Ann. Rev. Nutr. 1986. 6:273–97

METABOLISM, NUTRITION, AND FUNCTION OF CAROTENOIDS

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INTRODUCTION

Carotenoids represent one of the most widespread groups of natural pigments. They are synthesized de novo by all plants and some microorganisms but they are also found throughout the animal kingdom, where they accumulate either

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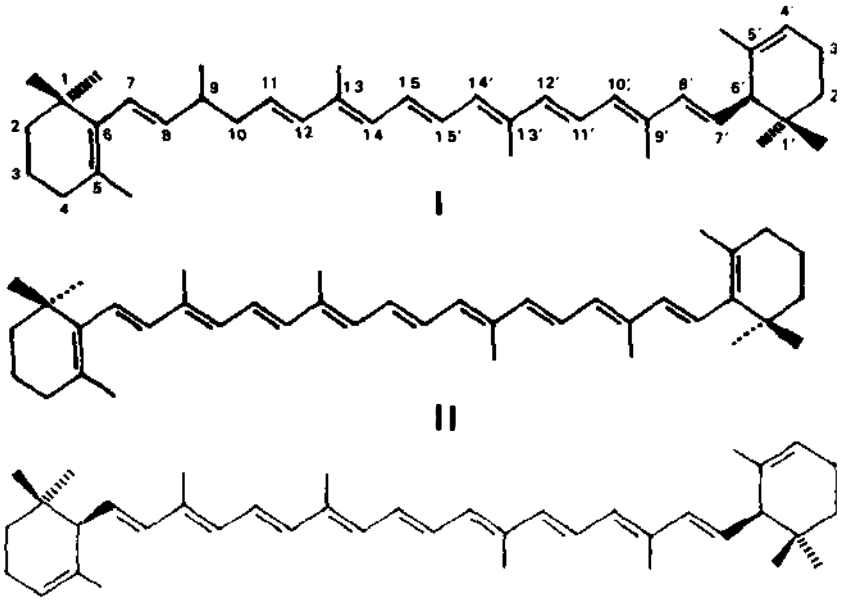
unchanged from the diet or are metabolically modified. Modification is most apparent in the lower animals (39). Over 500 carotenoids are now known to occur in nature but only a small number concern us in this review. They are all based on the hydrocarbons α-carotene (β,ε-carotene) (I), β-carotene (β,β-carotene) (II), and ε-carotene (ε,ε-carotene) (III). The numbering of the carotenoid molecule is given in Formula I, where it will be noticed that a chiral center exists; the structure indicated is (6'*R*)β,ε-carotene, while that of Formula III is (6*R*,6'*R*)ε,ε-carotene. All these are the structures of the plant carotenes.



I

II

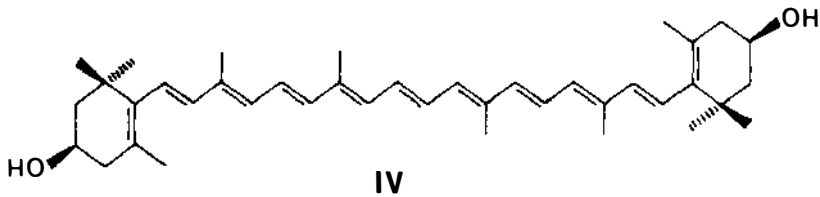
III

Carotenoids that contain oxygen functions are termed xanthophylls and the main components considered here are (3*R*,3'*R*)-zeaxanthin (IV), (3*R*,3'*R*,6'*R*)lutein (V), and (3*S*,3'*S*)astaxanthin (VI)[1]. As with the carotenes mentioned, these are the chiral species found in plants. It was previously

[1]The *R*, *S* convention is the internationally accepted way of denoting chirality. It depends on "priorities" of the groups attached to the chiral center. This can make for confusion, for example the *absolute configuration* at C-3' of lutein (V) is opposite to that at C-3 although application of the priority rules leads to both being designated *R*. Similarly 3*R*,3*R*' zeaxanthin (IV) has the same absolute configuration as 3*S*,3'*S*-astaxanthin (VI). When ambiguity might arise the structures are given in full. See (40) for a simplified explanation of the application of the *R*, *S* convention.

thought that when these pigments were found in animals they had the same chirality as those from the ultimate dietary source, plants (39). It is now clear that sometimes they have and sometimes they have not the same chirality, in fact almost all possible epimers have been found somewhere in the animal kingdom.

The recent penetrating studies, discussed in detail later, on carotenoid metabolism in lower animals have revealed many new aspects and destroyed some cherished dogmas about carotenoid nutrition. The scientific aspects of using carotenoids to treat diseases involving photosensitization is also considered here, together with the evidence suggesting that carotenoids can delay the onset of some tumors. Many other aspects of carotenoid chemistry and biochemistry are not considered because of shortage of space, but the reader is referred to recently published monographs (5, 38, 39) and to the published proceedings of the last three triennial Symposia on Carotenoids, held in Maidson, Liverpool, and Munich, respectively (11, 24, 37).



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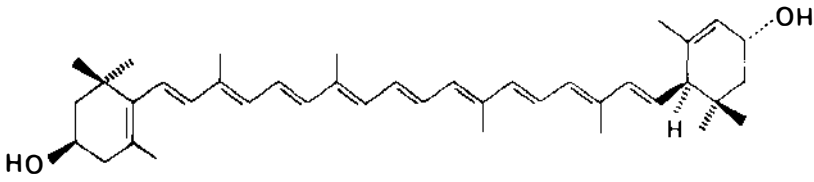

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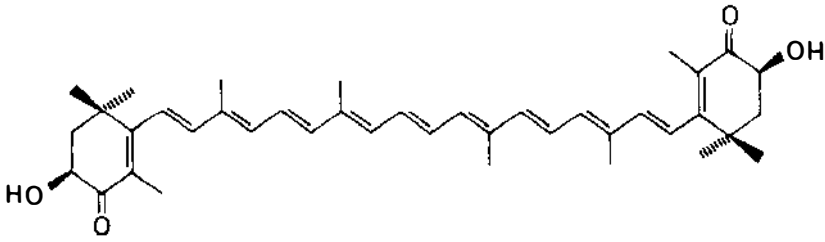


