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METABOLISM AND NUTRITIONAL SIGNIFICANCE OF CAROTENOIDS

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INTRODUCTION

The carotenoids represent the most widespread group of naturally occurring pigments in nature. They are present without exception in photosynthetic tissue, occur with no definite pattern in non-photosynthetic tissues such as roots, flower petals, seeds, fruits, vegetables, and are found sporadically in the *Protista,* including the fungi—yeast, molds, mushrooms, and bacteria. Likewise, the yellow, orange and red pigments in the skin, flesh, shell, or exoskeleton of animal species such as salmon, lobster, prawn, carp, and flamingo are due to these pigments. Although animals are thought incapable of a de novo synthesis, they are able to make some alterations of the basic chemical structure. The most significant aspects of carotenoids from a purely anthropocentric consideration is the color they impart to our food and environment and the fact that they represent the major source of vitamin A in the diet.

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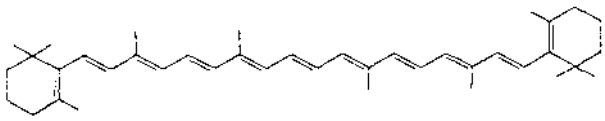
Vitamin A deficiency is a worldwide nutritional problem often associated with protein-energy malnutrition, parasitic infestation, and infectious and/or diarrheal diseases (130, 131, 135). Because of the difficulty in determining vitamin A status, the deficiency is generally not diagnosed until the symptoms become acute. One aspect of the problem, nutritional blindness, has been well studied in Indonesia (76). A conservative estimate from that study predicts that 60,000 Indonesian children develop corneal xerophthalmia each year. The mortality rate among these children was estimated to be very high. Indonesia is the most intensively studied example of a vitamin A-deficient area, but the deficiency state is not limited to that country. Extrapolations on a worldwide basis could well reach the hundreds of thousands of cases of vitamin A deficiency blindness each year.

The carotenoids are very conspicuous in nature and thus various functions have been ascribed to this class of compounds (89, 90). In animals, the carotenoids are often associated with reproductive organs, but evidence of a universal role is lacking. Georgiev (52) showed that the fertility of pigmented trout eggs was greater. Lotthamer and co-workers have published a series of papers (4, 106–108, 119, 150) that show vitamin A-unrelated importance of β-carotene in the fertility of cattle. Carotene deficiency in cattle is likely to result in a higher incidence of still estrus, lower conception rate, higher incidence of embryonal death, and poor composition of colostrum. β-Carotene was reported to have a specific effect on spermatogenesis (172); whether it reflects the deficit of carotene or of associated nutrients is not proved.

As stated above, the carotenoids are the natural precursors of vitamin A. Thus, it is germane in this review to consider the pigments that have provitamin A activity, their biosynthesis, analysis, and stability in food, and their absorption and metabolism.

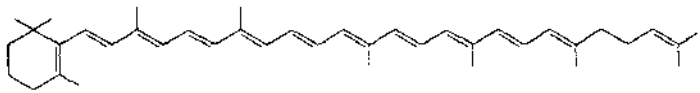
COMMON PIGMENTS

The structures of some important carotenoids are shown in Figure 1. β-Carotene (I) is a very common pigment and possesses full vitamin A activity. Lycopene (II) is a biosynthetic precursor of β-carotene, but it has no vitamin A activity. γ-Carotene (III) (one ring) and β-cryptoxanthin (IV) (one OH group) have diminished vitamin A activity. Lutein (V) is a common pigment in fruits, vegetables, eggs, and green leaves and astaxanthin (VI) is a very common animal pigment. Neither has vitamin A activity itself, but when ingested, each reduces the need for Vitamin A in some fish. The most recent listing of natural carotenoids has been compiled by Straub (163) and the distribution of carotenoids has been reviewed by Goodwin (62).

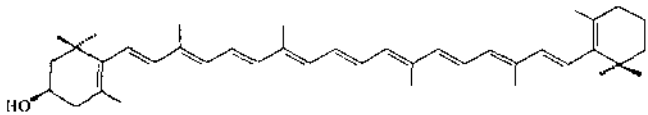


β-Carotene I

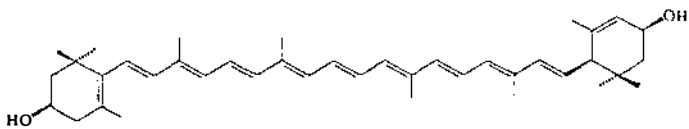
Lycopene II



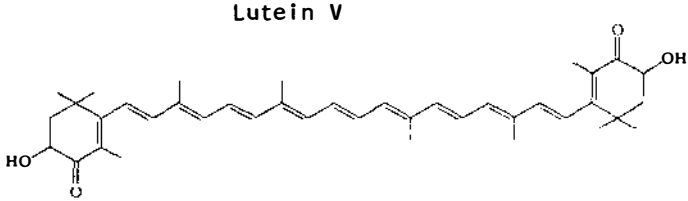
γ-Carotene III



β-Cryptoxanthin IV



Lutein V



Astaxanthin VI

Figure 1 Structures of important carotenoids.

BIOSYNTHESIS

Recent reviews detailing the synthesis and biosynthesis of carotenoids have been published by several authors (22, 23, 35–37, 58, 102, 123, 155).

