62] has not been substantiated[53,63] and the suggestion, made at the same time, that because of this cryptoxanthin acts as a vitamin in its own right, must be rejected. Patel, Mehl and Deuel[60] have shown that it is converted into vitamin A (in the intestinal wall) and used as such in the chick. There are no reports of examinations of hens' faeces but Seybold and Egle[64] found that the carotenoid distribution in goose droppings was very little different from that in the food (nettle leaves).

Usually between 15–25 per cent. of the total ingested carotenoids[51,65] are deposited in the body tissues, although it is claimed that up to 40 per cent. are deposited when grass is the source ;[66] only 2·5–7·0 per cent. of the alimentary carotenes are stored unchanged, presumably owing to the major portion being converted into vitamin A.

As is the case with mammals, carotene may be better absorbed by adult hens from an oily medium[55] but chicks appear to utilize carotene in grass better than in oil.[54] It is, therefore, important to note that carotene *per se* is of paramount importance in chick rearing and this is emphasized by recent work which indicated that in young chickens, at least, preformed vitamin A in the form of cod liver oil is not utilized.[7] On the other hand, bob-white quails utilized vitamin A better than carotene, fed either as lucerne meal or as β-carotene in cotton-seed oil.[67] High doses of vitamin A reduce the carotenoid levels of the plasma and liver of chickens[24] and turkeys.[68] A similar phenomenon has been noted with shank pigmentation[69,70] although, in this case, it is not certain whether the " pigment-depressing " factor is vitamin A or not. This fall is in both cases probably due to the fact that in the presence of large amounts of unsaturated lipids (as occur in cod liver oil) the body stores of xanthophylls as well as of vitamin E are used up, for Goldhaber, Zacharias and Kinsey[71] found that supplements of crude xanthophyll extracts fed to chicks on a vitamin E deficient diet prevented the appearance of signs of vitamin E deficiency in about 50 per cent. of the birds. The explanation given is that the xanthophylls exert an antioxidant effect. Food protein levels are important in carotene assimilation for Mann[7] found that on a low protein diet (13 per cent.) chicks begin to utilize carotene 22 days after hatching, whilst on a high protein diet (17 per cent.) utilization is delayed until 35 or 42 days after hatching.

During the development of the embryo the carotene[72] and the

xanthophylls[7] of the yolk do not appear to be used up in any way At hatching the liver of the chicks contains about 8 per cent. of the total body carotenoids.[7] The liver and plasma carotenoid levels are high at hatching but rapidly decrease during the first week of life.[68] Wald and Zussman[20] investigated the eyes of developing embryos; pink droplets (containing astaxanthin) appeared on the 19th day of incubation and the yellow droplets (sarcinene) somewhat later. This shows that these pigments must either be totally synthesized in the retina, or formed by oxidation of the yolk carotenoids in a unknown site and are then transported to the retina.

The second alternative is much more probable, for such a process has been demonstrated in the production of plumage carotenoids. Brockmann and Völker[16] produced white birds when canaries were reared on a carotenoid-free diet. Only xanthophylls were precursors of the canaryxanthophyll, for pigmentation of the feathers only occurred when lutein (xanthophyll) or zeaxanthin, but not carotene or lycopene, were included in the diet. Violaxanthin did not produce pigmentation but its failure was ascribed to its instability in the gastric juice of canaries, although it may not have been absorbed (*see* p. 259).

In bishop birds, Kritzler[37,38] found that lycopene was a precursor of pigment R_2; captive birds produced only small amounts of R_2 unless fed tomatoes. Capsanthin was carried to the feathers unchanged.

FUNCTION

It has been noted that carotene functions in birds by acting as a vitamin A precursor, and it is converted into vitamin A with considerable efficiency. The biological functions of the xanthophylls which birds so assiduously store are, on the other hand, still obscure.

Palmer and Kempster[46] considered that in hens at least xanthophylls are of no physiological importance and that mobilization from the shanks to the eggs is merely due to the fact that eggs are a convenient excretory route for fat-soluble substances. They also managed to rear perfectly normal chickens with normal fecundity and fertility on xanthophyll-free diets. Recent work by Schumacher, Scott, Hughes and Peterson[73] and by Bohren, Carrick and Andrews[74] confirms this.

Other workers, whilst not denying all biological function to xanthophylls are, with one exception, convinced that they have no vitamin A activity in the chick.[75-80] Euler and Klussman,[75A] however, believed, in 1931, that lutein (xanthophyll) was indeed a vitamin A precursor in the chicken. It has been suggested that lutein (xanthophyll) is converted into an essential growth factor differing from vitamin A but

somewhat similar in action.[78,81-83] Ferrand and Bohren[84] have recently stated that inadequate intake of lutein reduces the "sperm competetive ability" of some species of domestic fowl, although by all chemical tests the spermatozoa from cocks fed on the lutein-free diet were identical with those from cocks on the lutein-rich diet. Hens were artificially inseminated with a mixture of spermatozoa from the two groups of cocks. In the case of the New Hampshire and the White Plymouth breed, a greater percentage of chicks which hatched out were sired by the normal cocks; in other words, the "sperm competetive ability" was reduced on the lutein deficient diet. No such reduction was observed with Barred Plymouth Rocks.

In birds with nuptial display feathers, carotenoids undoubtedly play a positive rôle which is possibly a true sexual function. The nuptial display plumage of male bishop birds is rich in carotenoids, whilst the post nuptial "henny" feathers are almost devoid of carotenoids, which are at that time stored in the body fat and to a certain extent in the liver. The carotenoids can be readily mobilized into the post nuptial plumage of males when they are injected with pregnant mare's serum after removal of the henny plumage by plucking. The functional nature of the stored carotenoids is emphasized by the fact that they remain present in considerable amounts even after birds have been on carotenoid-free diets for as long as three months.[37,38] In hens carotenoids are mobilized into the blood by heavy doses of oestrogens,[85] and this is probably how the pigments are transferred to the eggs when the hens come into lay.

It is worth noting an unconfirmed report which states that carotene and lutein stimulate the dehydrogenase activity of pigeon breast muscle.[86]

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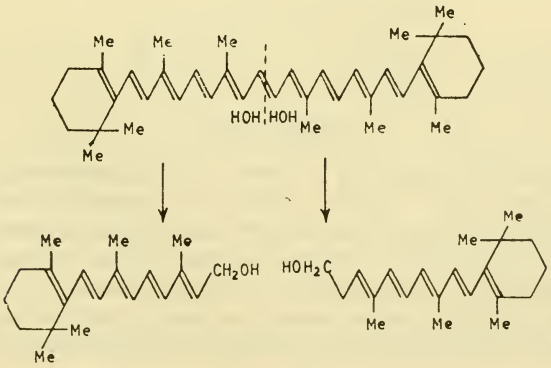
CHAPTER XI

CONVERSION OF CAROTENOIDS INTO VITAMIN A

The occurrence of vitamin A is, as far as is known at present, confined to mammals, birds, fish and crustacea. In the case of mammals, birds, and crustacea, if vitamin A is not eaten preformed it is produced by the *in vivo* conversion of " biologically active " carotenoids into vitamin A. In fish, although conversion of carotenoids into vitamin A can take place, there is a growing belief that fish vitamin A is provided preformed in crustacea. Almost certainly fish do not synthesize vitamin A *de novo*.

At this point it may be useful to condemn the phrases " biologically inactive " and " biologically active " as usually applied to carotenoids ; these terms are generally used in the sense that the pigment under discussion is either capable or incapable of being converted into vitamin A. This implies too narrow a concept of carotenoid function ; " biologically inactive " carotenoids are " biologically active ", for example, in such a function as chromatophore response in fish (*see* p. 196). When discussing the conversion of carotenoids into vitamin A the terms " vitamin A precursors " or, less good, " provitamins A," should be used.

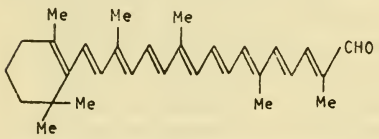
After a considerable amount of pioneer work by various investigators which has been ably summarized by numerous authors including



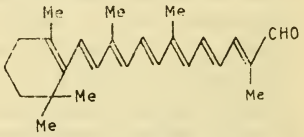
Sherman and Smith,[1] it was Moore[2] who unequivocally demonstrated the conversion of β-carotene into vitamin A in rats which was stored in the liver. It was soon realized that not all carotenoids were vitamin A precursors and when, as a result of the classic work of Karrer and Kuhn and their collaborators, the structures of many carotenoids and of vitamin A were established. the relation between structure and vitamin A activity became clear.

β-Carotene is twice as active as is either γ- or α-carotene,[3-5] and can be considered, for the moment at least, to be converted into vitamin A by hydrolytic fission, as shown on the previous page.

α-and γ-carotenes being about one half as active as β-carotene as vitamin precursors[6-9] and lycopene being inactive, it is obvious that a β-ionone residue is a first essential for activity. The inactivity of zeaxanthin and of lutein (xanthophyll) indicates that the β-ionone residue has to be unsubstituted. Unilateral oxidative degradation of the β-carotene molecule with the production of apocarotenes[10,11] does not destroy activity as long as the vitamin A side chain remains intact. For example, β-apo-8′-carotenal and β-apo-12′-carotenal are still as active as α-carotene.

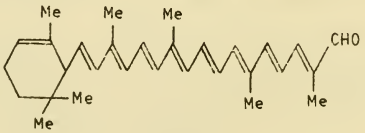


β-apo-8′-carotenal



β-apo-12′-carotenal

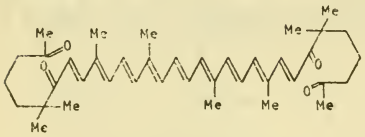
The apo-α-carotenals are inactive because only an α-ionone residue remains, *e.g.*, apo-8-carotenal[12]:—



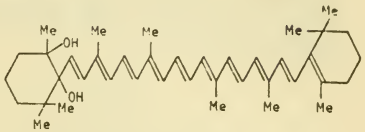
α-apo-8-carotenal

Oxidation which opens the β-ionone ring without reducing the number of carbon atoms destroys activity, for example, β-carotenone[13] is inactive but β-hydroxycarotene[14] and β-semicarotenone[15] have activities of the same order as α-carotene because they still contain one unsubstituted β-ionone residue. Dehydrogenation of β-carotene to isocarotene[16] (dehydro-β-carotene) removes its activity because there also occurs a rearrangement of double bonds with the production of

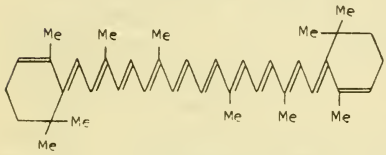
two α-ionone residues.[16] Alteration of the side chain such as occurs in β-dihydro-carotene[17] destroys vitamin A activity.



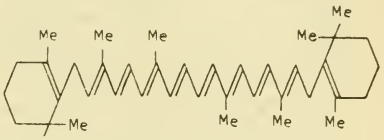
β-carotenone



β-hydroxy-carotene

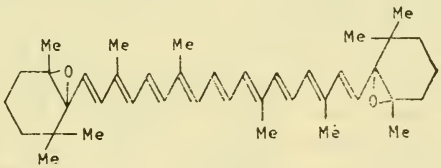


Isocarotene



β-dihydro-carotene

Recent work by Karrer and his associates on the production of carotenoid epoxides, has led to the necessity of modifying somewhat the statement than an unsubstituted β-ionone residue is necessary for activity because 5 : 6-diepoxides, *e.g.*, β-carotene-5 : 6–5′ : 6′-diepoxide are vitamin A precursors ;[18] the body can presumably convert the epoxide into β-carotene.

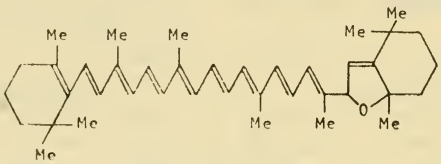


β-carotene-5 : 6–5′ : 6′ diepoxide

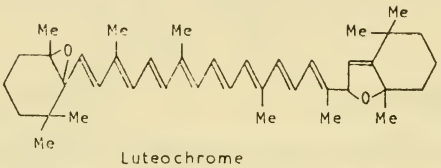
However, when 5 : 6-epoxides are isomerized to 5 : 8-epoxides such compounds, *e.g.*, aurochrome, are inactive.[19] The presence of a furanoid grouping does not interfere with activity of a molecule which contains in addition an unoxidized β-ionone or a 5 : 6-epoxy group, for example, mutatochrome[19,20] and luteochrome[17] are active : see next page.