VI

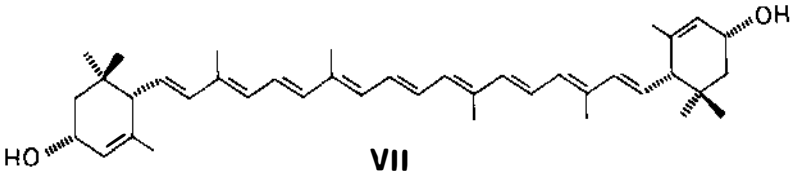
METABOLISM IN FISH

Nature of Pigments

The bulk of the yellow and red colors in fish skin are xanthophylls that are generally esterified (39). According to early investigators, the main pigments were astaxanthin, lutein, and taraxanthin, a pigment of unknown structure that has gradually disappeared from current literature (39). The generalization concerning astaxanthin and lutein is still true but stereochemically imprecise. The separation by high performance liquid chromatography (HPLC) of diastereomeric camphanates of xanthophylls (103, 112, 127) has made possible chiral analysis of carotenoids. Thus it emerged that lutein from goldfish, trout, and salmon is not "plant" lutein (V) but the 3'-epimer (3*R*,3'*S*,6'*R*)-epilutein (structure illustrated in Figure 2) (17, 93). It is significant that so far epilutein has been reported only once in plants, in the petals of *Caltha palustris* (16). The reported widespread occurrence of lutein in fish (see 39) needs now to be reassessed. In marine fish, C-6' lutein epimers, i.e. (6'*S*)-lutein, are reported with configurations 3*R*,3'*S*,6'*S*-, 3*R*,3'*R*,6'*S*-, and 3*S*,3'*R*,6'*S* (88). It is of considerable evolutionary significance to discover whether in general 6'*R*-luteins are confined to freshwater fish and 6'*S*-luteins to marine fish.

The "astaxanthin" from the flesh of Atlantic and Pacific salmon consists of three isomers with 3*S*,3'*S*-(VI),3*R*,3'*R*- and 3*R*,3'*S*- configurations; the relative amounts in both species were 8:1.5:0.5, respectively (115). In contrast, samples of plant astaxanthin so far examined contain essentially only the 3*S*,3'*S*-isomer (108, 109).

Tunaxanthin, first isolated from *Thunnus orientalis* (53) and shown to be an ε,ε-carotene-3,3'-diol (21), has now been found in a number of Japanese fish in three chiral forms: tunaxanthin A (3*S*,6*S*,3'*S*,6'*S*) (IX), tunaxanthin B (3*R*, 6*S*, 3'*S*, 6'*S*) (VIII) and tunaxanthin C (3*R*,6*S*,3'*R*,6'*S*) (VII) (91, 92, 111). These pigments are identical with oxyxanthin 45, oxyxanthin 51, and oxyxanthin 58, respectively (92), pigments isolated from the Southern Californian *Oxiulis californica* (8). There is also one report of the presence of (3*S*,6*R*,3'*S*,6'*R*)-chiriquixanthin B (X), a C-6, C-6'-epimer of tunaxanthin A, in the fish *Sebastes flavidus* (8). The chiriquixanthins, which have the *R* configuration at C-6 and C-6', are so called because they were first isolated from the frog *Atelopus chiriquiensis* (7).



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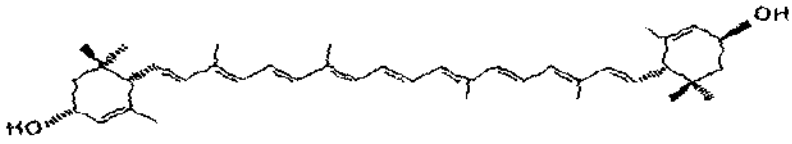

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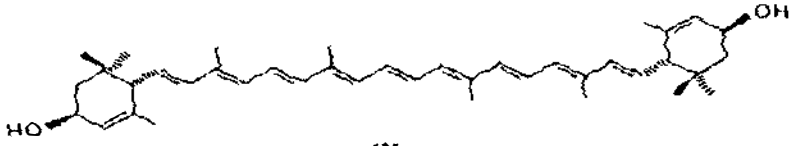

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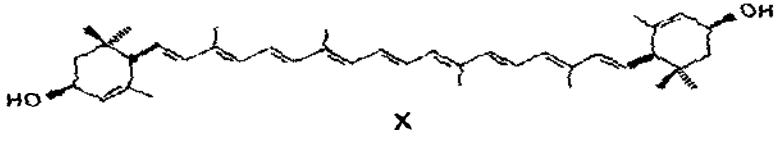


X

Astaxanthin Formation

FRESHWATER FISH β-Carotene can be converted into 3*S*,3′*S*-astaxanthin in goldfish and the probable steps in the conversion are indicated in Figure 1. The proposed intermediates isocryptoxanthin, echinenone, and canthaxanthin all yield astaxanthin when fed to pigment-depleted fish (110); 4′-hydroxyechinenone (Figure 1) is the only proposed intermediate that has not been detected in the fish (110). Other workers suggest that this is only a minor pathway and that zeaxanthin (IV) is a key intermediate (47). However, in the fish *Tilapia nilotica* zeaxanthin is oxidized to rhodoxanthin (4′,5′-didehydro-4,5′-retro-β,β-carotene-dione) (XI) (89). Incidentally, the pathway followed in prawns (59) and lobsters (60) is that outlined in Figure 1.

XI

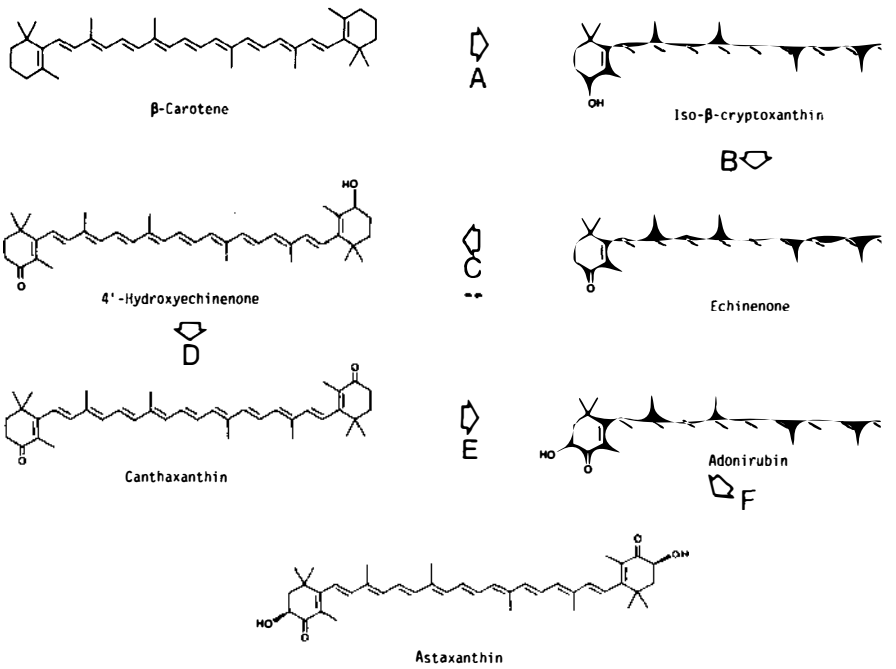


Figure 1 Possible pathway of conversion of β-carotene into astaxanthin in goldfish (adapted from 15). Reproduced with permission.