The carotenoids are chemically related to the more general class of compounds known collectively as terpenes and terpenoids. The basic feature of these compounds is the repeating isoprenoid (methyl-butadiene) units. This isoprenoid-like carbon skeleton is also found in such diverse compounds as steroids, bile acids, squalene, sex hormones, ubiquinones, natural rubber, essential oils, phytol (in chlorophyll), and the side chains of vitamins E and K. Figure 2 details the basic biosynthetic route by which two carbon compounds are converted to complex carotenoid structures. The ubiquitous compound, acetyl CoA, is the basic carbon source. Mevalonic acid is the pivotal compound in the synthesis. In most systems, once this compound is formed, the pathway cannot be reversed as it is committed to terpene synthesis. Isopentenyl pyrophosphate is also a key compound, because it serves as the building block for successive C-5 units. Animals are capable of forming the C-15 structure and dimerizing it to form squalene, and hence the steroids. On the other hand, to our knowledge, only higher plants and the *Protista* can further elongate the C-15 structure to form the C-20 and the C-40 phytoene. Although phytoene is colorless, successive dehydrogenations and cyclizations result in the colored and ringed structure, β-carotene. The introduction of oxygen generally occurs on the more unsaturated carotenes and, for most animals, results in a loss of vitamin A activity.

Change in the pattern of carotenoid levels has been altered through genetic breeding [tomatoes (70, 71, 78, 138, 154); peaches (42); carrots (18); cf. Cerdá-Olmedo & Torres-Martinez (29)], bioregulators [tomatoes (31, 79)], by environmental factors [mangoes (93, 167); peppers (87)], and during maturity and storage [tomatoes (99, 104, 169, 170); peaches (66); carrots (86); soybeans (12)].

Splitting the ingested carotene molecule has been the most studied transformation in animals, but it is not the only change made by them. Although it can be assumed that the carotenoids isolated from the *Protista* are the result of complete biosynthesis, no such statement can be made in regard to animals. The observed pigments may have been absorbed directly from the diet, and in some cases, metabolically changed. This distinction can only be made through feeding studies that are not often done. The lower animal forms can make many changes, whereas the higher forms may be limited to esterification of the ingested carotenoid (cf 61, 155).

The conversion of lutein to astaxanthin in the goldfish represents several unique metabolic steps. Carbon-14 data showed that lutein carbon was transferred to astaxanthin carbon in the goldfish; this led to the proposal

of a biosynthetic pathway (75, 146, 147), which Andrewes et al (7) questioned since lutein and astaxanthin are of different chiral forms and thus would require a unique chiral change. Eugster (46) showed that the lutein in the goldfish was altered to 3'-epilutein with the same absolute configuration as astaxanthin. The transformation of lutein to astaxanthin thus required two rare metabolic steps: conversion of an α-ring to a β-ring and change in chiral form. Recently, 3'-epilutein was isolated from the flowers of *Caltha palustris* but was not found in egg yolk, where lutein is the major pigment (25).

DEGRADATION

Chichester et al (32) and Simpson et al (156) reviewed the metabolism of carotenoids, in addition to other pigments, in senescent tissues. Pure solutions of carotenoids are destroyed or altered by acids and, in some cases, by alkalies. Acids, particularly in the presence of light, cause the formation of *cis-trans* isomers from the usual all-*trans* structure. There are some naturally occurring *cis* isomers, such as the central *cis* phytoene (60) and the poly *cis* pigments in tomatoes (143). Generally, the formation of *cis* structures are degradative steps and result in the lowering of vitamin A activity. It has been calculated that lycopene, with 11 conjugated double bonds, could, in theory, exist in 1056 different forms. Fortunately, only 72 sterically unhindered isomers exist in lycopene and less exist in β-carotene.

The observation by Bickoff et al (15) of 40 chromatographic bands from dehydrated lucern meal in contrast to the 12 carotenoids isolated from fresh lucern would be consistent with what one would predict from model systems. The stability of the carotenoids was found to be greater in the alfalfa leaf protein than in the dehydrated alfalfa meals (105).

Data are not extensive on the random formation of *cis* isomers in senescent or stored tissues during processing, cooking, or storage of carotene-containing fruits or vegetables. However, under conditions that involve elevated temperatures, low pH, and light, one would expect that *cis* isomers would be produced, thus altering their vitamin A activity (13, 49, 151, 165).

Although little work has been reported on the biological significance of *cis* isomers, the introduction of a *cis* bond can greatly reduce the potency of β-carotene as a vitamin A precursor (6). Some *cis* isomer analogues of vitamin A have been reported to have vitamin A activity, although it is low in comparison to the parent compounds [e.g. 13-*cis* retinoic acid (38)].

It has been postulated that the initial step in the breakdown of carotenoids occurs through the formation of epoxides (cf 156), although a number of 5,6-epoxides exist naturally and are part of the violaxanthin cycle. Some epoxides of β-carotene and zeaxanthin are given in Figure 3.