The work on the apo-carotenes revealed another interesting fact that, from the point of view of vitamin A activity, it matters little whether the terminal group is aldehydic, carboxy, or carbonyl. This ability of the body to deal with varying terminal groups has been further demonstrated recently with derivatives of vitamin A itself :

retinene (vitamin A aldehyde),[21] vitamin A ethers,[22-24] and vitamin A acid,[25-27] are all active. The position of the hydrocarbon corresponding to vitamin A (so called axerophthene) is not settled ; Karrer and Benz[28] claim it is active whilst Meunier and his associates state that it is inactive.[29,30]

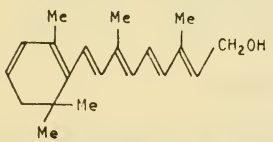


Mutatochrome



Luteochrome

Vitamin A_2 occupies a very interesting position from the point of view of structural criteria for vitamin A activity. It is active *per se* in the rat for it replaces vitamin A completely without in any degree being converted into vitamin A. Its relative activity is, however, only 40 per cent. that of vitamin A.[31] The structure of vitamin A_2 is now known, for Jones and his associates[32] have shown by synthesis that it is 3-dehydrovitamin A, thus confirming the suggestion of Morton, Salah and Stubbs[33] and refuting those of Karrer, Geiger and Bretscher[34] and Shantz.[35] This work on vitamin A_2 shows that vitamin A activity is still possible with a dehydro-β-ionone residue. The origin of vitamin A_2 in fish has been considered on p. 174.

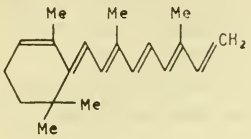


Vitamin A_2

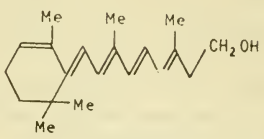
Anhydrovitamin A,[36] obtained by treating vitamin A with alcoholic HCl, has a double-bond system similar to that of iso-carotene (*see* p. 271) which is inactive. Shantz,[37] is, however, convinced that anhydrovitamin A has slight but definite activity. Most of it is stored in the liver as a compound containing an hydroxyl group. This material, named rehydrovitamin A,[37] is found to be much more active than anhydrovitamin A.

The effect on biological activity of altering the side chain of vitamin A, is not of major interest here because the compounds examined have been

synthetic and do not occur naturally. Goodwin[38] has recently reviewed this work.



Anhydrovitamin A



Rehydrovitamin A

Another factor which controls the vitamin A activity of carotenoids is spatial isomerism. It will be recalled (*see* p. 9) that carotenoids generally occur in nature in their all-*trans* forms. Deuel and Zechmeister and their collaborators[39-50] have assayed many *cis*-isomers of α- and β-carotenes for vitamin A activity (*see* Zechmeister[51] for a review); all these isomers except one (*see* below) are less active than are the corresponding parent all-*trans* compounds; confirmatory results have recently been obtained in India.[52] To account for this loss of activity these investigators suggest that a *trans* —→ *cis* rotation will result in a carotenoid losing its straight shape; this will then have difficulty in fitting on to the enzyme system(s) necessary to convert it into vitamin A. The importance of this straight chain is illustrated in the case of neo-β-carotene U; in this compound only one peripheral double bond has undergone *trans* —→ *cis* rotation but its activity is somewhat less even than that of α-carotene, which can be considered to be derived from β-carotene by migration of one terminal double bond out of conjugation.

An apparent deviation from this rule is pro-γ-carotene. This *cis* isomer which occurs naturally (*see* p. 30) is now considered as active a vitamin A precursor as all-*trans*-γ-carotene, both being more active than neo-γ-carotene P.[46] Originally it was thought that pro-γ-carotene was the most active of the series.[50] In this case Deuel, Zechmeister *et al.* consider that by undergoing the maximum number of *trans* ⟶ *cis* rotations a carotenoid molecule can recover a straight chain shape. Pro-γ-carotene is a poly *cis*-carotenoid and in all probability is *3 : 5 : 7 : 9 : 11*-penta-*cis*-γ-carotene (the numbers refer to the double bonds) thus:

3 : 5 : 7 : 9 : 11-penta-cis-γ-carotene

There remains the possibility that only the all-*trans*-forms have vitamin A activity and that other stereoisomers are active only in so far as they are rearranged, probably in the digestive tract, to all *trans*-β-carotene. Kemmerer and Fraps[53] have produced evidence that, in the case of neo-β-carotene U, this rearrangement does in fact take place, and recently it has been shown that in chickens pro-γ-carotene and lycopene undergo considerable stereoisomerization in their passage down the intestinal tract.

For example, 71 per cent. of the recovered γ-carotene had been isomerized into a number of pigments including the all-*trans* form.[50] *Cis*-isomers of vitamin A also vary in their biological activity (*see* Goodwin[38]).

The Conversion of Carotene into Vitamin A

The mechanism of the conversion of carotene into vitamin A is obscure. Biological assays generally reveal that the relative molar potencies of β-carotene, α-carotene, and vitamin A are 2 : 1 : 2. If the 2 : 1 activity of β-carotene and α-carotene is explained by the fact that conversion occurs by fission at the central double bond, then one molecule of β-carotene should give rise to 2 molecules of vitamin A and on a molar basis, β-carotene should be twice as active as vitamin A. This has only been reported twice (*vide infra*)[54,55] compared with numerous reports of lesser activity.

One is, however, loath to discard the theory of symmetrical fission in favour of fission at points other than the central until all the factors, considered below, have been fully investigated. The failure of β-carotene normally to be as effective as a symmetrical fission would suggest, is probably due to three main reasons :

(*a*) the poor efficiency of absorption of carotene from the gut compared with vitamin A (*see* p. 250). Even at very low doses (1–2 μg./day) up to 20 per cent. can be lost in the faeces.[56]

(*b*) the stability of β-carotene (and other carotenoids) in the intestinal lumen may be much less than that of vitamin A. Destruction is probably due to non-specific oxidation which can be reduced in the presence of anti-oxidants such as the tocopherols (*see* p. 252). In fact, Koehn[54] has recently conducted experiments using " optimum " amounts of α-tocopherol, in which he increased the relative activity of β-carotene to a value approaching that required by symmetrical fission and this has recently been confirmed by Burns, Hauge and Quackenbush,[55]

who found that with 1·0 μg. of tocopherol/day, no biological difference was apparent between 1·0 μg. of vitamin A and 1·0 μg. of β-carotene. Johnson and Baumann,[57] however, could not detect any visible affect of added tocopherol when the amount of vitamin A stored in the liver after a dose of carotene was measured; it should be noted that compared with Koehn, Johnson and Baumann used much higher doses of carotene.

(*c*) Factors such as thyroxin (*see* p. 279) may have a much greater effect on carotene than on vitamin A metabolism.

According to Johnson and Baumann[58] more vitamin A is formed from a given amount of carotene in hyperthyroid than in normal rats, and Cama and Goodwin[59,60] have shown that this is due primarily to the action of the thyroid on the absorption of carotene. Thiouracil reduces and desiccated thyroid increases carotene absorption (*see* p. 281). This has been recently confirmed in cows and goats.[60A]

Implicit in the assumption of a symmetrical fission is the fact that the activity of any β-carotene derivative in which one β-ionone residue is intact should be the same as that of α-carotene. This is not always the case, *e.g.*, semi-β-carotenone, although allowance has never been made for variations in absorption. Apart from differences which may result from the biological methods of assay in different laboratories and which are often spurious, real differences in activity may exist. These are probably due to one or a number of the factors just discussed rather than to some inherent property of the molecules. The problem is, however, by no means solved and recent important work by Johnson and Baumann[58,61] has opened up new possibilities; they found that cryptoxanthin is as active as β-carotene when assayed by the vitamin A liver storage test and twice as active when assayed by the growth method. When α-carotene is compared with β-carotene the situation is reversed.

It is very likely that the breakdown of β-carotene into vitamin A takes place in two stages; the first stage involves an oxidative scission with the production of vitamin A aldehyde (retinene) and the second a rapid conversion of retinene into vitamin A.[62]

Site of Conversion of Carotene into Vitamin A

Moore[2] in 1929 first unequivocally demonstrated that in mammals carotene was converted into vitamin A which was then stored in the liver. Since then it has, until recently, been tacitly assumed that the conversion takes place in the liver. *In vitro* experiments undertaken

to justify the assumption that the liver was the site always produced equivocal results. Ahmad,[63] Olcott and McCann,[64] Parienti and Ralli[65] and Euler and Klussmann[66] using various techniques claimed to have produced traces of vitamin A by incubating a colloidal suspension of β-carotene with minced liver. Woolf and Moore[67] critically discussed these results and pointed out the uncertainty in detecting vitamin A in the small amounts in which it was claimed to have been produced. Rea and Drummond,[68] and Drummond and MacWalter[69] were unable to demonstrate the conversion, and later experiments of Ahmad[70] were also negative.

Vitamin A, but not carotene, stimulates the growth of fibroblasts ;[71] Willstaedt[72] claimed that carotene in the presence of liver tissue was active in improving growth rate of the fibroblasts and concluded that the liver tissue had converted carotene into vitamin A.

In vivo experiments in which carotene was administered parenterally have been almost equally inconclusive. Wolff, Overhoff and van Eekelen[73] and Ahmad, Grewal amd Malik,[74] noted an increase in the liver vitamin A levels of rabbits after the intravenous injection of carotene colloidally suspended in isotonic dextrose ; Ahmad *et al.* could not, however, repeat the observations using rats and dogs. Similar experiments by Rea and Drummond[68] were also negative. Drummond, Gilding and MacWalter[75] showed that carotene introduced intravenously is stored in the liver primarily in the Kupfer cells. In further experiments in Drummond's laboratory, a colloidal solution of carotene was injected directly into the portal vein and the disappearance of the stored carotene from the liver was followed by partial hepatectomy ; the disappearance of carotene was not accompanied by a concomitant rise in vitamin A levels in the liver.[76] Similar experiments recently carried out by Vinet, Plessier and Raoul[77] did not produce results sufficiently significant to warrant the authors' conclusion that vitamin A was produced from carotene in the liver.

The results of intramuscular injections of carotene can usually be given a negative interpretation ; in mammals any vitamin A effects noted being very considerably less than the effects produced by a similar dose given *per os.*[78,79] This is also true for chickens.[80] Similarly, subcutaneous administration of carotene is ineffective ; Greaves and Schmidt[81] failed to elicit a " 100 per cent. biological response," and Rokhlina, Balakhovski, and Bodrova[82] found that the vitamin A activity of subcutaneously injected carotene was nil. irrespective of whether the vehicle was oil or water. Using the technique of fluorescence microscopy, Popper[83] never detected vitamin A in livers of depleted rats after parenteral administration of carotene. It should

be noted in passing that one claim exists that subcutaneously injected carotene is effective.[84]

A thorough re-examination of the problem by Sexton, Mehl and Deuel,[85] has left no doubt as to the ineffectiveness of parenterally administered carotene, irrespective of whether the injection is intraperitoneal, intravenous, intrasplenic, or intracardiac. In fact after intrasplenic injection of carotene into vitamin A-deficient rats the deficiency symptoms persisted although substantial amounts of carotene reached the liver and were stored there. Sexton *et al.*[85] pointed out that their results strongly suggested the intestine as the site of conversion. This was the first firm suggestion that the intestine was involved, although it was mooted by Verzar and McDougall in 1936[86] and Wagner and Vermeulen had considered this possibility in whales.[87] However, a preliminary *in vitro* experiment in which Sexton *et al.*[85] dosed vitamin A-depleted rats with carotene and then removed the intestines and incubated them for 6–24 hours, provided no evidence of vitamin A formation.

Meanwhile, investigations were being carried out at Liverpool which all pointed to the intestine as the site of conversion. This was implicit in the results of the work of Goodwin, Dewar and Gregory,[88] who could not demonstrate the presence of carotene in either the portal or systemic blood of living sheep and goats even after very high doses of carotene and after by-passing the rumen by feeding the carotene directly into the duodenum via a duodenal cannula. Further, Ball, Glover, Goodwin and Morton,[89] and Glover, Goodwin and Morton,[62] demonstrated the conversion of vitamin A aldehyde (retinene) into vitamin A in the intestinal wall; this was very significant for, in all probability, retinene is an intermediate in the conversion of β-carotene into vitamin A.

It was not surprising then that *in vivo* reports of the conversion of carotene into vitamin A in the intestinal wall of rats soon appeared first from Liverpool[90,91] then almost immediately afterwards from California.[92-94] Meanwhile, Thompson, Ganguly and Kon[95,96] had, using a slightly different technique reached the same conclusion for pigs. Goodwin and Gregory's[97] experiments with goats were successfully concluded when they demonstrated a rise in the concentration of vitamin A in thoracic lymph after feeding β-carotene. The failure previously to find an increase in the vitamin A blood plasma level after feeding β-carotene was due to the dynamics of the situation; the vitamin A produced in the intestinal wall accumulated in the thoracic lymph in amounts which were easily detectable; when this lymph was delivered into the systemic blood stream it was

so quickly diluted that the resulting small increase in the blood level could not be detected (*see* Fig. 33).