Rather unexpected is the clear demonstration over the past few years of the conversion of (3*R*,3'*R*)-lutein (V) into (3*S*,3'*S*)astaxanthin (VI) in goldfish and fancy red carp (see 121). The difference of chirality at C-3' in lutein and astaxanthin first put this result in question (15) but the discovery that "lutein" in skin is in fact 3'-epilutein (16) removed this difficulty and so it is reasonable to assume that as a first step in the formation of astaxanthin in goldfish (3*R*,3'*R*)-lutein is inverted to 3'-epilutein via 3'-O-didehydrolutein (*A*, *B* in Figure 2). 3'-O-Didehydrolutein has been otherwise observed only in the petals of *Caltha palustris* and egg yolk (16) and probably in some moths, particularly *Philosamia cynthia* under the name philosamiaxanthin, although the chirality of this pigment is not known (see 61). Lability of this compound to alkali suggests that it may be more widespread than so far reported because alkali treatment is frequently an essential early step in purifying carotenoid extracts.

[^{14}C] Lutein is converted into α-doradexanthin (54), β-doradexanthin (54), and astaxanthin (54, 105) in goldfish and fancy red carp, although in other experiments with goldfish the conversion appeared to stop at α-doradexanthin (*C* in Figure 2) (47). However, the conversion of β-doradexanthin into astaxanthin (*E* in Figure 2) is well documented (47, 49). The only doubtful step is *D*, which involves the isomerization of a β-ring into an ε-ring. This is much more

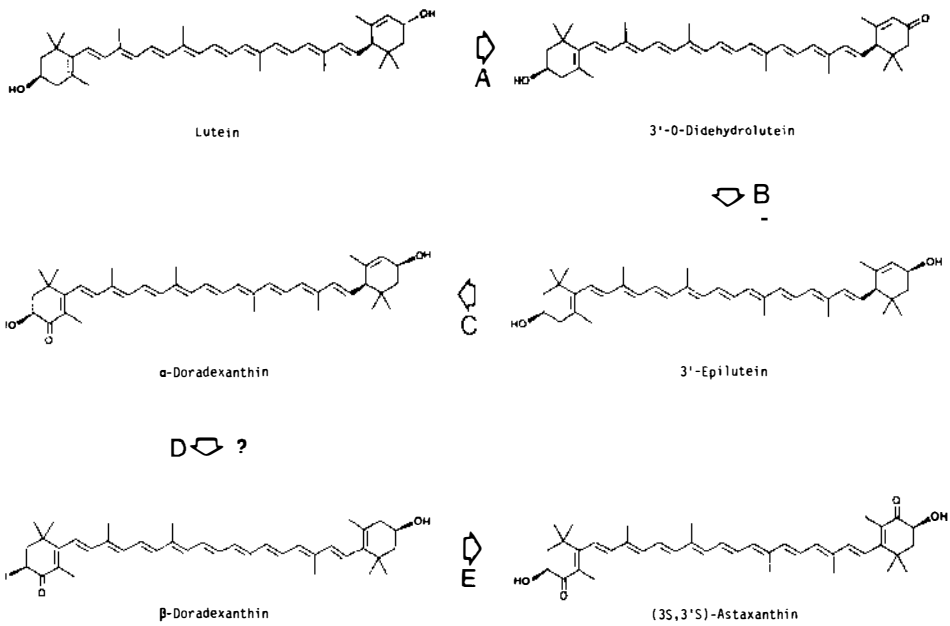


Figure 2 Possible pathway of conversion of 3*R*,3'*R*-lutein into astaxanthin in goldfish (adapted from 15). Reproduced with permission

likely than was thought a few years ago and has been established in chicken retinas (see next section); it does not occur in plants (38). Support for α-doradexanthin as an intermediate comes from the demonstration that its configuration at C-3 and C-3' is the same as that in the major astaxanthin epimer present (15).

(3*R*,3'*R*)-Zeaxanthin (IV) is also converted into (3*S*,3'*S*)-astaxanthin (49, 50) and a possible intermediate, idoxanthin (105), has recently been shown to have the appropriate absolute configuration [(3*S*,3'*S*,4'*R*)-trihydroxy β,β-carotene-4-one] (96). The exact pathway is not known but it will be recalled (Figure 1) that zeaxanthin is not involved in the conversion of β-carotene into astaxanthin.

The explanation of the existence of small amounts of (*3R*,3'*R*)-astaxanthin and (3*R*,3'*S*)-astaxanthin in fish is not yet apparent. In some cases they may be supplied preformed in the small crustacea in the diet of *Salvelinus alpinus* and, possibly, *Salmo trutta* (125). This, of course, only pushes the problem one step further back and the question whether or not algal chiral carotenoids are stereochemically pure needs to be addressed.

SALMON AND RAINBOW TROUT Salmon and rainbow trout differ from goldfish for they cannot oxidize 3,3'-dihydroxy carotenoids (e.g. zeaxanthin)

to astaxanthin (48). Because the absorption of dietary β-carotene is almost zero (116), this pigment also cannot be a precursor and one must conclude that astaxanthin in these fish is essentially of dietary origin.

Xanthophyll Formation in Marine Fish

Two biosynthetic problems are posed by the existence of the widely distributed tunaxanthins: (*a*) how are pigments with two ε,ε-rings formed, and (*b*) how is the 6*S*' chirality achieved? A pathway from β-carotene has been suggested (15) but as yet there is no accompanying experimental evidence. On the other hand astaxanthin is converted into the tunaxanthins in the yellowtail *(Seriola quinqueradiata)* (32, 99, 100), and the red Sea Bream (33). A thorough study, including the determination of chirality, of the carotenoids found in the eggs of the dolphin *(Coryptiaena hippurus)* and flying fish *(Prognichthys agoo)* (90) led to the proposal of a putative pathway for the formation of tunaxanthin A, B, C from (3S,3'*S*)-astaxanthin via (3*R*,3'*R*)-zeaxanthin, (3*R*,6'*S*)-3-hydroxy-β,ε-caroten-3'-one, and (6*S*,6'*S*)-ε,ε-carotene-3,3'-dione.