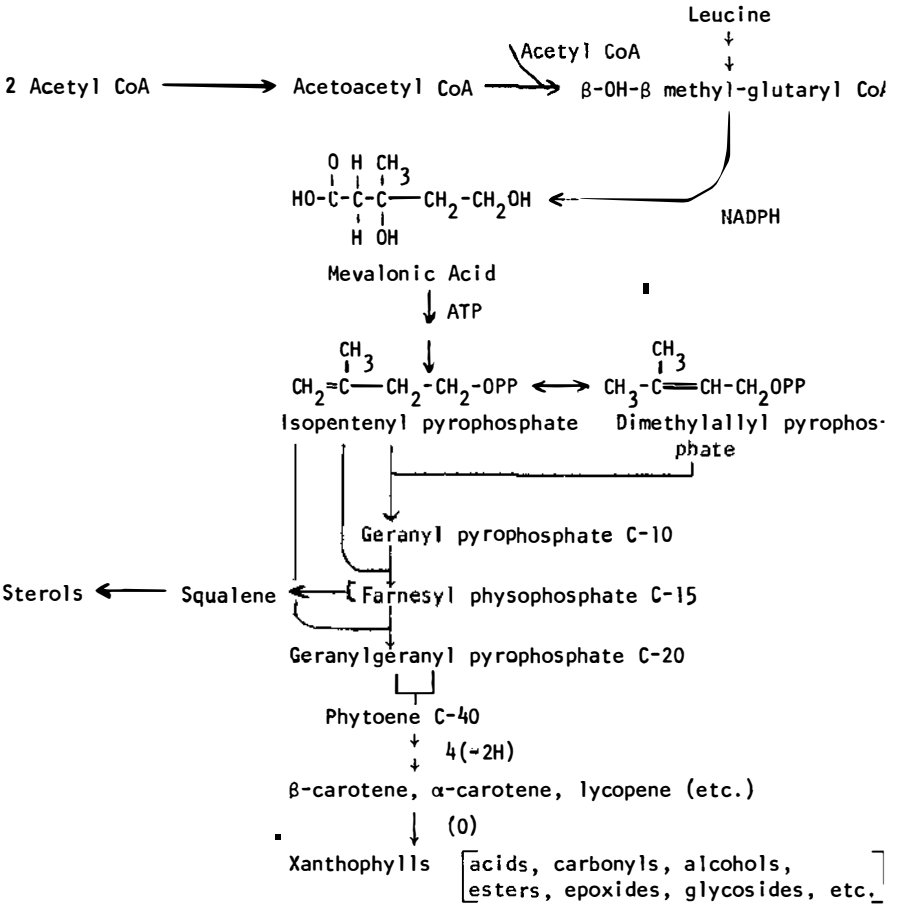


Figure 2 Biosynthetic formation of carotenoids.

Britton and co-workers (14, 24) found many acyclic and cyclic epoxides in tomatoes. They suggested that the epoxides may be the result of injury, but that they may also be early products in oxidative degradation of senescing tissue. The latter suggestion was based on their unpublished observations that epoxides may occur in greater quantities in overripe tomatoes, reducing vitamin A activity.

El-Tinay & Chichester (44) studied the oxidation of β-carotene in toluene at various temperatures. The rate of β-carotene disappearance indicated a zero order reaction in the presence of excess oxygen. The initial site of attack was the 5,6-double bond, leading to a number of mono- and di-epoxides. They found that a free radical inhibitor stopped the loss of β-carotene.

Carnevale et al (27) showed that fluorescent light catalyzed the autoxidation of β-carotene in fatty acid solvents (saturated > unsaturated) and was inhibited by ethoxyquin. Sulfite, a chemical widely used in foods, produces free radicals on its aerobic conversion to sulfate. The process was shown to result in the free radical destruction of β-carotene (134).

Cyronak et al (34) observed that 13,14- and 11,12-monoepoxycanthaxanthins are readily split to apocarotenals on magnesia, a commonly used material for chromatographic separations. The epoxide function is directed toward the chain when the 4 position is substituted. Although the apo derivatives of canthaxanthin would not be expected to be vitamin A active in man, the epoxide to apo-carotenal route has been postulated as a means of synthesis of retinal (cf 54, 55) and a number of apo-carotenals have been shown to possess vitamin A activity (cf 13). Yokoyama & White (173) suggested that β-apo-10'-carotenal and β-apo-8'-carotenal are natural degradation products of β-carotene in citrus fruits.

The carotenoids have long been known to be substrates for lipoxygenase and other enzymes. The major work on enzymatically catalyzed oxidation of the carotenes has been concerned primarily with the lipoxygenase system from soya (171). In these systems the carotenes act as antioxidants or secondary substrates to the fat oxidizing systems (156). Lipoxygenase has been separated into two isoenzymes that differ in their ability to oxidize carotenoids (65). Discoloration of red fish occurred at refrigeration temperatures in the dark and homogenates from the skin, muscle, and liver were unable to degrade astaxanthin, tunaxanthin, and β-carotene to colorless compounds. Tsukuda (168) isolated and partially purified a heat-labile lipoxygenase-like enzyme from the skin of red fish (*Sebastes thompsoni* and *Chelidonichtys kumi*) that decolorized carotenoids in the presence of linoleic and linolenic acids. Vitamin A could be expected to undergo the same degradative reactions. The initial products of these enzymatic processes could generally be characterized as apo-carotenals.

The stability of vitamin A palmitate in flour after accelerated storage (103) and on extrusion cooking (98) was high; extrusion cooking resulted (98) in a relatively higher destruction of β-carotene.

Kanner et al (82) stressed the effect of the water content of dried paprika on the solubilization of the ascorbic acid-Cu^{2+} antioxidant system. The effects of available water and antioxidants were reported in model systems (8) and in dehydrated carrots (9, 121).

Food processing generally results in a slight loss of carotene [papaya puree (30); prunes (19); red peppers (174); loquat fruits (88); green leafy vegetables (85); apricots (20)]. Very few controlled experiments on the losses of carotenoids in cooking have been reported. When Sood & Bhat (160) cooked various green leafy vegetables in the "traditional method" (in India the vegetable is boiled in an open vessel until the water is evaporated),

a greater loss of carotene resulted than with other methods of cooking. Another study reporting on "traditional" methods of cooking in Africa and Southeast Asia reported slight losses of β-carotene. These results, as well as others (142), led to conclusions that carotenoid cooking losses are lowest with minimal time, water, and temperature. However, what data exists reflects wide variability.