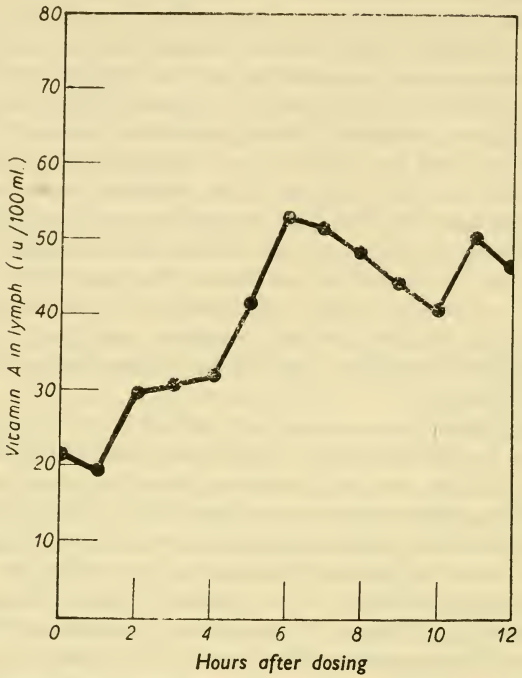


Fig. 33.—Showing the rise in the vitamin A in the thoracic lymph of goats after feeding carotene. (*From Goodwin, T. W., and Gregory, R. A. (1948)* **43,** 505.)

Krause and Pierce[98] have demonstrated the intestinal conversion of carotene into vitamin A in rats in which the liver was tied off at the portal vein. In retrospect the explanation of Popper's[83] observation that vitamin A fluorescence sometimes appeared in the intestine before the liver after the oral administration of carotene is now obvious.

Since these first investigations, many confirmatory reports have been published. Using the same technique as that employed by Goodwin and Gregory,[97] Kon and his associates [99,100] and Alexander and Goodwin[101] have confirmed the intestinal conversion in the rat. Other animals in which this conversion have been demonstrated are chicks,[99,100,102] sheep[99,100] and dairy cattle[103,104]

Patel, Mehl and Deuel[105] have also demonstrated the conversion of cryptoxanthin into vitamin A in the intestinal wall of the chick,

thus disproving With's[106] suggestion that cryptoxanthin exerts its vitamin A activity *per se* in the chick (*see* p. 265). Patel *et al.*[105] also showed that chickens with sterile intestinal lumena (sterilized by treatment with sulphasuccidine) were able to carry out the conversion, thus finally ruling out the intestinal flora as the possible agent responsible for the conversion.

Thus we see that in normal animals the conversion of β-carotene into vitamin A has been clearly established to take place in the intestinal wall. Although it has not been proved conclusively that the liver cannot effect the conversion, it is highly probable that it cannot.[85] Recently, however, it has been claimed that cow liver can accomplish the conversion,[103] but this has been denied.[104] Injection of carotene into the portal vein of dogs is stated to result in the accumulation of vitamin A in the liver.[107] Bieri[108] has found that intramuscular injections of carotene into eviscerated rats, produces in the blood plasma a material with an absorption spectrum similar to that of vitamin A.

Factors Controlling the Conversion of Carotene into Vitamin A

Apart from the role of the tocopherols in preventing the oxidation of carotenoids in the intestinal lumen (*see* p. 252), not a great deal is known of other factors which may control the conversion of carotene into vitamin A. Considerable attention has been focused on the thyroid gland in this connection. Kunde[109] in 1926 noted the appearance of vitamin A deficiency in thyroidectomised rabbits which were fed carotene, and somewhat later in 1932–3 Fellenberg and Greuter,[110] and Fasold and Heidemann[111] claimed that carotene appeared in the milk of thyroidectomised goats. Abelin[112] at the same time noted that in guinea pigs the administration of thyroxin adversely affected the metabolism of carotene and vitamin A equally. The clinical aspects of the subject have been reviewed by Drill.[113]

After Abelin's work there was a considerable gap before the subject was again investigated. Drill and Truant,[114] using the remission of xerophthalmia in rabbits as the criterion of vitamin A production, failed to demonstrate its formation from carotene in thyroidectomised animals. A considerable objection to this work is that the carotene was injected and, as stated on p. 277, there are serious doubts whether injected carotene is utilized to any great extent. Canadell and Valdescas[115] appear to confirm Drill and Truant's work, but Remington, Harris and Smith[116] state that eye symptoms are cured by the oral administration of carotene to thyroidectomised animals. Di Bella[117-118] found carotene effective, but with reduced efficiency. Carotene is also

converted into vitamin A in thyroidectomized goats,[119] but with what degree of efficiency is not known. Barrick, Andrews, Beeson and Harper[120] consider that very high doses of thiouracil inhibits the conversion in feeder lambs and this appears also to be true with sheep.[121]

Johnson and Baumann,[57] using the liver storage of vitamin A as criterion, found that the same dose of carotene produces less liver vitamin A in thiouracil-treated animals than in controls, and that rather surprisingly, controls stored less than did rats dosed with desiccated thyroid. Administration of thyroxin and thiouracil together produced normal liver storage ; this indicated that the action exerted by the thiouracil, causing reduced vitamin A storage, was anti-thyroid and that the effect was not due to another (unknown) pharmacological action. Kelley and Day,[122] using the same criterion have confirmed Johnson and Baumann's observations. Wiese, Mehl and Deuel[123], in an important contribution, emphasized that in assessing the effect of thiouracil on carotene by means of biological assays involving measurements of weight increases, allowance must be made for the growth-inhibiting action of thiouracil itself. That this growth inhibition was due to thiouracil *per se* and not to its action on carotene metabolism was demonstrated by the fact that normal growth in thiouracil-treated animals could be elicited by the addition of desiccated thyroid but not by massive doses of vitamin A. Wiese *et al.* overcame this difficulty by evaluating the amount of carotene required to produce one-half the maximum growth attained in the control and thiouracil-treated groups. In this way they found that β-carotene was equally as effective in treated animals as in controls. These results probably explain the " reduced efficiency " of carotene in thyroidectomized animals noted by Di Bella.

Goodwin,[124] and Cama and Goodwin[59,60] acting on the assumption that the thyroid does have an action on carotene metabolism, pointed out that none of the work just discussed indicates a possible mode of action of the hormone. They considered that three possibilities existed :

(*a*) the enzyme " carotenase " is inhibited ;

(*b*) thiouracil reduces the intestinal stability of carotene ;

(*c*) intestinal absorption of carotene is reduced.

If the first possibility were correct the carotene should traverse the gut wall and appear in the general circulation ; a similar situation possibly occurred in the unconfirmed report that thyroidectomized goats' milk is yellow,[111] although it appears that this is incorrect for

neither in England[125] nor in America[119] has thyroidectomy resulted in the appearance of carotene in the blood and milk of goats. Goodwin[124] and Cama and Goodwin[59,60] could never demonstrate the presence of carotene in the systemic blood of rabbits fed a carotene-rich diet together with large doses of thiouracil. These results appear to rule out the first possibility and there is good reason also to reject the second possibility, for Cama and Goodwin[126] have shown that thiouracil has no effect on carotene stability *in vitro*. A group of Italian workers, however, found that thyroxin retarded the destruction of colloidal solutions of β-carotene when the yellow colour of the solution was taken as criterion but accelerated it when the colour with $SbCl_3$ was measured.[127] Cama and Goodwin did not find this to be so.[126] That thiouracil exerts its action by reducing the absorption of carotene from the gut wall (possibility C) has been made highly probable by the results of a recent investigation by Cama and Goodwin.[59] Under controlled dietary conditions rats treated with thiouracil excrete a greater percentage of a given dose of β-carotene than do control rats; desiccated thyroid on the other hand increased absorption. (*See* Table 48). This has recently been confirmed in cows and goats by Owen and his co-workers,[128] who also found that the ratio vitamin A : carotene in the milk was increased by thyroxine and decreased by thiouracil. One can now offer an explanation of the apparently opposite results of Johnson and Baumann[57] and Wiese *et al.*[123] In the latter experiments very small doses were used and the

TABLE 48

Illustrating the Effect of Thiouracil and Desiccated Thyroid on the excretion of β-carotene by Rats

DIET	Amount of carotene (μg./day) excreted by		
	Controls	Thiouracil-fed Animals	Thyroid-fed Animals
Ether extracted food cubes	4·89 3·34	9·32 3·81	3·04 3·41
Extracted food cubes + 30 μg. of β-carotene/day ..	10·14	15·90	8·72
Carotene free diet + 50 μg. of β-carotene/day	7·07	9·53	—

From Cama, H. R., and Goodwin, T. W. (1949), *Biochem. J.*, **45**, 236.

result of small variations in absorption would not be detected by the biological assay. In Johnson and Baumann's experiments, on the other hand, larger doses were used and a 10–15 per cent. loss would easily be noted in the variations in the amount of vitamin A stored in the liver.

A further interesting point in connection with the relationship between the thyroid gland and carotene metabolism is the claim that thyroglobulin[129,130] and iodinated casein act as enzymes for the *in vitro* conversion of carotene into vitamin A. Careful investigations of this claim have proved it to be in all probability incorrect.[126,131,131A] No evidence of an increased rate of conversion has been obtained *in vivo* using hyperthyroidic calves,[132] although recently such evidence has been submitted in the case of hyperthyroidic cows and goats.[60A]

A different aspect of the carotene-thyroid relationship was investigated by Smith and Perman;[133] they found that carotene inhibited the action of thyroid extracts in increasing the oxygen consumption of cats. It will be recalled that Rokhlina[134] claimed that carotene antagonizes the thyrogenic stimulation of axolotl metamorphosis.

Samaras and Hingerty[135] consider that blockage of the reticulo-endothelial system in normal rats increases the efficiency of conversion of carotene into vitamin A. Recently, Vavich and Kemmerer[136] have reported an investigation which indicates that small rats utilize β-carotene better than do large rats. An unconfirmed report exists claiming that the conversion of β-carotene into vitamin A is stimulated by insulin.[137] Pregnant rats utilize carotene more efficiently than non-pregnant ones.[138]

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CHAPTER XII

CONCLUSION

It is not easy to attempt a general assessment of significance of the considerable amount of knowledge concerning the carotenoids which is now available and which has been presented in the foregoing pages. With regard to the occurrence, distribution and identification of caroteniods, the situation is very satisfactory but with regard to formation and function, knowledge is rudimentary.

Most of the pigments described have been unequivocally identified although, inevitably, there are doubts concerning some. Perhaps the most common defect met in the literature (and not only in carotenoid studies) is the branding with fresh names of pigments, the uniqueness of which is open to considerable doubt; in other words, the possibility that a pigment has previously been described has not been eliminated completely. More than one name for the same thing can not only cause considerable confusion but also sometimes lead to the perpetuation of errors—for usage rather than appropriateness often determines survival in scientific literature.

It is now well established that carotenoids are manufactured *de novo* only in the plant world. Animals take in these pigments as part of their food and then deal with them in one or more of a variety of ways, viz.: the pigments may be unselectively absorbed; they may be selectively absorbed; they may be altered in some way, generally by oxidation, before storage in special organs; or they may, if their structure permits, be converted into vitamin A.

The tendency to dispose of carotenoids in a particular way depends to a considerable extent on the position of the animal in the evolutionary tree. Mammals, birds, some amphibia and probably fish, possess the specialized ability to convert certain carotenoids into vitamin A. Frogs and probably primates, including man, are unusual in that they tend broadly to absorb carotenoids unselectively. Cows and horses, are typical of a group of species which in the main selectively absorb carotenes without altering them. Birds and fish, on the other hand, tend to store unchanged xanthophylls rather than carotenes. In both these groups, however there is a tendency to oxidize a small

fraction of the ingested lutein (xanthophyll) and to store the altered products in the feathers and the skin respectively. However, it is only when we come to invertebrates, in particular the marine invertebrates, that the ability to produce highly oxygenated carotenoids is very marked, but even in this class there are some exceptions.

It is perhaps not for the biochemist to speculate on the evolutionary significance of the variations which are played on the carotenoid theme; his primary aim should be to enquire into the mechanisms whereby carotenoids are altered and into the functions in the animal economy of the resulting products.