Direct biochemical evidence still remains to be reported but it should be emphasized that the proposals involve a *reductive* pathway of metabolism for astaxanthin; other such pathways of astaxanthin are considered in the next section.

Reductive Metabolism of Xanthophylls in Fish

A number of reports of reductive pathways of metabolism of fish carotenoids have recently appeared. The formation of tunaxanthins, just discussed, is a case in point. Reductive reactions in freshwater fish are of the utmost importance leading as they do to a new source of vitamin A in the food chain. Thorough investigations with labelled (3*S*,3'*S*)-astaxanthin revealed a number of metabolites in the skin of rainbow trout *(Salmo gairdneri)* (Figure 3); the skin is the main site of accumulation in the trout (116).

The pathway of reduction involves the stepwise removal of the keto groups at C-4 and C-4' to form zeaxanthin, which then appears to undergo more conventional degradative processes beginning with epoxidation. Adonixanthin and (3*R*,3'*R*)-zeaxanthin, which are normally present in trout skin, are thus probable metabolites of dietary astaxanthin obtained from small crustacea (117). Feeding of adonirubin and canthaxanthin (Figure 3) confirmed the elimination of the keto groups at C-4,4' but again, in the case of adonirubin, there was no elimination of the C-3 hydroxyl group. This means that only canthaxanthin, not normally a dietary carotenoid, can give rise in the skin to β-carotene, the vitamin A precursor.

In Atlantic salmon *(Salmo salar)* dietary astaxanthin and trout are deposited more efficiently in the flesh than in the skin, in contrast to the rainbow trout (116). The reductive metabolic pathway of the two pigments is the same in both

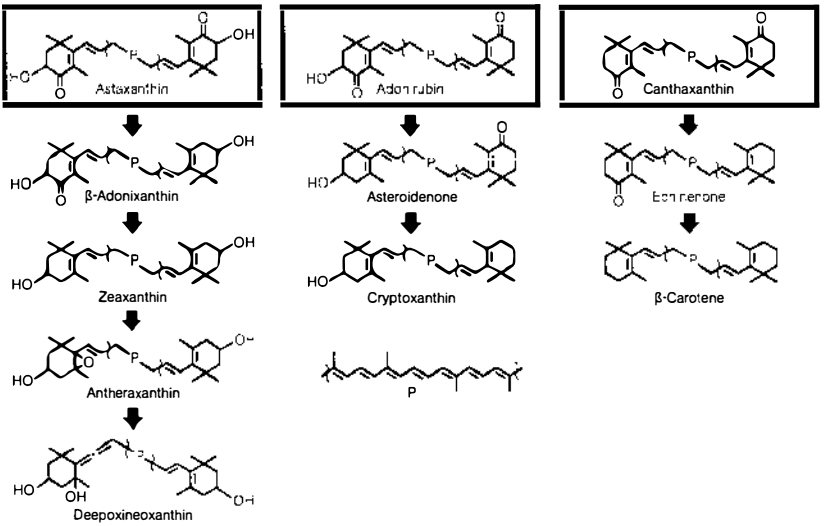


Figure 3 The metabolism of astaxanthin, adonirubin, and canthaxanthin in skin of rainbow trout (116). Reproduced with permission.

species although idoxanthin, a putative intermediate between astaxanthin and adonixanthin, was found in salmon (116). Similar results have been reported in *Oncorhynchus keta* (63), and in the freshwater fish *Heteropneustes fossilis* astaxanthin is converted into β-carotene and crustaxanthin (β,β-carotene, 3,4,3',4'-tetraol) (41). (3*S*,3'*S*)-Astaxanthin is also reduced to (3*R*,3'*R*)-zeaxanthin in eggs of mackerel *(Pneumatophorus japonicus)* and yellowtail *(Seriola quinqueradiata)* (90). (3*R*,3'*R*)-Zeaxanthin is the isomer present in plants (38) but recently the (3*S*,3'*S*) diastereomer was obtained from trout skins (116). As dietary astaxanthin is not epimerized at C-3,3' during absorption and metabolism in the trout (30, 116), the conclusion must be that (3*R*,3'*R*)-astaxanthin, present in small amounts in the crustacean food, is the direct precursor of (3*S*,3'*S*)-zeaxanthin (116).

Parasiloxanthin, 7',8'-dihydrozeaxanthin, and 7,8-dihydroparasiloxanthin are xanthophylls with reduced in-chain double bonds. They are reported in the Japanese catfish *Parasilurus asotus* (95) and are said to arise in vivo by reduction of zeaxanthin; similarly lutein is reduced to 7,8-dihydrolutein (94).

Carotenoids as Vitamin A Precursors

Nutritionists first became aware of the importance of carotenoids as the ultimate source of vitamin A when Moore (101) showed conclusively that β-carotene is a precursor of vitamin A (retinol) in mammals. The essential structural requirement in the carotenoid molecule is one unsubstituted β-ring attached to an intact conjugated polyene structure from C-7 to C-15 (see Formula I); thus β-carotene

(II), with two essential structural units, is the most potent pro–vitamin A. The main site of conversion is the intestinal mucosa and two enzymes are involved: the first is β-carotene-15,15'-oxygenase, which splits the molecule at the central (C-15,15') double bond to yield vitamin A aldehyde, retinal; the second is retinal reductase, an NADPH-dependent enzyme that converts retinal into retinol (see 39).

It has been known for some time that animals, particularly those of the lower orders, can oxidize dietary carotenoids to hydroxy and keto carotenoids. The latest work in this area was discussed in previous sections. However, the reductive pathways recently discovered (see previous section) open up the possibility that xanthophylls can be precursors of vitamin A by virtue of their conversion into β-carotene.