PROVITAMIN A COMPOUNDS

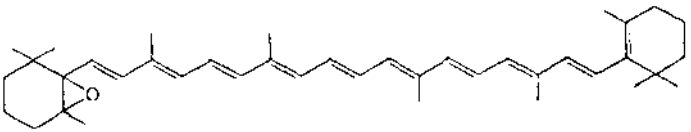
The number of carotenoids that have now been described exceeds 400. Of this number, most are xanthophylls—that is, have an oxygen group either on the ring or in the chain. These oxygen-containing groups include hydroxyl, carbonyl, carboxylic acid, ester, epoxide, glycoside, and ether.

Structurally, different forms include acrylic compounds, simple rings, and rearranged rings (e.g. cyclopentane, aryl), the absence of methyl groups (nor), split rings (seco), and shortened chains (apo). Other changes in the basic structure include retro-bond formation, *cis-trans* isomerization, inchain acetylenes and allenes, extension of the rings to form C-45 and C-50 higher carotenoids, and the formation of stereochemical isomers (cf 163).

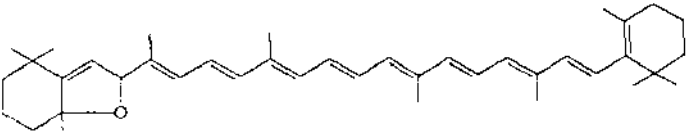
In general, the retinyl structure is responsible for the vitamin A activity and changes invariably result in lower activity. The provitamin A precursor activity of a carotenoid or apo derivative has been assessed by various means: (*a*) the compound could be fed to an animal under prescribed bioassay and conditions; (*b*) in vitro methods have been developed using the cleavage enzyme, 15,15'-dioxygenase, and noting the formation of retinal; (*c*) activity by analogy—compounds that possess an active portion are presumed to be active; and (*d*) adhesion assay of spontaneously transformed mouse fibroblasts in culture (cf 38).

Several recent reviews have appeared that list provitamin A precursors (13, 129, 157) and the activity of retinoids (161). Bauernfeind (13) and Singh & Cama (157) reference the original literature. Olson & Lakshmanan (129) list 19 and Bauernfeind (13) lists 32 provitamin A carotenoids and apo-carotenals. On the basis of structure alone (see above), the number of provitamin A precursors would be between 50 and 60 carotenoid and apo-carotenoid compounds. Table 1 gives a partial list of carotenoids and apo-carotenals that illustrate the typical types of provitamin A precursors.

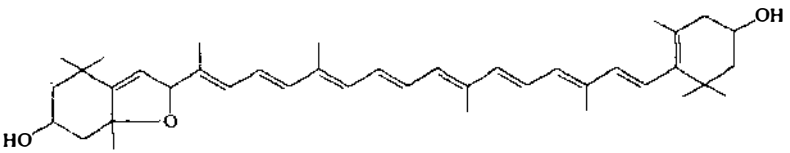
β-Carotene (I) is a symmetrical molecule that, in theory, yields two molecules of retinal. It contains two β-ionone rings extended with a 22-carbon polyene fragment. This very common pigment occurs in fairly high concentration in a number of yellow and green vegetables and fruits. α-Carotene (XI), in theory, is half as active as β-carotene due to the 5',6'-double bond shift to the 4',5'-position.



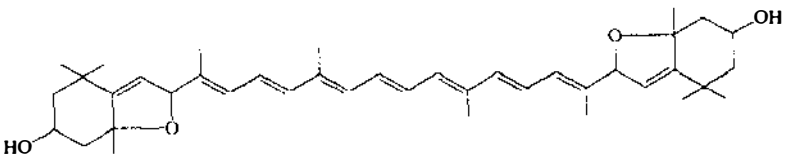
β-Carotene-5,6-epoxide VII



Mutatochrome VIII



Aurochrome IX



Mutatoxanthin X

Figure 3 Epoxides of β-carotene and zeaxanthin

The metabolism of α-carotene results in the formation and subsequent storage in the liver of vitamin A and α-vitamin A (118). The α-vitamin A appears to have a vitamin A sparing effect for some functions rather than low activity (159). α-Vitamin A does not combine with RBP (122). It is capable of inducing hypervitaminosis A as it is not delivered to the tissues (136). γ-Carotene (XII) has only one ring and is about one half as active as β-carotene. β-Zeacarotene (XIII) has a point of saturation (7',8') in the acyclic portion and is less active than γ-carotene.

Additional unsaturation in the ring in the 3,4- or 3',4'-position (XIV) lowers the activity but does not make the compound inactive. The 5,6- (VII) or 5,8- (XV) mono- or diepoxides lower activity but, again, do not eliminate provitamin A activity.

The 5,6-epoxyretinoic acid isomer of retinoic acid has been tested for growth-promoting activity. It has been reported to be 157 and 80% as active as retinyl acetate in growth promotion of rats (109). A more recent finding (0.5%) questions the earlier reports (177). The opening of one ring (seco) markedly decreases all activity but does not eliminate it. Several apocarotenoid (XVII,XVIII) structures are active, including 8, 10, and 12. The 14 appears not to be active (cf 157).