Investigations of the carotenoids on such specifically biochemical lines are woefully few and, except in the case of mammals, virtually nothing is known of the ways in which carotenoids are modified or of the factors controlling the processes. It is natural that the energies of biochemists interested in carotenoids should recently have been canalized to elucidate the conversion of carotene into vitamin A in mammals. Advance in general biochemical knowledge of the carotenoids has undoubtedly been hampered by this narrowing of the scope of the investigations and it is hoped that now the fundamental features of the carotene —→ vitamin A conversion are reasonably well understood, investigators will look further afield. However, studies on carotenoids in mammals have sometimes had some bearing on the wider problems of comparative carotenoid biochemistry. We know why mammalian species differ in the degree to which they store carotene in their adipose tissues; the deciding factor is the efficiency of the animals' intestinal "carotenase" in converting carotene into vitamin A. The fatty tissues of a goat, for example, are free from carotene because of the extreme efficiency of its intestinal enzyme system which does not allow any pigment to spill over into the blood; humans and cows, on the other hand, being inefficient converters, allow this overspill. There is as yet no evidence that such variations between species can be fitted into a functional pattern.

To turn away from mammals, not a single recorded fact throws light on the mechanism of, for example, astaxanthin formation in some insects and in lobsters, or of pectenoxanthin production in the scallop; many other similar cases could be cited. Although there are no hard facts there are plausible ideas as to how such processes could occur. However, when the biochemical problems are posed in questions such as: "What is the special need of the lobster which calls for astaxanthin?" or "What is the function of pectenoxanthin in the scallop?" it then emerges that we have neither facts nor ideas—so far these problems baffle us.

In birds, some work has been carried out in identifying precursors of one or two characteristically avian carotenoids, but of this problem too, only the surface has been scratched.

The state of knowledge is a little different in plants, for they have the ability to produce carotenoids *de novo*. In the case of phanerogams, environments which produce healthy plants will also result in high yields of carotenoids. Apart possibly from some work on tomato fruit, there is no evidence which compels assent to any hypothesis concerning the rôle of micronutrients or of light in carotenoid production. The evidence concerning possible carotenoid precursors in phanerogams is flimsy ; it is equally so in the cryptograms, even when one considers the fungi in which the absence of chlorophyll must simplify the problem. Work on these organisms is now under way in a number of laboratories.

With respect to the functions of carotenoids, it will be noted that the ability to absorb visible light is the common factor in all well-established effects. In plants, carotenoids possibly function in photosynthesis and probably do in photokinesis.

In fish they are constituents of the xanthophores and thus play a vital part in phototropism ; in many invertebrates the photoreceptor substance in the eyes is almost certainly astaxanthin. Apart from these two fields, knowledge of the participation of carotenoids in the biological processes of the animal kingdom is slight. The mediation of carotenoids in these two functions may be more widespread in the animal world than is usually appreciated, yet there are numerous species, especially marine, which are well provided with carotenoids, but in whose life light plays a very minor rôle. We have no inkling of any function which can be ascribed to the carotenoids in these animals.

The argument that the presence of a carotenoid necessarily points to a function is repugnant to many ; but when an animal absorbs ingested carotenoids, alters them in a very specific manner, and stores them preferentially rather than excretes them unchanged, it is surely not irrational to proceed on the assumption that, until a function is apparent, knowledge is incomplete. The author hopes that there will emerge a comprehensive theory of carotenoid function embracing the whole animal and plant kingdoms. The absence of carotenoids from some species (especially mammals) should not be taken to indicate that such a general hypothesis is unattainable. The absence of carotenoids from a number of species would be linked up with some metabolic idiosyncrasy of the species and the apparently fortuitous distribution of carotenoids would no longer appear so, but would fall into the general pattern.

At the moment such a generalized conception of the place of carotenoids in the living world may seem very remote, but the wealth of data, which has been presented in this book, must at some point be holding out a master clue if only we had the wit to recognize and grasp it.

It has been thought that such a comprehensive theory may emerge from a closer investigation of the relationship between carotenoids and the reproductive processes, and the author recently fully reviewed the evidence for this.[1]

Eggs of a large number of animal species contain carotenoids, very often xanthophylls. When xanthophylls are present in eggs they are always unesterified and this, by analogy with vitamin A is a hint that they are present in a functional form. Furthermore, egg carotenoids are often present attached to proteins ; this recalls that in some sea-urchins the pigment echinochrome when attached to protein may be active in attracting spermatozoa. Body carotenoids can undergo changes during the sexual cycle, for they are often mobilized into and metabolized by the gonads ; β-carotene is actively metabolized during the development of fertilized locust eggs.

Sexual differentiation of carotenoid distribution is not confined to the animal kingdom for we find fungi in which there is a marked quantitative and often qualitative differentiation of carotenoids between the male and female gametes ; further, in the algae, participation in sexual processes has been noted with the crocetins which, if by the definition adopted in this book, are not " true " carotenoids, are almost certainly derived from such compounds.

Speculation can be pushed too far, but it may not have been inappropriate to conclude this monograph on a somewhat speculative note by considering the territory open to conquest and possible lines of attack. With the fundamental chemical data on the identification and distribution of the carotenoids so well founded and so considerable, the time appears propitious for investigations aiming at the integration of carotenoid biochemistry in a sound and comprehensive theory of carotenoid metabolism. This demands an attack on a broad front with the comparative approach as the spearhead. If such an attack is carried out using all the weapons available to modern biochemistry then it is possible that the next ten years will see advances sufficiently great to allow wide generalizations concerning the biological role of the carotenoids.

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APPENDIX I

Carotene Content of Plants

Note : Only values obtained using well-authenticated chemical methods of analysis are tabulated. Values for processed foodstuffs are not included.

(*A*). *GREEN TISSUES.*

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Agathi	*Sesbania gangeticus*	14·3–	1		
Alkali drop-seed	*Sporobolus aeroides*	2·7		4·1–	
		7·5[b]	86	18·9[b]	86
Amaranth	*Amaranthus gangeticus*	11·0	1		
		2·5–11·1	79		
Andropogon	*Andropogon annulatus*	4·4–11·8	2		
Asparagus	*Asparagus acutifolius*	0·58	3	3·29–7·47	3
(Green)		3·58	80	7·25	4
		1·12	82	7·47	82
Aster	*Aster indicus*	6·30	91		
Australian beard-grass	*Andropogon intermedius*			14·0	5
Bahia grass	*Paspalum notatum*			27·1	5
Bajri (Indian Millet)	*Pennisetum typhoideum*	4·5–5·6	2		
Banana (Plantain)	*Musa paradisiaca*	0·7	1		
Barley (grass)	*Hordium vulgare* (*sativum*)	9·6	83	41·5	6
				37·6	83
,, (whole plant)		3·5	7	17·1	7
Beet (tops)	*Beta vulgaris*	6	8	32·9–44·2	10
		5–10	9	30–60	9
		10·5	11	57·2	12
		5	80		
Bengal gram	*Cicer arietinum*	8·1	1		
		18·4	76		
Bermuda grass	*Cynodon dactylon*			18·7–41·3	13
Berseem (Egyptian clover)	*Trifolium alexandrinum*	8·4	2	28·6	5
				18·8–36·2	10
				15–40	14
Big blue stem	*Andropogon provincinalis*	0·3—7·7	83	0·3[a]	5
		0·54–10·7[b]	86	10·3	5
				0·4–22·1	83
Biri	*Biri* spp.	13·0	2		
Black finger	*Chloris cucullata*			11·9–16·5	13
Bird's foot trefoil	*Lotus corniculatus*			52·6	75
Black grama	*Bouteloua eripoda*			0·54–12·57[b]	15
Blue grama	*Bouteloua gracilis*	0·48–		0·51	
		1_3·9[b]	86	–26·9[b]	86
Blue grass	*Poa arachnifera*	12–18	16	1·14[a]	5
Blue joint	*Calamagrostis canadensis*	0·44		0·54	
		–5·3[b]	86	–16·1[b]	86
Bottle gourd	*Lagenaria vulgaris*	9·7	76	52·9	5
Bracken	*Pteridium aquilinum*	0·8	91		
Bristle grass	*Setaria macrostachys*			0·25[a]	5
				15·8	5
Broad red clover	*Trifolium pratense*			22·8–59·4[b]	19
				16·0–41·1	20
				49·2	75
				5–92	34
Broccoli		0·6–1·1[b]	17	21·3	3
		4·65	80	45·3–51·7	18
		1·58	82	10·67	82

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Brome grass	*Bromus* spp.			28·9	13
				72·6	20
				36·2–57·5	84
				3·69	82
Brussel sprouts	*Brassica oleracea*	0·4	21		
		0·12–0·36	22		
		0·58	82		
Buffalo grass	*Buchloe dactyloides*	0·7–12·6	83	9·9–25·4	5, 13
		0·55–7·8[b]	86	1·55[a]	
				0·9–28·2	83
Buttercup	*Ranuculus* spp.			45·13	5
Cabbage					
(outer leaves)		3·9	79	14·6	10
(heads)		0·032	79	0·5	10
Cactus (spineless; Bokhara clover)	*Melilotus alba*			41·3	13
Canadian Brome		4·2–10·4	83	7·7–53·3	83
Carpet grass	*Axonopus affinis*			9·4–25·7[b]	23
Carrot (tops)	*Daucus carota*	17·4	24		
		11·2	21		
		14·8	76		
Cauliflower (leaves)	*Brassica oleracea*			40	10
(head)		0·038	79		
Celery leaves	*Apium graveoleus rapaceum*	5·76	79		
		0·21	85		
Cenchrus	*Cenchrus cilieris*	0·05–7·35	25	0·06–41·3[b]	25
Chinese cabbage	*Brassica chiensis*	7·8	85		
Chloris	*Chloris babata*	4	2		
Chrysanthemum	*Chrysanthemum conorarium*	3·8	91		
Cocksfoot (orchard grass)	*Dactylis glomerata*	3·8–6·8	83	13·7–59·6[b]	19
				38·5[b]	73
				14·2–37·7	75
				7·5–15·4	83
				34·4–57·5	84
Collards	*Brassica oleracea*	3·28–4·64[b]	17	29–46	17
	var. *acephala*	2·86–7·92[b]	11	51·1	12
		1·74	80		
Combs' Paspalum	*Paspalum album*			19·8	5
Coriander	*Coriandium sativum*	30	1		
		11·6	76		
		12·6	79		
Cow pea (plants)	*Vigna catjang*	11·4–16·1	2		
Crab grass	*Digitaria sanguinalis*			1·68[a]	5
				16·9–23·1	5
Cress		4·5	21		
		0·9	22		
Cryptotaenia	*Cryptotaenia canadensis*	5·6	91		
Curly Mesquite	*Hilaria belangeri*			11·2–22·2	13
Curry leaf	*Murraya koenigi*	24	1		
		12·63	79		
		5·3	85		
Cynodon	*Cynodon plactostachyum*			0·59[a]	5
				7·2	
Dakota brome		3·8–8·6	83	6·6–33·4	83
Dallis grass (Italian blue grass)	*Paspalum dilatum*	26	2	22·7–49·5	13
				18·7	5
				11·3–46·4[b]	23
Dandelion	*Taraxacum officinale*	8·7	26	69	82
		9·66	82		
Dhub grass	*Cynodon datylon*	11·1	1		
		24·6	6		
Digitaria	*Digitaria eriantha* v. *stolonifera*	0·12–10·9[b]	25	0·13–39·2[b]	25
Dock	*Rumex crispus*			85·6	12
	R. obtusifolius			58·3	75
Dolichos (Broad bean)	*Dolichos lablab*	13	2		
Drumstick grass	*Moringa oleifera* (*aptera*)	32	1		
Early white clover	*Trifolium repens*			27·3–67·0[b]	19