Claims in the older literature (see 39) that astaxanthin is a precursor of vitamin A, particularly in crustacea, were not seriously pursued for 25 years until feeding experiments strongly indicated that astaxanthin, canthaxanthin, and isozeaxanthin (β,β-carotene-4,4'-diol) were all converted into both vitamins A and A_2 (3,4-didehydroretinol—which generally accompanies retinol in freshwater fish) in guppies and platies (45). However, ^{3}H-crustaxanthin, a suggested intermediate from astaxanthin, was not active in carp (9). Definitive isotope experiments have now unequivocally demonstrated in rainbow trout the conversion of astaxanthin, canthaxanthin, and zeaxanthin into vitamins A and A_2 as they cross the intestinal wall (116). Similarly [^{14}C]-β-carotene, -zeaxanthin, -lutein, and -canthaxanthin are all converted into both vitamins in goldfish.[2]

In trout the conclusion must be that, because carotenoids with or without oxygen functions at C-3 and C-4 are active, the common intermediate in the formation of retinol is β-carotene. As already indicated (Figure 3), canthaxanthin, which is not a usual dietary constituent in trout, is metabolized in the skin to β-carotene, but this is not so with astaxanthin, so specific enzymes must exist in the intestinal mucosa to reduce astaxanthin to β-carotene (116). This investigation emphasizes that the basic structural requirement for vitamin A activity in carotenoids in trout remains the same as in mammals. The key difference between the two groups is that mammals do not possess a reductive pathway for xanthophyll metabolism. Furthermore the major site of conversion of β-carotene into vitamin A is the same in both groups—the intestinal mucosa—and presumably the key enzyme that splits the chain is a 15,15'-oxygenase in both cases. Eventually the determination of the degree of homology between the various oxygenases will be important for students of biochemical evolution.

In the experiments on trout the specific activity of the isolated retinol (A) was always greater than that of dehydroretinol (A_2) which demonstrates that vitamin

[2]B. H. Davies, B. W. Davies, Univ. Coll. Wales, Aberystwyth, personal communication.

A is the precursor of vitamin A_2 and confirms the pioneering feeding experiments in 1939 (102) and similar later investigations (51, 67). The same relationship was found in the A and A_2 in the liver and intestine of goldfish fed labelled carotenoid precursors, the ratio of specific activity of A/A_2 being 4:1 (however, the ratio in the eye is 1:1).[2] No explanation is yet forthcoming for this observation.

A series of feeding experiments on an Indian freshwater fish *(Heteropneustes fossilis)* indicates the lutein may be the specific precursor of vitamin A_2 (3, 4, 41, 42), with anhydrolutein (3',4'-didehydro-β-β-carotene-3-ol) as an intermediate that is cleaved at the center of the molecule to give 3,4-didehydroretinol (A_2) and 3-hydroxyretinol. β-Carotene apparently does not give rise to A_2 in this fish (4). In the goldfish experiments just cited, lutein behaved similarly to other carotenoids in apparently making vitamin A_2 from A_1. β-Cryptoxanthin is converted into retinol in freshwater Indian fish that normally accumulate retinol, and into 3,4-didehydroretinol and 3-hydroxyretinol in fish that normally accumulate dehydroretinol (42, 43).

Regulation of Vitamin A Synthesis

In the trout experiments discussed above (116), almost no vitamin A could be detected in the liver of very young fish (50 g) raised on a diet containing the vitamin, but the amount increased rapidly in fish weighing 200 g or more. Carotenoids, poorly absorbed in young fish, are rapidly taken up by vitamin A–depleted fish nearing sexual maturity and are converted into vitamin A. After administering labelled astaxanthin, as much as 17% of the radioactivity in the liver was in the vitamin A fraction. The purified vitamin furthermore had almost the same specific activity as the precursor, that is the molecular specific activity was approximately one half that of the administered astaxanthin. However, when fish saturated with vitamin A were used in a similar experiment, no significant conversion of astaxanthin into retinol was observed. Similar results were obtained with labelled canthaxanthin: 7.4% of the label was recovered in the vitamin A from fish already on a diet containing it, in contrast to 54% in fish that were vitamin A–depleted. On the other hand, accumulation of vitamin A itself (fed as retinyl acetate) was not significantly influenced by the vitamin A status of the fish. Clearly an important negative feedback control is operating on the activity of the 15,15'-oxygenase.

Coloration in Artificially Reared Fish

The great commercial expansion in aquaculture in recent years has stimulated considerable interest in the cosmetic aspect of carotenoid biochemistry. The aquaculturist must produce trout and salmon the appearance of which approximates to that of wild fish in order to meet customer expectation and acceptance. The considerable technical literature on this subject has been most thoroughly

reviewed (121); suffice it to say here that natural canthaxanthin either in shrimp meal (18–20) or added to diets in the form of pure synthetic material stabilized in beadlets (118) is currently the pigment of choice (122). The low level of the pigment in natural shrimp meal, which itself has a low digestibility because of its high chitin and calcium carbonate content, has led to industrial extraction of the pigment with hot soybean oil. The colored extract, containing some 155 mg pigment/100 g oil, is well utilized by trout when it is fed either alone or incorporated into a solid diet at a level of 6–9 mg/100 g feed (123). The stabilized beadlets fed at a level of 190 mg/kg feed produced after 31 weeks rainbow trout with a flesh color similar to that of red salmon (118).

METABOLISM IN BIRDS

The characteristic carotenoid pattern in birds is the accumulation of xanthophylls to the almost complete exclusion of carotenes in all tissues except the retina (14, 39). Most nutritional and metabolic studies have been carried out on poultry and a comprehensive review on carotenoids in poultry feeds has recently appeared (72). Here we concentrate on recent investigations in chickens, particularly of their eggs and retinas.

Absorption, Storage, and Metabolism—General

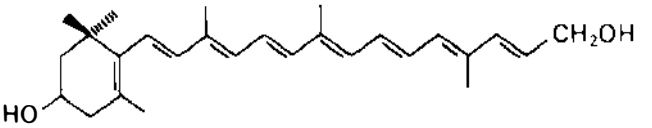
On a maize (corn)-based feed lutein and zeaxanthin are equally well absorbed by chickens and stored in the egg yolk, whereas only traces of β-carotene reach the yolk (114). Canthaxanthin and astaxanthin are also well absorbed from shrimp meal (72) but zeaxanthin is three times better absorbed than astaxanthin (114).

Experiments with [^{3}H](3*R*,3'*R*)-zeaxanthin and [^{3}H](3*S*,3'*S*)-astaxanthin showed that both appear in blood, muscle, liver, fat, skin, and feathers of young birds; the stores in the muscle and skin are transferred to the ovaries with the onset of sexual maturity. In the laying hen, 50% of the total body zeaxanthin is localized in the ovaries and 20% of the ingested pigment is eliminated in the egg yolk (116). (3*R*,3'*R*)-Zeaxanthin is metabolized in the yolk by oxidation, first at C-3 and then at C-3' accompanied by allylic rearrangement that yields (3*R*,6'*S*)β,ε-caroten-3'-one and 6*S*,6*S*'-ε,ε-carotene-3,3'-one (114). Thus we have the first clear demonstration of the formation of an ε-ring from a β-ring. Lutein, on the other hand, is converted into (3*R*,6'*R*)-3-hydroxy-β,ε-caroten-3-one and (6*S*,6*R*)-ε,ε-carotene-3,3'-dione (114); thus the β-ring is metabolized as in zeaxanthin whereas the preformed 3'-hydroxy-ε-ring is simply oxidized to the corresponding 3'-one. (3*R*,3'*R*)-Zeaxanthin is slightly better absorbed by chickens than is the (3*S*,3'*S*)-diastereoisomer, but the absorption of the meso (3*R*,3'*S*) form is only 40% of that of the chiral forms (114); this suggests the involvement of an active transport process in zeaxanthin absorption.