With the exception of some poly *cis* compounds, the various geometric *cis-trans* isomers are less active than the all-*trans* (41). The addition of dimethyl groups to the 2,2'-position or replacement of the 1- or 1,1'-methyl with ethyl groups reduces, but does not destroy, activity (164).

Anhydrolutein (XIX) has been shown to be converted to vitamin A_2 in the rodent and in fish (67, 149), but not in the human. The information to date suggests that the metabolism of carotenoids involves oxidative changes, however. Gross & Budowski (68) report an increase of vitamin A_1 and A_2 on feeding astaxanthin, isozeaxanthin, canthaxanthin, or β-carotene to a fish. Barua et al (11) reported the liver storage of 3'-dehydroretinol on feeding lutein to the freshwater fish *Saccobranchus fossilis.*

Boonjawat & Olson (21) prepared [H^3]-crustaxanthin and administered it to both the carp and the rat. No direct conversion of crustaxanthin to retinol was demonstrated, although Gross & Budowski (68) suggested that the tetrahydroxy compound was an intermediate between astaxanthin and retinol.

Our knowledge is fairly extensive for mammals on the conversion to vitamin A of β-carotene, other β-ionone ring compounds, and oxygen-substituted derivatives. Fish, in particular, seem to perform "strange" transformations, which have not been well studied.

ABSORPTION AND UTILIZATION

The carotenoids of fruit, vegetables, and animal products are usually fat soluble and are associated with the lipid fractions. They may also be esterified or complexed with protein. The protein-carotenoid complex may be broken by cooking (e.g. lobster) or by the action of proteolytic enzymes. The carotenoids may be part of a larger structure, such as chloroplast in green tissue, dissolved in fat as in margarine, or nearly in a crystalline state as in lycopene in the tomato.

During the digestion process, the action of esterases, lipases, and proteases release the carotenoids, which are then solubilized by the bile salts. The carotenoids penetrate the plasma membrane. Within the mucosal cell β-carotene is acted upon by carotenoid dioxygenase(s), resulting in oxidative cleavage, which in theory would result in two molecules of retinal. The

Table 1 Representative types of carotenoids with provitamin A activity

Carotenoids	Activity[a] (%)
β-Carotene (I)	100
α-Carotene (XI)	50–54
γ-Carotene (XII)	42–50
β-Zeacarotene (XIII)	20–40
3,4-Dehydro-β-carotene (XIV)	75
β-Carotene-5′,6′-monoepoxide (VII)	21
β-Carotene-5′,8′-monoepoxide (XV)	50
β-Semicarotenone (XVI)	active
β-apo-8′-Carotenal (XVII)	72
β-apo-8′-Carotenoic acid (XVIII)	active
Anhydrolutein (XIX)	21

[a] From Bauernfeind (13).

retinal is reduced to retinol, esterified, and transported to the liver for storage (cf 129). On the other hand, an oxidative cleavage, starting at one end of the molecule, results in the formation of apo-carotenoids, which may also have vitamin A activity.

The absorption and/or conversion to retinal is enhanced by bile salts (43), lipids (77, 129), protein

mental temperature has a minor effect on the utilization of β-carotene by the rat when other factors for the conversion are optimal (158). The effect of temperature changes on the utilization or nonutilization of β-carotene by rainbow trout can be dramatic (137).

El-Gorab et al (43) studied the roles of bile salts and other detergents on the uptake of retinol and β-carotene by rat everted gut sacs. Although retinyl esterification was seen with all detergents, β-carotene cleavage and conversion to retinyl esters occurred only with the bile salts. β-Carotene uptake was nearly linear in relation to concentration, thus indicating a diffusion-limited mechanism. Retinol absorption was suggested by these authors and others as an active, energy-requiring process 7–30 times faster than β-carotene uptake.

Sweeney & Marsh (165) fed rats various *cis-trans* isomers of β-carotene and α-carotene. The results showed a consistent isomeric composition of the carotene (high in the all-*trans* isomer) recovered from the feces, suggesting a conversion to the all-*trans* in the acid environment of the stomach.

Lakshmanan et al (92) partially purified the carotenoid cleavage enzyme from rabbit intestine and determined its specificity toward various carotenoids and apo-carotenoids. The apo-carotenoids gave a decreasing substrate specificity with increasing length (β-apo-10′-carotenal > 16-oxotorulene).

Interestingly, the mono- and diepoxide were not cleaved, although they have been shown to be effective precursors of vitamin A.

Sharma et al (153) further studied the cleavage enzymes on β-carotene and apo-carotenal substrates. Soluble and particulate fractions were isolated from rat and chicken livers. Retinal was only oxidized by the soluble fraction, whereas the 8'- and 12'-apo-β-carotenals were oxidized mainly by the particulate fraction of the homogenate. When β-carotene was fed to either rats or chickens, 8'-, 10'-, and 12'-apo-β-carotenals and retinal were isolated. When the 8'-apo-β-carotenal was fed, the 8'- as well as the lower 10'- and 12'-apo-β-carotenoic acids were isolated from the gut. Sharma et al (153) proposed a scheme that is consistent with these data (Figure 4). β-Carotene is cleaved mainly to retinal, which is either oxidized to retinoic acid or reduced to retinol. The latter compound is esterified and contributes to the liver stores. Other products of β-carotene cleavage include the 8'-, 10'-, and 12'-apo-β-carotenals. The 8'-,10'-, and 12'-apo-β-carotenoic acids were isolated in significant amounts from rat intestine when 8'-apo-β-carotenal was fed. These authors suggest that the acids are degraded to retinoic acid, even though retinoic acid was not isolated. It is still not altogether clear whether the intestine contains an unspecific splitting enzyme that forms retinal and a series of apo-compounds or a more specific cleavage enzyme that also yields retinal and a series of apo-compounds. Figure 4 shows the complexity of the interconversions. The method of analysis adds to the problem of interpretation. Sweeney & Marsh (165) used liver storage rather than growth of test animals as a means of assessing the conversion of stereoisomers to retinol. They found a higher level than would have been predicted by other studies. It is interesting to speculate whether or not the *cis* isomers were split to yield retinal rather than apo-carotenals, thus increasing liver storage. More apo-acids would lead to retinoic acid with no storage, yet would support growth.