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Earthnut	*Conopodium denudatum*			48·8	75
Eastern grama	*Tripsacum dactyloides*			3·19[a]	5
				18·9–22·5	5
Egyptian clover (*see* Berseem)					
Elephant grass	*Pennisetum purpureum*	2·8–10·0	2	20·4	6
Eragrostis	*Eragrostis plana*	0·05–8·06[a]	25	0·05–14·7	25
Erichloa	*Erichloa sericea*			19	5
Fahli clover				33·7	10
Flax	*Linum usitatissimum*	0·65	27		
Garagway grass		12·6	2		
Garlic	*Allium sativum*	4·2	76		
		0	79		
Goosegrass	*Eleusine indica*			10·3	5
Goosefoot	*Chenopodium album*	12·4	76		
Gordura grass	*Melinis minutiflora*	8·8	2		
Grapevine mesquite	*Panicum obtusum*			13·6	13
Green clover				10·8–33·4	28
Groundnut (stem and leaves)	*Arachis hypogaea*	1	2		
Guajillo	*Acacia berlandieri*			10·5	13
Guinea grass (Hairy " buffels-gras ")	*Panicum maximum*	4·9	2		
		0·07–7·0	25	0·11–30·85	25
Hairy grama	*Bouteloua hirsuta*	0·2–14·9[b]	86	12·2	13
Hamburg parsley		12·5	21		
Horse grama	*Dolichos biflorus*	13·0	2		
Indian grass	*Sorghastrum nutans*	1·0–13·2[b]		4·1[a]	5
				13·2	5
				37·3	13
Indigofera	*Indigofera annaphylla*	5·2	2		
Ipomea	*Ipomoea hispida*	8·8	2		
	I. sepiaria	10·8	76		
Italian blue grass (*see* Dallis grass)					
Italian rye grass	*Lolium italicum* (*multiflorum*)			11·9–45·6[b]	19
				37·2	13
Japanese peppers	*Xanthoxylum piperitum*	4·4–13·7	91		
Jews mallow	*Kerria japonica*	7·9	91		
Johnson grass	*Sorghum halepense*	3·2	2	36·6	13
Jowar	*Andropogon sorghum*	4	2	21·4	6
June grass	*Koeleria cristata*	3·7–8·3[b]	86	6·1–18·3	86
Jute	*Corchorus capsularis*	10·8	76		
Kachnar ka patta	*Bauhinia variegata*	8·4	76		
Kalai	*Phaseolus radiatus*	4·6	2		
Kale	*Brassica oleracea*			87	29
Karki	*Celtis caucasica*	4·6	2		
Kentucky blue grass	*Poa pratensis*	3·6–7·2	83	4·1–21·4	83
				19·5–43	84
Knotgrass	*Paspalum distichum*			22	13
Kohlrabi (leaves)				36–38·8	30
Kollukatti grass	*Pennisetum cenchroides*			14·9	6
Lang's paspalum	*Paspalum langei*			0·85[a]	5
				23·5	14
Lathyrus	*Lathyrus sativus*	15·5	76		
Leek	*Allium porrum*	0·65	85		
Lettuce	*Lacuta sativa*	0·77–2·23[b]	11	74	12
		6.8	76		
		3·7	85		
		2·1–2·4	79	21–36	10
		0·61	80		
Lindheimer's Panicum	*Panicum lindheimeri*			11·0	5
Little blue stem	*Andropogon scoparius*	2·7–6·7	83	4·3–15·6	83
Lofa	*Malva verticillata*	7·5	76		
Love grass	*Eragrostis curvulla*	0·44–5·7[b]	86	12·9	5
Love lies bleeding	*Amaranthus caudiatus*	14·8	76		

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Lucerne	*Medicago sativa*	6·7–10	30	15·3	5
				21·7–28·3	84
		8·5	4	11–35·5	10
		29·6	1	80 (max.)	31
		3·2–8·8	83	30–53	30
		16·8	2	30·1–43·2	18
				8·3–29·2	83
		9	72	70	7
Maize (leaves)	*Zea mais*	12·2–18	32	56·3	20
		3·7	2		
,, (whole plant		0·9	7	3·4	7
Mallow	*Malva* spp.			49·7	10
Mangold leaves				111·3	10
Mautitius	*Panicum muticum*	10	2		
Meadow fescue	*Fescuta elatior*			28·6–54·7	84
Mesa dropseed	*Sporobolus flexulosus*			0–13·86[b]	15
Mesquite leaves	*Prosopis chilensis*			4·4	13
Methi	*Trigonella*	5·8	2		
(Fenugreek)	*foenum-graecum*	9·1	76		
Mint	*Mentha viridis*	8·7	76		
Mugwort	*Artemisia vulgaris*	6·7	91		
Mustard		1·93	80		
Napier (*see* Elephant grass)					
Needlegrass	*Stipa spartea*	1·2–11·1[b]	86	1·6–23·6[b]	86
Neem	*Azadirachta indica* (*Melia azadirachta*)	14·3	1		
Nettle	*Urtica dioica*	4·50	79		
		14	21	38·4	19
				34·8	10
Northern reed grass	*Calamagrostis inexpansa*	0·57–4·8[b]	.. 86	0·79–12·3	86
Oak	*Quercus* spp.			5·5–11·4	13
Oats	*Avena sativa*			22·6–64·7	10
				38·7	20
				33·3–49·8	33
				52·5	18
Onion (leaf)	*Allium cepa*	2·0	76	17·5	10
Onion	*A. fistulosum*	0·3	91		
Onion	*A. odoratum*	2·4	91		
Orchard grass (*see* Cocksfoot)					
Pan (Betel)	*Piper betel*	13·0	1		
		7·27	79		
		13·7	85		
Panicum	*Panicum fasiculatum*			49·5	13
Panicum	*Panicum milare*	10·4	2		
Parsley	*Petroselum sativum*	10–11	16	38·8	10
		5·6	85		
		9·4	21		
		3·2	79		
Parwar	*Trichosanthes dioica*	13·4	76		
Paspalum	*Paspalum stramineum*			40·7	13
Pea (leaves)	*Pisum arvense*	8·0	2		
		15·5–24·4	76		
(stem)		6·8	76		
Penicillaria	*Penicillaria* Spp			8·5–11·8	10
Peppers	*Capsicum annuum*	10·2	91		
Perennial rye grass	*Lolium perenne*			14·4–43·0[b]	19
Perilla	*Perilla ocimoides*	6·3			
		12·4	91		
Petastines	*Petastines japonica*	6·6	91		
Plantain	*Plantago lanceolata*			40·6	19
Pooin	*Basella viridis*	4·8	76		
Portulaca				19·1	10
Potato (leaves)	*Solanum tuberosum*	12·2	76		
Prarie dropseed	*Sporobolus heterolepis*	0·26		0·33	
		–8·2[b]	86	–19·3	86
Prickly pear	*Opuntia* spp.			0·6	13

Common Name	Botanical Name	Carotene content (mg./100 g)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Pumpkin (leaves)	*Cucurbita maxima*	10·1	76		
Purple top grass	*Sieglingia (Triodia) flava*			10·8	5
Quack grass		12	26		
Radish	*Raphanus sativus*	7·4–9·8	76, 91	69·8	12
Ragi	*Eleusine coracana*	9·6	2		
Ragwort	*Senecio jacobea*			39·9	19
Ranmatki	*Phaseolus trilobus*	8·8	2		
Rape	*Brassica napus*	8·8	76	79·2	12
Reana	*Reana luxurians*	5·4	2		
Red Pigweed	*Amaranthus rubrum*	11·5	76		
Red top	*Agrostis alba*	3·4–5·7	83	5·4–24·1	83
Rescue	*Bromus catharticus*			40·6	13
Rhodes grass	*Chloris gayana*	3·0	2	28·3	13
			25	16–21	5
					25
Rhubarb (leaves)	*Rheum officinale*	6	8		
,, (stalks)		0·1	21		
Rye		9·5–10·7	33	25·8–37·8	3
		5·5–8·4	83	25·1–30·6	83
				20·6–40·6	35
Sainfoin	*Onobrychis cristagalli*			3·5[a]	5
St. Augustine	*Stenotaphrum secundum*				
Salt grass	*Distichlis stricta*	0·75–17·0[b]	86	1·1–17·2	86
Sand dropseed	*Sporobolus cryptandrus*	0·48–11·5[b]	86	0·53–30·4[b]	86
Sand reedgrass	*Calamorilfa longifolia*	0·93–14·1[b]	86	1·1–43·3[b]	86
Sandhill bluestem	*Andropogon halii*	1·0–13·4[b]	86	1·3–51·2[b]	86
Seresia	*Seresia lespedeza*			28·1	20
Sesbania	*Sesbania grandiflora*	20·2	2		
Setaria	*Setaria italica*	12·0	2		
,,	*Setaria lindbergiana*	0·4–9·41[b]	25	0·55–33·7[b]	25
Sheeps fescue	*Fescuta ovina*			44·7	75
Shevari		11·2	2		
Side oats grama	*Bouteloua curtipendula*	0·75–13·0[b]	86	0·80–28·3[b]	86
Slough grass	*Spartinia pectinata*	1·36–9·6[b]	86	1·7–27·1[b]	86
Sorghum	*Sorghum versicolor*	7·6	2		
	S. purpureo-sericeum	9·0	2		
Sotol (leaves)	*Dasylirion texanum*			4·2	13
Soya bean (leaves)	*Glycine soja*	15–29	36	43·2	32
		11·4	2		
Spear grass	*Heteropogon contortus*	3·4	2		
Sowa	*Peucedanum sowa*	15·0	76		
Spear grass	*Stipa leucotrida*			27·4	13
Speedwell	*Veronica chamaedrys*			35·6	19
Spinach	*Spinacia oleracea*	3·2	11	12·25–13·0	16
		5·0	37	65	12
		6·1	76	12·4–44	10
		2·63	79	44–56[c]	3
		3·12	70	41·4–57·4	38
		7·58	82	94·6	82
		4·8	85		
Spring onion	*Allium* spp.	5·6	85		
Stick grass	*Panicum antidotale*			1·86[a]	5
				21·8	5
Sudan grass	*Holcus (Sorghum) sudanensis*	5·8	2	43	32
		2·8	7	7·2–18	10
				13·5	7
Sugar beet (leaves)		6	8		
Swede (leaf)	*Brassica campestris*	2·6	91		
Sweet clover				24·7	32
Sweet potato (leaves)	*Ipomoea batatas*			60.8	10
Swiss chard	*Beta vulgaris.* v. *rapa*	5·9	8	46–62[c]	8, 12
		3·4	80		

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Switch grass	*Panicum virgatum*	0·2–9·0[b]	86	0·21–33·3[b]	86
Tall dropseed	*Sporobolus asper*	0·85–13·1[b]	86	1·1–33·8[b]	86
Tall oatgrass	*Avena elatios*			10·6–67·0[b]	19
Tea, black	*Thea sinensis*	2·54	79	47·3	39
				71·5	40
,, green				76·1	39
				150·7	40
,, Oolong				78·4	40
,, Touchang				168·1	40
Teosinte	*Euchlaena mexicana*	3·8	2		
Thistle	*Carduus* (*Cirsium*) spp.	1·33	27	32·7	19
Thorny pigweed	*Amaranthus spinosus*	26·8	76		
Timothy	*Phleum pratense*			11·0–58·2[b]	19
				11·2–27·5[b]	73
Tufted hair grass	*Aira caespitosa*			44·4	75
Tobacco leaves		13·6	1	59·88	11
Tulip	*Tulipa gesneriana*	4·3	91		
Turnip	*Brassica rapa*	2·9–5·1	16	88·7	12
		5·10	9		
		3·26	80	30–60	9
Vetch	*Vicia sativa*			0·02	35
				13–43	10
Vite	*Vitis quadrangularis*	0·27	41		
Water hyacinth	*Eichhornia crassipes*	5·8	2		
Watercress	*Nasturtium officinale*	3·2	85		
Western needle grass	*Stipa comata*	0·74–9·1[b]	86	0·94–13·9[b]	86
Western wheat grass	*Agropyron smithii*	4·3–10·2[b]	86	5·1–32·5[b]	86
Wild rye	*Elymus canadensis*	1·5–6·2[b]	86	4·7–21·0[b]	86
Wheat		6·6	83	17·9	83
Wild white clover	*Trifolium repens*			27·4–66·9[b]	19
				14·3–55·2	73
				36·1	75
Yarrow	*Achillea millefolium*			37·9	19
Yorkshire fog	*Holcus lanatus*			13·9–60·4[b]	19

(*B*). *FRUITS, SEEDS AND TUBERS*

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Actinida	*Actinidia chiensis*	0·035	41	0·190	41
Apple	*Pyrus malus*	0·05	41	0·311	41
		nil	79		
		0·08	80		
Apricots		3·5	41	24	11
		10·0	80		
		1·05	11		
Asparagus	*Asparagus officinalis*	0·6	91		
Avocado		0·710	80		
Bamboo	*Dendrocalamus flagellifer*	0	85		
Bananas	*Musa sapientum*	0·05	41		
		0·1	76		
		0·8	80		
		1·0	85		
Barley	*Hordium vulgare* (*sativum*)			0·17–0·24	35
				0·11	43
Beet	*Beta vulgaris*	0	85		