The metabolism of astaxanthin in chickens contrasts with that of zeaxanthin in a number of ways: (*a*) as indicated above it is considerably less well absorbed; (*b*) it is not esterified in the body whereas most of the zeaxanthin is esterified and stored in the liver; and (*c*) it is not oxidatively metabolized but reduced to idoxanthin and crustaxanthin, which are rather quickly eliminated from the liver (114).

Metabolism in Retina and Embryo

The colored oil droplets of the cones of birds' retinas yield a mixture of conventional carotenoids, including lutein, zeaxanthin, and astaxanthin (39, 97, 98). In addition there are also unequivocally present in hen and turkey retinas a carotene, 6*S*,6*S*'-ε,ε-carotene, (22) galloxanthin, (3*R*-10'-apo-β-carotene-3,10-diol (XII), and ε-galloxanthin (3*R*,
diol) (22[3]).



XII

Recent analysis by the most sophisticated methods of turkey retina extracts yielded a complex mixture of carotenoids, with many components exhibiting unexpected chirality.[4] Full assessment of this detailed investigation is not yet available but there is no doubt that the situation is less simple than previously thought. A compound present in the retinas was originally reported to be 14'-apo-β-caroten-14'-ol (22, 106), but was eventually revealed as an artefact formed by the reaction of retinal with acetone during the extraction procedure (23). The concentration of carotenoids in the retinal droplets is high (56) and particularly in the droplets from turtle retinas, which have cones similar to those in birds, where it can reach molar levels (71). The precursor of ε,ε-carotene, galloxanthin, and astaxanthin, which are not normal constituents of birds' diet, has not been fully settled but preliminary experiments with [^{14}C]zeaxanthin, a putative precursor, injected into the sub-blastodermic fluid of fertile chicken eggs showed that mobilization of radioactivity into the retinal lipid coincided with the appearance of the characteristic oil droplet carotenoids (25). Recently that activity has been associated with galloxanthin, ε-galloxanthin, and astaxanthin.[5]

[3]See also B. H. Davies, S. Pollard, R.-J. Lewis-Jones, A. Akers, A. Lachenmeir, H. Pfander, Univ. Coll. Wales, Aberystwyth, personal communication.

[4]B. H. Davies, B. W. Davies, A. Akers, K. Schiedt, Univ. Coll. Wales, Aberystwyth, personal communication.

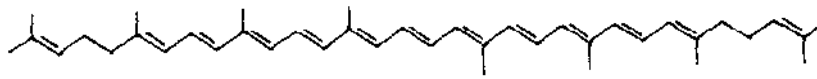
[5]B. H. Davies, Univ. Coll. Wales, Aberystwyth, personal communication.

Nutrition

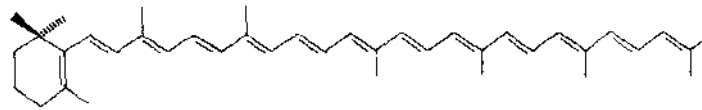
Most of the many papers on carotenoids in poultry feeds are concerned with providing broilers whose appearance is attractive to the public and providing eggs with an acceptable depth and shade (72). Important as these investigations are to the food industry they have little fundamental nutritional content. Perhaps of more importance have been the efforts to provide satisfactory diets to improve and maintain the plumage color of birds kept in captivity. Space is available only to mention the classical work of the late D. L. Fox on carotenoid metabolism in flamingoes (31), the spin-off from which is the world-wide improvement in plumage color of captive flamingoes. Canthaxanthin appears to be the carotenoid of choice to be added to the diet of many birds to obtain optimum coloration (72).

METABOLISM IN INSECTS

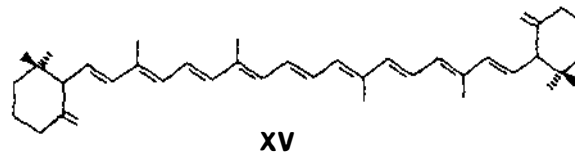
As is the case with fish and birds, many insects accumulate carotenoids that are absent from their food (39, 61). Most important insect carotenoids are astaxanthin (Locusta), various carotenoids uniquely substituted at C-2 with both *R* and *S* configurations (stick insects—Phasmida), and hydrocarbons such as lycopene (ψ,ψ-carotene) (XIII), torulene (XIV), and γ,γ-carotene (XV).



XIII



XIV



XV

Although no experiments with labelled substrates have been reported, the conversion of β-carotene into canthaxanthin and astaxanthin appears well established (46, 70, 104), thus confirming very early work in developing locust eggs, where β-carotene the major pigment in newly laid eggs gradually disappears and is replaced by astaxanthin (36). Lutein may be converted into astaxanthin via a route similar to that observed in fish. However, the chirality of

presumed intermediates has yet to be determined. In particular the following questions remain: (*a*) Is endogenous insect "lutein" really lutein or is it 3'-epilutein? (*b*) Has philosamiaxanthin the same absolute configuration as 3'-O-didehydrolutein? (*c*) Is the configuration of papilioerythrin from *Papilio xuthus* (61) the same as that of α-doradexanthin, as claimed (46)?

The new 2-hydroxy and 2-oxy carotenoids found in stick insects are derived from β-carotene as shown by isotope experiments in *Carausius* (61), but these experiments and kinetic studies during embryonic development of *Ectatosoma tiaratum* (61) did not settle unequivocally the sequence of reactions involved. In particular it is not known whether the first step is hydroxylation or the formation of a ketone. The question of how *R* and *S* isomers arise is also not solved. Different insects metabolize the 2-substituted carotenoids in various ways (62).