Some carotenoids are directly absorbed and pass into the blood where their composition reflects the diet (16, 53, 81, 96, 127). They may be deposited in the liver (48, 128) or elsewhere, such as the fat depots, adrenal, cortex, or atheromatous plaques (17), in various organs (114), in flesh (28, 97, 155), in milk (26, 126), and in eggs (2, 47, 50, 69, 84, 120, 125, 127). There is some question as to whether the liver may be able to split carotenoids to yield retinal (17).

The carotenoids may also be deposited in the skin, especially in the diabetic (72), on high carotenoid intake [lycopenemia (144); carotenemia (72, 112, 117)], or with treatment of erythropoietic protoporphyria (56, 114–116, 176).

Carotenoid metabolism was not impaired in the diabetic (141). In carotenemia (113), the low- and high-density lipoprotein plasma levels increased

in patients given 180 mg of β-carotene per day. No significant difference was observed in the cholesterol fraction. A few studies have attempted to compare the formation of plasma retinol in animals fed various fruits and vegetables with those fed β-carotene in oil (1, 132, 133, 140). In each case, the plant source was inferior to pure β-carotene supplementation. This difference may be due to biological unavailability of the natural carotene or to overestimation of the β-carotene level in the plant (cf 166).

Carotenoid and retinol levels in the blood of patients with ulcerative colitis were only slightly lower than those of the controls (152).

β-Carotene lowers the serum cholesterol level in the rat, whereas when the non-provitamin A carotenoids, canthaxanthin (5) and lycopene (45), were fed, the serum cholesterol increased.

Krinsky et al (91) separated carotenoids from human plasma into three fractions on alumina. These were identified as β-carotene, lycopene, and lutein. Fraction 1 is assumed to contain only β-carotene (16), but it should contain other hydrocarbons, probably in a proportion related to the dietary intake.

METHODS OF ANALYSIS IN FOODS AND PLASMA

There are numerous methods suggested for the separation of carotenoids from foodstuffs and plasma. These may or may not result in complete resolution of the carotenoid mixture into purified components that can be quantified by spectrophotometric analysis.

The classic separation method, used by many investigators for resolution and identification of the various carotenoids, relies upon chromatographic separation of the various carotenoids. This method requires extraction with a suitable solvent, saponification, transfer to a developing solvent, and chromotography under vacuum or pressure. The column is developed with a gradient of solvents, and the pigments are separated by elution or are cut from the column and extracted. Often rechromatography is required of some bands, followed by verifying chemical reactions. The quantity of each purified fraction is determined by spectroscopy. The method requires several hours, and in some cases artifacts (*cis-trans* isomers, epoxides, etc) are formed due to the long exposure to solvents, adsorbents, light, and oxygen. Although the method does give a separation of provitamin A precursors from inactive carotenoids, it is time consuming and requires skilled technical operators. Thus, the method is not suitable for analysis of the large number of food items for a table of food composition.

The much more simple Association of Official Analytical Chemists (AOAC) method (10) chromatographically separates the carotenes from their oxygenated compounds, but it does not separate individual carotenes,

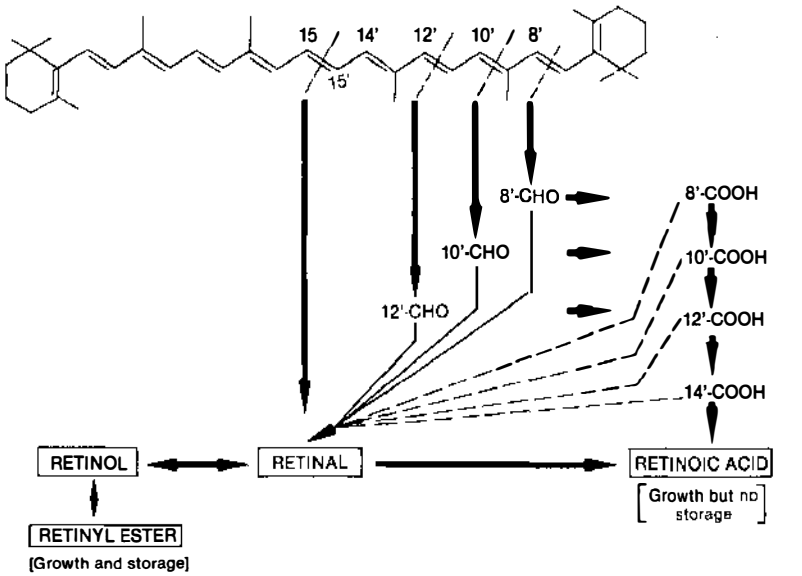


Figure 4 Proposed mechanism for the conversion of β-carotene to vitamin A. (From 153.)

their *cis* isomers, or carotenoid esters. The method thus tends to overestimate provitamin A activity, especially if β-carotene represents only a small part of the total. The values for vitamin A reported in the USDA Agriculture Handbook No. 456 (3), and similar handbooks for use elsewhere [Leung et al, East Asia (101); Leung et al, Africa (100)], were determined by the AOAC method.