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Bengal gram (husks)	*Cicer arietinum*	0·316	79		
		1·93–3·1	88	0·310	41
Black Bengal gram	*Phaseolus mungus*	0·064	79		
Blackberry	*Eugenia jambolana*	0·03–0·24	42		
				0·11	43
Blimburg	*Averrhoa bilimbi*	0·32	85		
Blueberry		0·06	87		
Canary grass seed	*Phalaris canariensis*	0·28	27		
		0·12–96	78		
Canistel	*Lucuma nervosa*	0·95–7·4	42		
Carambola	*Averrhoa carambola*	0·12	85		
Cantaloupe (yellow)		4·56	80		
„ (green)		0·39	80		
Cape gooseberry	*Physalis peruviana*	3·4	76		
Carrot [d]	*Daucus carota*	13·6	32	111	32
		2·0	79	30–130	44
		8·5	44	82–130	45
		6·6	11	73–86	46
		16·2	1	17·5–160·5	77
		3·3–22·6	77	98–104	89
		2·7–73·8[c]	21		
		7·7	76		
		0·8	85		
		0·02–11·7	47		
		6·5–14·4	48		
		0·7–17	49		
		10·8–12	22		
		14	72		
		4·8	50		
		4·13	80		
Cherries		0·59	42		
		0·30	41		
Chinese persimmon	*Diopyros kaki*	0·21	85		
Cluster bean	*Cyamopsis psoralioides*	1·2	76		
Coconut	*Cocos nucifera*	nil	42, 79, 85		
Colocasia	*Colocasia antiquorum*	0·04	79		
Coriander (seeds)	*Coriandrum sativius*	0·157	79		
		0·045–(0·055)[e]	24		
Cow peas	*Vigna catjang*	0·3–0·7	76	17·0–20·5	10
		0·025–0·040[e]	52		
		0·6	76		
Cranberry		0·24	11		
Cucumber skin	*Cucumis sativus*	0·10	91		
flesh		nil	91		
Custard apple	*Anona squamosa*	nil	76		
Dolichos (Broad beans)	*Dolichos lablab*	0·32	85		
Egg plant, skin	*Dolichos melanogena*	0·10	91		
flesh		nil	91		
Elephant apple	*Feronia elephantum*	trace	76		
Fenugreek (seeds)	*Trigonella foenicumgraecum*	0·24	11		
Figs	*Ficus hispida*	0·3	76		
		0·052	80		
Four-angled bean	*Psophocarpus tetragonolobus*	0·53	85		
French beans	*Phaseolus vulgaris*	0·19–0·42	54	4–4·9[c]	3
		1·1	85		
		0·22	79	2·36	53
		5·31	80		
		2·26	80		
Ginger	*Zingiber officinale*	0·1	85	1·9–3·8	54
Gourd (bitter)	*Momordica charantia*	0·21	79		
		0·16	85		
		5·0	1		
Grape, skin	*Vitis vinifera*	0·1–0·4	91		
flesh		0·01	91		

Common Name	Botanical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Grape fruit	*Citrus decumana*	nil	42		
Green gram	*Phaseolus radiatus*	0·158	79		
Ground nut seeds	*Arachis hypogoea*	0·063	79		
Guavas	*Psidium guajava*	0·16–0·28	13		
Hemp seeds	*Cannabis sativa*	0·98	27		
Hog plum	*Spondis mangifera*	0·4	76		
		0·16	85		
Horse gram	*Dolichos biflorus*	0·158	79	55·3–73	10
Indian plum	*Zizyphus jujuba*	0·5	76		
Jackfruit	*Artocarpus polyphemia*	0·55	85		
Kang-kang	*Ipomea aquatica*	8·4	85		
Kwini	*Mangifera odorata*	2·45	85		
Ladies finger	*Hibiscus esculentus*	0·06	79		
		0·16	85		
		0·3	76		
Latarus	*Dioscorea alata*	0·4	76		
Lemon		0·120	41	1·0	41
Lentils	*Lens esculenta*	0·45	79		
Lima beans		4·3–6·6	55	45·4	20
Lime	*Citrus medica*	0·07–0·23	85		
Loquat	*Eriobotrya japonica*	0·4	76		-
Mace		0·172 (1·98)[l]	81		
Maize (yellow)	*Zea mais*	0·3	4	0·72	4
		0·32	85		
		0·11	44	0·20–0·94[c]	57
		0·9–1·1	59	0·039–0·73	35
,, (white)		0·042	79		
		0·039	80		
Mamey					
(S. Domingo)	*Mammea americana*	1·17–5·2	42		
(red)	*Achras zapota*	0·14–0·24	42		
		0·16	85		
Mandarin	*Citrus aurantium deliciosa* (*C. nobilis*)	0·40	81	3·201	41
		0·25	91		
Mangoes[f]	*Mangifera indica*	1·2–14[c]	58		
		2·6–6·0	76		
		3·4–11·0[c]	60		
(green)		trace	76		
		0·15	79		
Mangosheen	*Garcinia mangostana*	0	85		
Marking nut (orange cups)	*Semecarpus anacardium*	0·9	76		
Millet	*Andropogon* (*Holcus*) *sorghum*			0·165–0·22	35
Mulberry	*Morus indica*	0·2	76		
Musk, skin	*Curcubita moschata*	0·1–2·6	91		
flesh		0·2	91		
Musk melon (Gourd)	*Cucumis melo*	0·76	42	82·7	39
		0·5	76		
Mustard seeds	*Brassica juncea*	0·27	79		
Oats	*Avena sativa*	1·0	27	0·09	43
				0·065–1·41	35
Okra	*Hibiscus esculentus*	0·24	42		
		0·35	80		
Olives		0·23	80		
Onion	*Allium*	0·025	79		
Orange	*Citrus* spp.	0·150	81	1·15	41
		0·14–0·33	85		
,, (skin)		2·57	79		
Palm flesh	*Elaeis guineensis*	33	91		
Palmyra (mesocarp)	*Borassus flabellifera*	7·6	76		
Papayas	*Carica papaya*	0·128 (1·158)[k]	61		
		1·07	85		
(skin)		2·4–4·7	76		
(pulp)		0·4–2·8	76	1·84	79
Parsnip		0·03	79		
Passion fruit	*Passiflora laurifolia*	0	85		

Common Name	Botonical Name	Carotene content (mg./100 g.)			
		Fresh wt.	Ref.	Dry wt.	Ref.
Peach	*Prunus persica*	1·0	41	3·5	41
		0·35	80		
Pear	*Pyrus communis*	0·01	41	0·06	41
		0·014	79		
Peas (English)[g]	*Pisum sativum*	0·5	4	3·1–5·1	9
		0·47–0·71	63	2·0	82
		0·14	79		
		0·3	21		
		1·2	76		
Peas (country)	*Pisum arvense*	3·1	76		
		1·56	80		
Peppers	*Capsicum annum*	3·76–16·7[c]	62		
		0·26	85		
		2·18	80		
(green)		2·74	42		
		4·8	85		
Pineapple	*Ananas comosa*	0·26–0·74	79		
		0·42	85		
Poppy (seeds)	*Papaver somniferum*	0	27	0	27
Potato		0·056	79		
Pumpkin	*Curcurbita maxima*	0·084	79		
		0·16	85		
		1·27	80		
Radish	*Raphanus megalantha*	0·003	79	23–28	64
		0·0	85		
Ragi	*Eleusine coracana*	0·037–0·090	79		
Rambutan	*Nephelium lappaceum*	0	85		
Raspberries		0·35	41	0·54	41
Red cashew nuts	*Anacardium occidentale*	0·44–1·55	42		
Red gram (dhall)	*Cajanus indicus*	0·22	79		
		0·16–0·32	85		
Red sorrel (fruit)	*Hibiscus sabdariffa*	0·4	76		
Rice	*Oryza sativa*	nil	85		
Ridge gourd (loofah)	*Luffa acutangula*	0·4	76	11·9	64
		trace	85		
		0·06	79		
Rose apple	*Eugenia malaccensis*	0·11	85		
Rose hips	*Rosa spaldingi R. acicularis*	4·5	90	15	41
Sapodilla (*see* Red mameys)					
Snake gourd	*Trichosanthes anguina*	0·16	79		
		1·6	85		
Sotol bulbs	*Dasylerion texanum*			0·2	13
Soya beans	*Glycine soja*	0·018–0·705[b]	51		
		0·11	85		
		0·45–0·97	79		
Strawberries	*Fragaria* spp.	0·06	41	2·0	41
Sugar beet				0·05–0·15	31
Sweet potatoes	*Ipomoea batatas*	0·13–3·94[c]	65	42·3	12
		3·85–4·95	70	14	66
		0·05	85		
		0·08–0·15	91	13	28
				4–36·2[c]	73
Sword bean	*Canavalia ensiformis*	0·2	76		
Tamarind	*Tamarindus indicus*	0	85		
Tangerine	*Citrus nobilis*	0·75	47		
Tomatoes	*Lycopersicum esculentum*	1·4	44		
		0·58	42		
		0·9	1		
		4–5	67		
		0·1–19·1[e]	68, 69		
		0·51	80		
		1·0	85		
	L. pimpinellifolium	0·66–1·92	68, 69		
	L. peruvianum	0·07–0·36	68, 69		
	(*L. esculentum*, × *L. hirsutum*), × *L. esculentum*	0·07–6·75	68, 69		

Comman Name	Botanical Name	Carotene content (mg./100 g. Fresh wt.	Ref.	Dry wt.	Ref.
Vegetable marrow	*Luffa aegyptiaca*	0·2	76		
Vetch	*Vicia faba*	0·05	91		
Water chestnut	*Trapa bispinosa*	nil	76		
Water melon	*Citrullus vulgaris*	1·8	76		
		0·19	80		
		0·0	85		
Wheat		0·16–0·38	71	0·08	43
		0·108	79		
				0·24–0·84	35
White gourd	*Benincasa hispida*	0·5	91		
White turnips	*Brassica campestris*	nil	79	0·05–0·15	31
Wood apple	*Aegle marmelos*	0·1	76		
Yellow malanga	*Xanthosoma sagittifolium*	1·08	42		
Yam	*Dioscorea* spp.	0·434	79		
Yellow swedes	*Brassica campestris*			2·4–4·3	31
Yellow turnips	*Brassica campestris*			0·6–3·0	31

FOOTNOTES

a. Dormant forages.
b. According to season.
c. According to variety.
d. See Table 12 (p. 82) for further information.
e. mature plants.
f. See Table 11 (p. 81) for further data.
g. See Table 6 (p. 44) for further data.
k. Cryptoxanthin content.
l. Lycopene content.

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APPENDIX II

Recently, R. S. Harris and his colleagues have published an extremely comprehensive and important series of papers surveying the composition of foods grown in Central America, viz.:

Botanical name	Common name	Honduras		Guatelmala	
		Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)
A. EARTH VEGETABLES					
Allium ascalonicum	Shallot	79·8	0·006	—	—
Allium cepa	Onion	83·8–93·0	0·000	82·5–92·6	0·00 –0·028
Allium porrum	Leek	84·9	0·014	82·4–84·5	0·00 –0·020
Allium sativum	Garlic	—	—	61·3	0·002
Brassica rapa	Turnip	—	—	91·1–92·7	0·00 –0·29
Beta vulgaris v. *crassa*	Beet	90·1	0·00	85·5–90·4	0·003–0·015
Betavulgaris v. *rapacea*	Beet	—	—	—	—
Colocasia esculenta	Taro	—	—	—	—
Daucus carota	Carrot	87·2–90·6	0·643–5·65	88·9–90·0	3·42 –11·15
Dioscorea alata	Yam	—	—	—	—
Ipomoea batatas	Sweet potato	71·1	2·04	66·2–71·8	0·038–0·128
Manihot esculenta	Cassava (Manioc)	55·4–58·5	0·006–0·010	50·3–67·0	0·00 –0·009
Pachyrrhizus erosus	Yam bean root	89·5	0·000	—	—
Raphanus sativus	Radish	92·6–94·1	0·002–0·004	94·4–95·0	0·00 –0·007
Sechium edule	Chayote root	—	—	—	—
Solanum tuberosum	Potato	75·8–80·7	0·00 –0·004	70·4–79·4	0·00 –0·002
Tragopogon porrifolius	Salsafy	—	—	74·4–82·2	1·009
Xanthosoma violaceum	Malanga	61·1–58·3	0·002–0·007	—	—
B. HERBAGE VEGETABLES					
Acrocomia mexicana	Palm cabbage	—	—	—	—
Amaranthus chlorostachys		—	—	—	—
Amaranthus hybridus		—	—	85·8–86·8	5·84 –6·31
Amaranthus gangeticus	Tampala	84·4	1·80	—	—
Apium graveoleus		92·9	0·015	82·1–94·1	0·031–0·213
Bambusa arundinacea	Bamboo	90·7	0·016	—	—
Beta vulgaris v. *crassa*	Beet tops	90·6	0·018–3·02	—	—
Brassica campestris	Field Mustard	—	—	88·6–90·7	1·02 –5·03
Brassica caulorapa	Kohlrahi	91·9	0·007	88·0–92·0	0·002–0·035
Brassica juncea v. *foliosa*	Leaf mustard	—	—	87·4	1·07
B. oleracea v. *acephala*	Kale	82·1	5·45	—	—
B. oleracea v. *botrytis*	Broccoli	86·3–89·8	0·001–1·63	90·6–91·6	0·005–0·025
B. oleracea v. *capitata*	Cabbage	90·7–91·9	0·007–0·038	88·1–93·8	0·00 –0·059
B. oleracea v. *gemmifera*	Brussels sprout	—	—	85·2–83·0	0·079–0·351
Brassica pekinensis	Chinese cabbage	95·5–94·2	0·00 –0·158	96·8	0·009
Beta vulgaris v. *cicla*	Chard	—	—	90·7–93·5	0·026–2·72
Bromelia pinguin		—	—	—	—
Calandrinia micrantha		—	—	93·0	1·10
Calathea macrosepala		—	—	—	—
Chamaedorea graminifolia		—	—	—	—
Chamaedorea pacaya	Pacaya	—	—	—	—
*Chamaedorea tepejilote**		—	—	83·6–84·5	0·014–0·011
Chenopodium album		—	—	—	—
Chenopodium ambrosiodes		—	—	85·0	3·58
Chenopodium berlandieri	Lambs quarters	—	—	83·0	6·32
Chrysanthemum segetum		—	—	93·2	1·32
Cnidoscolus aconitifolius		76·4–83·2	5·96 –4·86	—	—
Coriander sativum	Coriander	—	—	81·6	2·82
Crotolaria longirostrata		81·8	6·85	81·1–83·0	0·158–9·36
Cucurbita pepo	Pumpkin (squash)	—	—	88·8–89·4	2·80 –1·12