Formation of Hydrocarbons

A new aspect of carotenoid metabolism has arisen from detailed studies on the aphid *Microsiphum liriodendri* (Hemiptera) (1, 128) and the ladybird beetle (ladybug) *(Coccinella septempunctata)* (Coleoptera) (12, 13). *M. liriodendri* exists in two dimorphic forms, one green and one pink; the pink form is characterized by carotenoids prominent in red yeasts, namely torulene, γ-carotene (β,ψ-carotene), lycopene, and 3,4-dehydrolycopene. As these carotenoids are not present in the aphids' food and as aphids are obligatorily associated with endosymbiotic microorganisms, it was concluded that these microorganisms, presumably fungi, are the source of the carotenoids that contribute the pink color to the aphids. Far less pigment is present in the green strains of the aphid but the components are still characteristically fungal; presumably the greens contain different and fewer colored symbionts.

The presence of lycopene in the elytra (wing cases) of ladybirds was reported nearly 50 years ago (see 39). Although this was recently confirmed, lycopene was by no means the only or the major hydrocarbon present (12, 13). The complex mixture was again characteristic of the carotenoids of red yeasts and contained partly saturated polyenes, such as phytoene (7,8,11,12,7',8',11',12'-octahydrolycopene), which are well established precursors in the de novo synthesis of colored carotenoids. The conclusion from these observations is that the ladybird pigments arise from the biosynthetic activities of symbiotic red yeasts and not to any great extent from the aphids they eat. Thus the food chain in insects can be more complex than appears at first sight.

DE NOVO SYNTHESIS IN ANIMALS

Although overwhelming evidence exists for the view that animals cannot synthesize carotenoids de novo, there has been a report that such a total

biosynthesis occurs in bovine corpora lutea (2), where the β-carotene levels reach very high values (see 39). Recently in a reinvestigation of this claim an enzyme preparation from bovine corpora lutea was obtained that will incorporate 65% of added [2-^{14}C]mevalonic acid (the specific precursor of sterols and carotenoids) into the unsaponifiable fraction of the preparation. The β-carotene isolated from this fraction after purification by HPLC, however, contained no radioactivity. Furthermore, no biosynthetic intermediates, such as phytoene, were detected.[6] A possible explanation of earlier positive results is that crystallization yields a less radiochemically pure product than does HPLC. Corpora lutea can synthesize retinol from β-carotene (35).

FUNCTIONS OTHER THAN AS VITAMIN A PRECURSORS

Invertebrates

The important contribution of carotenoids to the color pattern in invertebrates is well known and was touched on earlier in this review. They occur either as the free pigments or as carotenoproteins, which open up a much wider spectral range of light absorption than that achieved by free pigments. For example, the green of lobster eggs and the deep purple of their carapace are due to the presence of astaxanthin (orange in free state) attached to different proteins (10, 39). A clear metabolic function for carotenoids per se remains elusive—if one exists at all. It has been reported that only mussels with high concentrations of carotenoids in their nervous tissue can withstand extended period of anoxia. The absorbance at 450 nm in the nerves is increased in anoxia, twofold in 10–15 h, and decreased when the animals are allowed access to oxygen. The decrease in absorbance was attributed to the formation of in-chain epoxides, which would shift the absorption maximum to much lower wavelengths. The epoxides were considered to represent an oxygen reserve that could be utilized for ATP production in the anoxic condition, with the concomitant regeneration of the fully conjugated pigments (58, 129). This claim was reinvestigated using *Mytilus edulis* and sensitive analytical procedures, but no significant changes were noted in carotenoid pattern in mussels subjected to 116 hours of anoxia. Furthermore there was no indication of the presence of in-chain epoxides (107).

Vertebrates

FISH The important work discussed previously clearly establishes for the first time the pro-vitamin A activity of xanthophylls but it must be emphasized that in fish carotenoids do have a function per se. Many fish owe their bright yellow,

[6]B. H. Davies, A. Akers, Univ. Coll. Wales, Aberystwyth, personal communication.

orange, and red color patterns to the carotenoids concentrated in the integumentary chromatophores (39). The characteristic cryptic coloration for protection and the sexual dichroism that attracts females at spawning time are carotenoid based. In the Salmonidae, carotenoids are mobilized from the flesh and possibly the liver into the eggs at spawning time. Their function in eggs is primarily to provide the developing embryos with a source of pigments with which to produce the characteristic color pattern in the skin. There have been claims that high levels of carotenoids in salmonid eggs improve their viability; however, a thorough assessment of published data (20a) indicates no simple relationship between carotenoid content and viability although it appears that with eggs containing about 1–3 μg/g 80% hatchability can be expected. Below these levels the expectation drops below 50%. The evidence is not, however, conclusive (20a). Any other suggested biochemical functions for carotenoids per se (39) are all tenuously based and require critical assessment.

MAMMALS There is no well-established basic biological function for carotenoids per se in mammals, but they have become of medical value as drugs to treat diseases involving photosensitization and they may become increasingly important anticancer agents. Both aspects are discussed below but, in addition, effects of β-carotene on immunological responses have been reported; it is said to increase T-cell response generally (69) and particularly that to concanavalin A (6).

Protection Against Photosenitization

The development of carotenoids as drugs in treatment of photosensitization is an excellent example of the imaginative application of the results of basic research to treat a human disorder (77–79). It is well established that carotenoids can protect against photosensitization in photosynthetic organisms (see 38) and in nonphotosynthetic bacteria (74). Laboratory mice photosensitized by administration of hematoporphyrin were protected by injection of very large doses of β-carotene (3 mg per mouse) 18–24 h before administration of the hematoporphyrin (73). Because of the known lack of toxicity of β-carotene, large doses were tested on patients with erythropoietic protoporphyria and it was revealed to be an effective therapeutic measure in most patients; at least 180 mg/day is necessary for a positive response (79). The US Food and Drug Administration have approved the use of β-carotene in this treatment. It should be emphasized that carotenoids do not cure the disease. Full clinical information can be found in the work of Mathews-Roth (75–78).

Decrease in the number of lesions in Günther's disease (congenital porphyria) has been reported after carotenoid medication; similarly, patients with polymorphous light eruption also responded to carotenoid therapy (see 79). Carotenoids have, however, so far been ineffective in treating actinic reticu-

loid, porphyria cutanea tarda, solar urticaria, and hydroa aestivale (see 78–80). They do ameliorate skin lesions associated with feline solar dermatitis (55).