Gebhardt et al (51) estimated the provitamin A content of clingstone peaches by a modified AOAC method and chromatographic procedures that separated the individual components. The percent of USRDA for 100 g was much higher by the AOAC method than for the complete separation (60% vs 11% of the RDA for raw; 12% vs 6% for canned). DeRitter & Purcell provide a complete description of methods available (39).

Roels et al (148) published a standard method for carotene analysis in plasma. The method reports any pigment that absorbs light in 451 nm in hexane as β-carotene. Krinsky et al (91) separated plasma carotenoids on alumina into three fractions—"carotene," lycopene, and xanthophylls. The values obtained by Bjornson et al (16) for β-carotene in plasma were measurements of the carotene fraction. Since the blood carotenoids would be expected to reflect dietary carotenoids (57), the method would be expected to overestimate the provitamin A concentration in plasma and in some cases by very large factors.

Csorba et al (33) describe a thin-layer chromatographic (TLC) separation of chlorophylls and carotenes in which the quantity contained in the TLC

spots was estimated by a high-speed video-densitometric technique. Karawya et al (83) describe a TLC separation of carotenoids in which the compounds are scraped from the plate and further analyzed.

High pressure liquid chromatography (HPLC) has recently been applied to terpenoid analysis. Stewart (162) separated α,β- and β-cryptoxanthin from orange juice on a magnesia column. HPCL has been applied to the separation of β-carotene and retinol in margarine fats and oils (94, 110).

A reverse-phase HPLC method was developed by Zakaria et al (175) for the separation of the plant pigments lycopene, β-carotene, and α-carotene (Figure 5). Application of this method to tomato extracts separated β-carotene, γ-carotene, and lycopene from the xanthophylls (Figure 5). Reverse-phase HPLC has been found to be much more reproducible and rapid than other existing methods. An application of the method of Zakaria et al (175) was reported by Hsieh et al (74) for other fruits and vegetables.

Zakaria et al (175) compared the AOAC method with HPLC separation (Table 2). They reported that only 7% of the carotene value estimated by the AOAC method was actually β-carotene or provitamin A.

We have tried to recalculate the data of other workers and have found the results difficult to correlate with values that we have reported. Mathews

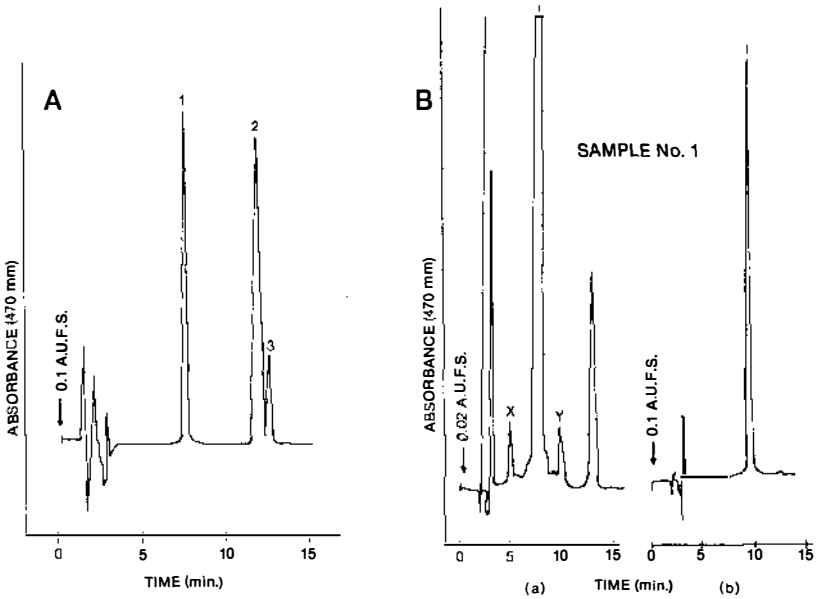


Figure 5 HPLC separation of carotenoids. (*A*) Separation of standards: 1, lycopene; 2, α-carotene; 3, β-carotene. (*B*) Separation of tomato extracts: a, 50 μl injected; b, 10 μl injected; 1, lycopene; 3, β-carotene. (From 175.)

Table 2 Measurement of provitamin A values in tomatoes: comparison of the HPLC and AOAC methods[a]

Methodology	Avg. value (μg/g)	Retinol equivalent (μg/g)	IU/g[b]
β-Carotene as obtained by HPLC	1.218	0.203	0.677
β-Carotene and lycopene obtained by HPLC	11.001	1.833	6.111
AOAC method	18.063	3.010	10.035

[a] In each case it is assumed that one sixth of the measured pigment(s) is converted to retinol. From 175.)
[b] 0.3 μg of retinol ≈ 1.0 IU.

et al (111) and Watada et al (170) analyzed various tomato samples by a "modified" AOAC method. The retinol equivalents that they report are about three times greater than the values reported by Zakaria et al (175) for β-carotene and about one third as great for lycopene and β-carotene. USDA Handbook 456 (3), for 100 grams of fresh, peeled tomatoes, gives a value of 790 IU or 16% of the RDA [based on 5000 (old value) IU/day], which obviously included lycopene. Watada et al (170) gives a value of 15% of the RDA per 100 g of fruit when lycopene was excluded (A. E. Watada, personal communication). By using their modified AOAC method, Mathews et al (111) estimated the provitamin A of the same tomato variety to be 10% of the RDA (not known if lycopene was included). The Food and Nutrition Board of the National Academy of Science (124) estimates that only one sixth of the β-carotene from a diet is converted to retinol in humans. The RDA for adult males is one thousand retinol equivalents. On this basis, the β-carotene in 100 g of tomatoes reported by Zakaria et al (175) represents 2% of the RDA/100 g (fresh weight); Watada et al reported 5.3% (170) and Mathews reported 7.4% (111) as compared to Handbook 456 (3), which estimates the level at 16%.