Honduras,[1,2] Guatelmala,[3,4,5] El Salvador,[6] Nicaragua and Panama,[7] Mexico[8] and Costa Rica.[9] The carotene values obtained during this work are recorded in the following table. A similar but much less comprehensive survey, not recorded here, has been made for Chinese foods.[10]

El Salvador		Nicaragua and Panama		Mexico		Costa Rica	
Moisture (%)	Carotenè (mg./100g.)	Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg.100g.)	Moisture (%)	Carotene (mg./100g.)
—	—	—	—	—	—	—	—
87·9–89·1	0·003–0·007	84·4	0·00	—	—	88·9–91·0	0·00 –0·08
—	—	—	—	715·5	0·05	—	—
—	—	—	—	—	—	62·0	0·002
93·4	0·003	—	—	—	—	90·8–92·1	0·00 –0·001
84·6–86·2	0·002–0·003	88·7	0·003	—	—	83·6–88·7	0·003–0·005
—	—	—	—	82·3	0·00	—	—
—	—	—	—	—	—	64·1–69·4	0·000
89·2–91·8	0·57 –4·23	91·7	1·47	91·1	5·14	83·4	7·88
73·5–78·7	0·00	—	—	—	—	—	—
73·2–79·0	0·084–0·289	65·0	0·008	60·7	0·45	65·3–66·6	0·43–0·044
60·5–68·3	0·007–0·014	67·8	0·002	—	—	60·5–64·9	0·001–0·002
83·0–89·3	0·002–0·005	—	—	—	—	—	—
94·0–95·0	0·003–0·005	94·8	0·005	—	—	94·5–94·6	0·000–0·003
—	—	—	—	—	—	80·6–84·5	0·002–0·018
80·1–85·1	0·006–0·007	75·9	0·006	—	—	82·4–84·2	0·00 –0·005
—	—	—	—	—	—	74·6	0·000
—	—	67·5	0·004	—	—	60·2–64·1	0·003–0·012
87·6	0·006	—	—	—	—	—	—
—	—	—	—	86·1	4·60	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
93·5	0·003	—	—	—	—	93·6	0·071
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	84·0	2·84	—	—
—	—	91·6	0·006	—	—	—	—
—	—	—	—	—	—	93·8	1·72
—	—	—	—	—	—	—	—
—	—	—	—	90·0	0·05	86·7–90·3	0·027–2·27
92·6–93·8	0·010–0·016	93·8	0·004	89·6	0·14	89·2–92·6	0·005–0·021
—	—	—	—	—	—	81·1–82·4	0·021–0·548
—	—	—	—	—	—	95·8	0·003
—	—	—	—	89·3	4·35	—	—
91·6–93·0	0·005–0·017	—	—	—	—	—	—
—	—	—	—	—	—	—	—
92·3	0·010	—	—	—	—	—	—
85·3	0·014	—	—	—	—	—	—
—	—	—	—	—	—	84·3	0·006
—	—	—	—	—	—	—	—
—	—	—	—	83·2–88·0	2·26–4·33	—	—
—	—	—	—	89·6	1·02	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	88·6	4·31	—	—
80·0–92·3	0·018–11·88	—	—	—	—	—	—
—	—	—	—	—	—	—	—

Botanical name	Common name	Honduras		Guatelmala	
		Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)
Dondia sufrutencona ..		—	—	—	—
Eryngium foetidum ..		—	—	—	—
Erythrina berteroana ..		—	—	82·1	0·159
Euterpa longipetiolata ..	Palm cabbage	—	—	—	—
Fernaldia pandurata ..		—	—	88.2	0·169
Geonoma edulis	Palm cabbage	—	—	—	—
Gliricidia sepium ..	Madre ..	86·3	0·030	—	—
Gnetum gnemon		65·6	10·27	—	—
Heteranthea reniformis ..		93·8	1·67	—	—
Hibiscus sabadariffa ..	Roselle ..	75·5–89·9	0·006–0·045	—	—
Ipomoea batatas ..	Sweet potato	87·8–84·8	3·34 –0·073	—	—
Jussiaea repens		87·5	3·94	—	—
Lactuca sativa vac. ..	Lettuce ..	95·5–95·8	0·014–0·00	94·5–94·9	0·042–0·287
Malva parriflora ..	Malva	—	—	77·2–84·9	6·32 –12·58
Malva silvestris ..		—	—	—	—
Malvum vactum ..		—	—	—	—
Manihot esculentum ..	Cassava ..	27·4–82·3	0·024–0·038	—	—
Mentha citrata ..		—	—	89·0	5·51
Nasturtium officinale ..	Water cress ..	—	—	—	—
Opuntia sativus	Cactus ..	—	—	—	—
Petroselenum crispum ..	Parsley ..	—	—	—	—
Piper auritum		83·9	1·91	—	—
Portulaca oleracea ..	Purslane ..	87·8–92·1	0·060–0·97	92·5	0·827
Rheum rhaponsticum ..	Rhubarb ..	—	—	94·6	0·039
Sechium edule	Chayote ..	—	—	—	—
Solanum nigrum ..		—	—	88·0	0·218
Sonchus oleraceus ..	Endive ..	—	—	—	—
Spathiphyllum phnynifolium		—	—	82·4	0·022
Spinacia oleracea ..	Spinach ..	—	—	89·4	4·12
Talinum triangulare ..	Phillipine spinach ..	90·3	0·041	—	—
Tetragonia expansa ..	New Zealand spinach ..	90·8–93·9	0·513–1·50	91·5–91·9	0·057–2·60
Yucca elephantipes ..	Yucca (flowers)	—	—	82·2–89·0	0·003–0·026
C. FRUIT					
Anona cherimolla ..	Custard apple	—	—	—	—
Achras zapota	Sapodilla ..	—	—	76·9–85·7	0·004–0·020
Acrocomia mexicana ..		47·9	0·107	—	—
Bnacardium occidentale	Cashew ..	—	—	—	—
Ananas comosus ..	Pineapple ..	77·8–84·6	0·004–0·027	83·6–87·0	0·002–0·049
Anona diversifolia ..	Ilama	—	—	—	—
Anona muricata	Guanabana ..	—	—	80·6	0·004
Anona reticulata		—	—	75·6	6·000
Anona squamosa ..	Sweetsop ..	69·8	0·005	71·1	0·007
Ardisia escallonioides ..		72·1	0·012	—	—
Artocarpus altilis ..	Breadfruit ..	79·3	0·004	—	—
Astrocarium stanolyanum		71·9	14·90	—	—
Bverrhoa bilimbi ..		—	—	—	—
Averrhoa carambola ..	Star apple ..	91·0–89·0	0·552–0·003	—	—
Bactris minor		—	—	—	—
Bactris subglobosa ..		—	—	81·8	0·345
Bouea macrophylla ..	Bandaria ..	85·2	0·043	—	—
Bromelia karatas ..		—	—	—	—
Bryosonema crassifolia ..		—	—	—	—
Calocarpum mammosum	Sapote ..	—	—	61·3–73·1	0·045–0·065
Calocarpum viride ..	Green sapote ..	—	—	68·1	0·069
Capsicum annuum var. ..	Sweet pepper	93·7–90·0	0·010–1·17	92·3–79·3	0·007–2·36
Capsicum frutescens ..		—	—	77·7–79·7	0·133–0·413
Capsicum pubescens ..	Hot pepper ..	—	—	88·6–90·0	0·441–0·249
Carica papaya	Papaya ..	—	—	86·8–89·3	0·006–0·241
Casimiroa edulis ..	White sapote	—	—	—	—
Chrysobalanus icaco ..	Icaco ..	86·4–84·6	0·007–0·006	—	—
Chrysophyllum cainito ..	Star apple ..	79·8–85·7	0·015–0·018	82·4–78·5	0·011–0·004
Citrullus vulgaris ..	Watermelon ..	—	—	93·6	0·029
Citrus aurantifolia ..	Lime	91·4–92·2	0·013–0·022	89·9–93·5	0·040–0·003
Citrus aurantium ..	Sour orange ..	—	—	77·8	0·071
Citrus limetta	Sweet lime ..	85·6	0·003	—	—

El Salvador		Nicaragua and Panama		Mexico		Costa Rica	
Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg.100g.)	Moisture (%)	Carotene (mg./100g.)
—	—	—	—	92·0	2·76	—	—
85·7	3·79	—	—	—	—	—	—
82·9–86·6	0·31 –2·17	—	—	—	—	—	—
—	—	—	—	—	—	90·0	0·020
—	—	—	—	—	—	—	—
—	—	—	—	—	—	88·2	0·006
85·7	0·137	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
94·4–95·4	0·124–1·10	95·2	0·226	96·6	0·61	92·4–95·4	0·348–1·03
—	—	—	—	82·9–92·4	2·4–8·5	—	—
—	—	—	—	92·0	4·80	—	—
—	—	—	—	91·2	4·31	—	—
73·4	0·683	—	—	—	—	—	—
8·90	5·51	—	—	—	—	—	—
94·7	0·71	—	—	93·3	1·04	93·4–94·5	0·38 –2·1
—	—	—	—	92·5	0·50	—	—
84·1	2·22	—	—	84·8	4·56	85·3	0·078
—	—	—	—	—	—	—	—
—	—	—	—	90·7	3·25	—	—
—	—	—	—	—	—	—	—
—	—	—	—	74·6–92·1	0·01–0·03	87·8	2·27
—	—	—	—	—	—	—	—
84·4–86·7	0·055–0·824	—	—	92·0	4·99	—	—
—	—	—	—	—	—	—	—
—	—	—	—	88·4	4·43	92·3	2·41
—	—	—	—	—	—	—	—
91·0–93·0	2·43 –2·81	—	—	—	—	91·6–93·1	2·24 –2·31
81·6	0·028	—	—	—	—	81·9–84·2	0·067–0·043
—	—	—	—	30·6	0·02	—	—
—	—	75·0	0·013	78·5	0·05	85·0–86·7	0·008–0·476
—	—	—	—	—	—	—	—
85·8–88·7	0·022–0·240	—	—	—	—	—	—
82·3–83·8	0·014–0·055	90·3	0·003	88·5	0·18	85·2–86·4	0·002–0·017
71·5	0·025	—	—	—	—	—	—
84·1	0·004	—	—	—	—	83·9	0·000
68·3	0·007	—	—	—	—	79·3	0·018
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	94·2	0·035	—	—	—	—
—	—	—	—	—	—	—	—
—	—	77·3	0·036	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
86·8–87·4	0·006–0·015	—	—	—	—	—	—
79·3–90·5	0·035–0·060	—	—	—	—	—	—
57·9–63·8	0·16 –0·67	62·9	0·518	71·4	1·46	55·3	0·051
—	—	—	—	—	—	69·5	0·031
77·2–93·3	0·063–1·08	87·6	0·349	—	—	90·7–91·5	0·012–0·219
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
86·9–91·6	0·004–0·208	—	—	—	—	85·9–89·2	0·19 –0·68
78·3	0·053	—	—	89·3	0·03	—	—
85·9–89·4	0·007	—	—	—	—	84·0	0·007
80·1	0·039	—	—	—	—	78·4	0·023
—	—	94·7	0·028	—	—	93·8	0·007
—	—	—	—	—	—	—	—
83·1	0·055	—	—	86·6	0·29	—	—
92·4	0·004	89·3	0·005	91·7	0·02	90·3	0·00