The possible toxicity of the high doses of carotenoids used has often been considered but no significant side effects, other than innocuous carotenodermia, have been reported (75, 77, 78). Large intakes of carrots are said to cause leucopenia but this is almost certainly due to constituents of the carrots other than β-carotene (see 78). Although carotenodermia has been known since 1917, the actual isolation of the pigment from the skin was only recently reported (68, 126); previously it had only been obtained from the horny layers scraped from the soles and knees of a patient (44). The carotenodermia is usually not a significant problem, the only likely effect is slight cosmetic worry. The excessive conversion of β-carotene into vitamin A could theoretically lead to the accumulation of toxic amounts of vitamin A in the liver, but early reports indicated no hypervitaminosis A in subjects on high carotene diets (77). A recent examination of biopsy and autopsy material from patients on a high β-carotene regime furthermore revealed no β-carotene in the liver and only traces in the brain. Hepatic vitamin A levels were also normal. Furthermore, no patients on carotene therapy showed enhanced blood levels of vitamin A (85). Obviously an effective feed-back mechanism is in operation. At the molecular biology level there are reports that β-carotene has no ill effects on the genome (52, 57).

Mechanism of Photoprotection

In plants and protista, carotenoids function as scavengers for the highly active singlet oxygen ($^1O_2$) produced mainly during photosynthesis. In the reaction, triplet (ground-state) oxygen is generated together with triplet carotene, which dissipates its energy to its surroundings and returns to its ground state. It is then ready to continue the reaction in cyclic fashion (38, 64):

$$^1O_2 + \text{car} \longrightarrow {}^3O_2 + {}^3\text{car}$$

$$^3\text{car} \longrightarrow \text{car} + h\nu.$$

Singlet oxygen was detected when human epidermis samples were illuminated (hematoporphyrin, a photosensitizer, had been added to the samples) (28). Similar results were obtained in vivo with hairless mice made porphyric with collidine (83). In this experiment production of $^1O_2$ in mice treated with either β-carotene or canthaxanthin was reduced compared with untreated controls. Inhibition of succinate oxidation in preparations from photosensitized mice was also reduced in carotene-treated animals (83). Although $^1O_2$ is strongly implicated in this type of photosensitization, the $^1O_2$ probe (1,3-diphenylisobenzofuran) used is not completely specific for this species, so the possibility of other mechanisms such as the formation of free radicals, which

are know to be intercepted by carotenoids (64, 65), cannot be excluded. Reactive oxygen species are, however, involved in lipid peroxidation observed in rat epidermal microsomes; this peroxidation is inhibited in the presence of β-carotene (26). β-Carotene also protects against in vivo lipid peroxidation (66).

CAROTENOIDS AS ANTITUMOR AGENTS

Many reports have appeared over the past 20–30 years on the antitumor activity of vitamin A (80, 124) and it was not unexpected that β-carotene would be tested for similar activity (see 84). A summary of the findings of the major investigations is given in Table 1. A general conclusion is that carotenoids slow down growth of tumors induced by UV-A, UV-B, BP, BP/UV-A, and 8-methoxypsoralen irrespective of their inherent ability or otherwise to act as precursors of vitamin A. However, the lack of effect of canthaxanthin compared with β-carotene on DMB-induced tumors suggests that in this case conversion into vitamin A is essential for activity (82). With UV-B-induced tumors, pigment administration after the appearance of the first tumors slows down the manifestation of later tumors (82).

Quantitative results show some variance; β-carotene when added to the diet in an alcoholic solution to give a concentration of 90 mg/kg diet decreased the incidence of DMBA tumors in rats (119) whereas the same amount in the form of stabilized beadlets was ineffective (82). When the beadlet dose was increased to 700 mg/kg however, protection was observed (82). These very high levels can only be obtained with commercial beadlet preparations. There is one report that topically applied β-carotene increased DMBA/croton oil or resin-induced tumors in hairless mice (120). This unexpected result might be due to the action of some product of β-carotene oxidation because on application to the skin the pigment rapidly bleaches (86).

Table 1 Activity of carotenoid in delaying development of skin tumors in mice

Carcinogen	Activity[a]			Reference
	β-carotene	canthaxanthin	phytoene	
DMBA[b]	+			27
UV-B	+	+	+	76, 80
UV-B	+			29
DMBA/croton oil	+	--	−	82
DMBA/UV-B	+	+	--	80
DBP[c]	+	+		113
BP/UV-A	+	+		113
S-Methoxypsoralen	+	+		113
UV-A	+	+		113

[a] +, positive effect: −, no effect: no symbol, not tested.
[b] 9,10-Dimethyl-1,2-benzanthracene.
[c] Benzpyrene

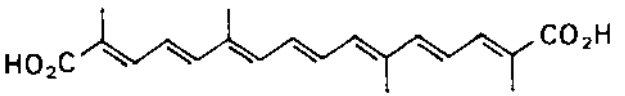
Because carotenoids quench singlet oxygen or free radical formation, they are clearly important in treating photosensitization. There are also indications that the pigments are functioning in a similar way in protecting against tumors induced by agents other than UV-B alone. Phytoene is much less effective than β-carotene or canthaxanthin in protecting against tumors induced by DMBA/croton oil or DMBA/UV-B, which correlates with the fact that β-carotene and canthaxanthin are much more effective quenchers of $^1O_2$ than is phytoene (87).

The water-soluble colored carotenoid crocetin (XVI) in the form of the unsaponifiable extract of saffron is reported to delay the onset of skin tumors induced by DMBA (croton oil) in Swiss Webster mice (34). However, pure crocetin was much less effective than β-carotene in DMBA (croton oil)-induced tumors in hairless mice and also had no discernible effect on UV-B-induced tumors (81).

A long-term investigation on the anticancer effect of β-carotene in humans is under way (84).

CONCLUSIONS

Two of the major recent developments in animal carotenoid metabolism are (*a*) the discovery of many chiral epimers of well-known plant carotenoids in fish, birds, and insects, and (*b*) the partial elucidation of the pathways by which the epimers are formed from dietary carotenoids. Xanthophylls, which are not vitamin A precursors in mammals, were found to be active in lower animals by reason of these animals' ability to metabolize them reductively to β-carotene, which is then converted into vitamin A. This discovery reveals the missing link in the vitamin A food chain of lower animals and is nutritionally the most important recent development in carotenoid biochemistry. The enzymes that carry out the conversion of β-carotene into Vitamin A are apparently very similar throughout the animal kingdom. The most significant development in mammals has little to do with basic carotenoid biochemistry but with the use of massive doses of β-carotene in ameliorating the distressing photosensitization associated with a number of diseases—a development that stemmed from the extrapolation of observations on the basic function of carotenoids in plants and bacteria. The antitumor action of carotene therapy appears to be well established in skin tumors in mice. Investigations in human beings are under way.



XVI

The future certainly holds more surprises as further details of chiral studies on the lower animals are brought to light. Furthermore, the techniques now available have the potential to allow investigators to study in detail the degradation of carotenoids in plants and stored foods; in particular, the exact fate of ingested carotenoids in mammals can now be investigated.

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