Botanical name	Common name	Honduras		Guatelmala	
		Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)
Citrus limonia	Lemon	93·7–94·3	0·003–0·031	91·6	0·021
Citrus maxima	Grapefruit	84·4–89·9	0·003	—	—
Citrus medica	Citron	—	—	87·1	0·009
Citrus nobilis	Mandarin orange	87·1–89·6	0·046–0·077	82·6–83·9	0·013–0·373
Citrus reticulata	Tangerine	—	—	—	—
Citrus sinensis	Orange	—	—	87·6–91·0	0·005–0·028
Coccoloba caracasana		—	—	—	—
Cocos nucifera	Coconut	62·8	0·004	52·2	0·000
Cola nitida		83·3	0·031	—	—
Cordia dentata		86·7	0·011	—	—
Crataegus oblonga		—	—	76·0	0·539
Crataegus pubescens		—	—	76·1	0·111
Cucumis melo	Melon	—	—	93·3	0·771
Cucumis sativus	Cucumber	91·5–95·2	0·013–0·032	05·2–96·7	0·020–0·002
Cucurbita ficifolia	Chayote	—	—	93·2	0·005
Cucurbita maxima	Pumpkin (squash)	—	—	95·2	0·001
Cucurbita pepo	Pumpkin (squash)	94·7–84·7	0·007–0·142	86·4–94·9	0·057–1·963
Cyclerantha pedata		—	—	93·4–93·8	0·011–0·062
Cydonia oblonga	Quince	83·9	—0·017	80·8–83·2	0·004–0·020
Cyphomandra betacea		—	—	87·8	0·371
Diospyros operaster	Black sapote	—	—	—	—
Doryalis hebecarpa	Ceylon gooseberry	81·9–83·6	0·125–0·356	—	—
Durio zibethinus	Durian	61·1	0·018	—	—
Elaeocarpus odorata		—	—	—	—
Elaeocarpus serratus		74·2	0·183	—	—
Eugenia dombeyana		85·3	0·039	—	—
Eugenia jambolana	Jambolana plum	85·6	0·004	—	—
Eugenia jambosa	Rose apple	85·1	0·123	—	—
Eugenia malaccensis		—	—	—	—
Ficus carica	Fig	—	—	86·8	0·013
Ficus glabrata	Deer fig	90·3	0·059	—	—
Fragaria vesca	Strawberry	—	—	88·4	0·014
Guiliebria gasipaes	Peach palm	36·4–49·6	0·835–2·76	—	—
Hibiscus esculentus	Okra	85·6–90·2	0·006–0·042	—	—
Hylocercus undatus		—	—	82·5–83·0	0·012–0·005
Lagenaria siceraria	Gourd	92·2	0·004	—	—
Licania platypus	Sapote	—	—	—	—
Lycopersicum esculentum	Tomato	89·2–94·9	0·018–0·942	85·4–91·7	0·173–0·692
Malphigia glabra		—	—	83·6	0·003
Malus sylvestris	Apple	83·1	0·051	94·6–86·5	0·004–0·010
Mammea americana	Mamey	—	—	85·5–87·7	0·150–0·089
Mangifera indica	Mango	80·8–88·8	0·28 –1·35	86·7–78·9	0·067–1·35
Mangifera odorata		79·9	0·388	—	—
Manilkara zapotilla	Sapodilla	73·1	0·024	66·0	0·001
Melicocea bijuga		—	—	—	—
Melicocea indica		—	—	—	—
Musa paradisiaca var.	Banana	59·7–75·4	0·001–1·65	61·7–76·8	0·010–0·121
Muntingia calabura		—	—	—	—
Noptalea cochenillifera	Prickly pear	—	—	83·4	0·002
Noronhia emarginata		78·0	0·011	—	—
Opuntia hyptiacanthus		—	—	—	—
Opuntia imbricata		—	—	—	—
Opuntia robusta		—	—	—	—
Passiflora ligularis	Sweet granadilla	—	—	69·9–76·5	0·000–0·001
Passiflora quadrangularis	Giant granadilla	—	—	—	—
Persea americana	Avocado	74·5–78·6	0·195–0·182	65·7–86·7	0·043–0·475
Persea gratissima		—	—	—	—
Persea schiedeana		—	—	—	—
Phyllanthus acidus	Otaheite gooseberry	—	—	—	—
Physalis aequata	Ground cherry	—	—	90·4	0·061–0·874
Physalis pubescens	Ground cherry	—	—	82·9–90·5	0·310–0·026
Polakowskia tacaco	Tacaco	—	—	—	—
Ponteria campechiana		—	—	—	—
Ponteria mammosa	Sapote	65·7	0·175	—	—
Ponteria viridis		—	—	65·6–72·7	0·061–0·099
Prunus armeniaca	Apricot	—	—	—	—

El Salvador		Nicaragua and Panama		Mexico		Costa Rica	
Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg.100g.)	Moisture (%)	Carotene (mg./100g.)
—	—	91·4	0·014	88·4	0·04	—	—
89·5	0·047	87·5–90·4	0·005–0·013	—	—	88·3	0·003
—	—	—	—	90·3	0·04	—	—
—	—	—	—	84·8	1·81	—	—
88·3	0·039	90·2	0·152	—	—	85·5	0·052–0·175
89·4	0·004	85·9–91·1	0·009–0·016	—	—	—	—
74·6	0·010	—	—	—	—	—	—
52·5–81·4	0·002–0·004	—	—	—	—	54·5	0·003
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	76·1	6·4	—	—
—	—	—	—	—	—	—	—
78·3	0·053	—	—	93·5	1·68	—	—
96·3	0·002	95·5	0·002	95·4	0·11	95·0–96·3	0·011–0·022
94·1	0·002	—	—	—	—	92·7–90·8	0·038–0·001
—	—	—	—	92·6	0·290	—	—
91·4–96·3	0·001–0·086	92·3–94·0	0·017–0·048	—	—	89·8–91·9	0·039–0·210
—	—	—	—	—	—	94·8–93·8	0·054–0·001
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	83·1	0·019	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
90·3–91·2	0·003–0·008	—	—	—	—	—	—
—	—	—	—	83·0	0·13	88·2–90·1	0·094–0·19
—	—	—	—	—	—	—	—
—	—	—	—	91·0	0·06	91·7–92·1	0·004–0·04
—	—	—	—	—	—	59·6–60·9	0·29 –1·73
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
67·4	0·273	—	—	—	—	64·6	0·157
91·0–94·4	0·289–1·56	94·3	0·253	94·6	2·54	93·0–93·9	0·091–0·48
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
85·0–86·3	0·043–0·250	—	—	—	—	—	—
83·2–86·4	0·025–1·87	—	—	79·6–83·3	1·17–1·96	81·7–88·3	0·089–1·82
—	—	—	—	—	—	—	—
74·3–77·4	0·012–0·036	—	—	—	—	—	—
82·5	0·044	68·8	0·020	—	—	—	—
—	—	—	—	—	—	—	—
65·5–73·4	0·006–0·377	62·0–69·9	0·015–0·17	78·6–60·2	0·28–1·95	58·8–74·0	0·433–0·00
77·8	0·019	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	86·2	0·08	—	—
—	—	—	—	83·5	0·05	—	—
—	—	—	—	82·0–89·3	0·01–0·12	—	—
79·1	0·000	—	—	78·5	0·25	71·9–76·0	0·011–0·02
78·4	0·019	—	—	—	—	—	—
79·8–85·4	0·034–0·188	79·9	0·130	79·7	0·18	83·6–87·7	0·075–0·15
—	—	—	—	—	—	—	—
76·5–77·6	0·033–0·003	—	—	—	—	63·9–69·2	0·034–0·13
91·9	0·019	—	—	—	—	—	—
—	—	—	—	92·5	0·19	—	—
—	—	—	—	—	—	—	—
—	—	—	—	83·4	0·02	—	—
62·9	0·382	58·3	2·63	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	83·4	2·54	—	—

Botanical name	Common name	Honduras		Guatelmala	
		Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)
Prunus capuli	Wild cherry	—	—	—	—
Prunus domestica	Plum	—	—	85·7–90·8	0·003–0·037
Prunus persica	Peach	—	—	85·5–84·3	0·002–0·000
Psidium guajava	Guava	—	—	78·9–77·9	0·063–0·008
Punica granatum	Pomegranate	—	—	86·4	0·000
Pyrus communis	Pear	88·9	0·003	85·9–83·6	0·000–0·004
Pyrus malus	Apple	—	—	—	—
Rheedia madruno	Madrono	86·1	0·003	—	—
Rubus glaucus	Loganberry	—	—	—	—
Rubus hondruensis	Blackberry	79·6	0·046	—	—
Sandoricum indicum	Sautol	87·0	0·003	—	—
Saurania panciscerata		—	—	—	—
Sechium edule	Chayote	90·1–93·4	0·001–0·034	88·3–92·6	0·00 –0·014
Sicania odorifera		—	—	—	—
Sizygium jambos	Rose apple	—	—	—	—
Sizygium malaccensis		—	—	—	—
Solanum muricatum	Pepino	—	—	90·2–92·1	0·136–0·003
Solanum melongena	Egg plant	92·3	0·011	91·8–90·8	0·009–0·003
Spondias mombin	Spanish plum	72·8	0·071	—	—
Spondias purpurea	Spanish plum	—	—	67·5–65·9	0·066–0·089
Theobroma bicolor		—	—	79·2	0·224
Vincentoxium salvinii		—	—	—	—
Vitis tilifolia	Wild grape	—	—	87·3	0·021
Zizyphus jujuba	Jujube	83·0	0·021	—	—
D. LEGUMES					
Arachis hypogaea	Pea nut	—	—	—	—
Cajanus cajan	Pigeon pea	65·4–73·7	0·005–0·064	67·6	0·064
Canavalia ensiformis	Jack bean	78·5	0·030	—	—
Dolichos lalab	Lablab bean	65·8	0·140	—	—
Hymonaea courabaril		—	—	—	—
Igna spp.		64·2–67·9	0·284–4·85	—	—
Phaseolus limensis		—	—	—	—
Phaseolus vulgaris	French (string) bean	87·6–91·5	0·016–0·154	92·5–53·5	0456·0·000
Pisum sativum	Pea	—	—	79·7–72·7	0·180–0·009
Vicia faba	Broad bean	—	—	78·8–56·5	0·120–0·18
Vigna unguiculata	Cow pea	78·3	0·025	—	—
E. CEREALS					
Zea mais	Maize (corn)	88·8	0·010	60·1–73·5	0·004–0·076

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APPENDIX II

El Salvador		Nicaragua and Panama		Mexico		Costa Rica	
Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg./100g.)	Moisture (%)	Carotene (mg.100g.)	Moisture (%)	Carotene (mg./100g.)
—	—	—	—	81·2	0·51	—	—
—	—	—	—	—	—	—	—
84·2–85·1	0·008–0·015	—	—	—	—	86·4	0·003
84·9	0·013	83·2	0·028	81·5	1·47	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	83·4	0·02	—	—
—	—	—	—	—	—	—	—
82·4–86·6	0·010	—	—	—	—	—	—
—	—	—	—	—	—	79·5	0·014
—	—	—	—	—	—	—	—
83·4	0·060	—	—	—	—	—	—
89·7–93·1	0·003–0·022	93·3	0·002	—	—	91·7	0·016
—	—	—	—	—	—	—	—
—	—	—	—	—	—	90·5	0·000
—	—	—	—	—	—	90·4	0·032
90·1	0·015	—	—	—	—	—	—
90·4–92·0	0·001–0·007	93·8	0·002	—	—	90·2	0·005
—	—	—	—	—	—	—	—
69·5	0·073	—	—	86·5	0·38	78·8	0·004
—	—	—	—	—	—	—	—
91·3–93·2	0·009–0·012	—	—	—	—	—	—
80·5	0·025	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	14·6	0·005
—	—	—	—	—	—	—	—
65·1–85·9	0·042–0·148	—	—	—	—	—	—
—	—	—	—	—	—	—	—
88·2–92·6	0·066–0·188	91·6	0·141	—	—	70·2–91·2	0·002–0·433
—	—	—	—	—	—	70·2–72·5	0·002–0·110
—	—	—	—	—	—	—	—
—	—	88·5	0·376	—	—	—	—
59·8–69·5	0·001–0·021	63·3–89·4	0·003–0·007	—	—	84·8	0·009

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* This normally indicates a mixture of 90–95% of β-carotene with 5–10% of α-carotene.