Since the long-term solution to the problem of vitamin A deficiency in the world embodies the consumption of sufficient dietary provitamin A, it is imperative that we know the dietary intake in terms of vitamin A equivalency. The data now available and the methods for estimating provitamin A activity of foods are inadequate. Goodwin & Goad reviewed the literature (63) on the carotenoid distribution in fruits. The carotenoid composition of over 100 fruits was listed in one table and a second table listed the β-carotene and total carotenoid concentration of about 40 fruits. These tables are at variance with many food composition tables and indicate the magnitude of the error of the tables. Thus, these and other compilations still suffer from the uncertainty of data reported by a large number of authors who

used a variety of purification methods. No equivalent review exists for the carotenoids of vegetables. In addition, the changes brought about in the processing, storage, and home preparation generally decreases the provitamin A activity in foods.

Eggs are often included as a source of provitamin A because of their rich color, but the major pigment of hen's eggs are lutein and zeaxanthin. β-Carotene represents less than 7% of the total (125). Fish flesh may be brightly colored, yet it is a poor source of carotenes, as the xanthophylls are those preferentially deposited (155). Both eggs and fish are examples of foods often recommended for their provitamin A content. The amount of vitamin A in eggs is low but variable depending on the season and the hen's diet (95, 125).

It would appear that much work needs to be done to standardize the techniques of measurement and the methods of reporting the vitamin A content of foods. Based on our past experiences, there is a need to develop further the newer HPLC methods of analysis and apply them to commonly eaten fruits and vegetables. In addition, the plasma level of provitamin A carotenoids should be correlated with the dietary intake of them.

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Literature Cited

1. Absar, N., Malek, M. A., Ahmad, K. 1976. Studies on dark green leafy vegetables and fruits. IV. Availability of carotene as a source of vitamin A. *Bangladesh Pharm. J.* 5:16–19
2. Abu-Serewa, S. 1976. Effects of source and level of fat in the hen's diet on the deposition of dietary oxycarotenoids in egg yolks. *Aust. J. Exp. Agric. Anim. Husb.* 16:204–8
3. Adams, C. F. 1975. Nutritive Value of American Foods in Common Units. *Agriculture Handbook No. 456,* ARS, USDA, Washington DC
4. Ahlswede, L., Lotthammer, K. H. 1978. A specific effect of β-carotene, independent of vitamin A on fertility in cattle. 5. Studies of organs (ovaries, corporalutea, liver, adipose tissue, uterine secretion and adrenals) and estimations of weights and contents. *Dtsch. Tierarztl. Wochenschr.* 85:7–12
5. Amen, R. J., Lachance, P. A. 1974. The effect of β-carotene and canthaxanthin on serum cholesterol levels in the rat. *Nutr. Rep. Int.* 10:269–76
6. Ames, S. 1958. Fat-soluble vitamins. *Ann. Rev. Biochem.* 27:371–402
7. Andrewes, A. G., Borch, G., Liaaen-Jensen, S., Snatzke, G. 1974. Animal carotenoids 9. On the absolute configuration of astaxanthin and actinioerythrin. *Acta Chem. Scand. B* 28:730–36
8. Arya, S. S., Natesan, V., Parihar, D. B., Vijayaraghavan, P. K. 1979. Stability of β-carotene in isolated systems. *J. Food Tech.* 14:571–78
9. Arya, S. S., Natesan, V., Parihar, D. B., Vijayaraghavan, P. K. 1979. Stability of carotenoids in dehydrated carrots. *J. Food Tech.* 4:579–86
10. Association of Official Analysis Chem. 1980. *Official Methods of Analysis,* ed. W. Horwitz, pp. 734–40. Washington DC: AOAC
11. Barua, A. B., Singh, H. T., Das, R. C. 1973. Conversion of lutein into dehydroretinol by the freshwater fish *Sacco-*

branchus fossilis. Br. J. Nutr. 30:1–12
12. Bates, R. P., Mathews, R. F. 1975. Ascorbic acid and β-carotene in soybeans as influenced by maturity sprouting, processing and storage. *Proc. Fla. State Hort. Soc.* 88:266–71
13. Bauernfeind, J. C. 1972. Carotenoid vitamin A precursors and analogs in foods and feeds. *Agric. Food Chem.* 20:456–73
14. Ben-Aziz, A., Britton, G., Goodwin, T. W. 1973. Carotene epoxides of *Lycopersicon esculentum*. *Phytochemistry* 12: 2759–64
15. Bickoff, E. M., Livingston, A. L., Bailey, G. F., Thompson, C. R. 1954. Xanthophylls in fresh and dehydrated alfalfa. *J. Agric. Food Chem.* 2:563–67
16. Bjornson, L. K., Kayden, H. J., Miller, E., Moshell, A. N. 1976. The transport of α-tocopherol and β-carotene in human blood. *J. Lipid Res.* 17:343–52
17. Blankenhorn, D. H., Freiman, D. G., Knowles, H. C. Jr. 1956. Carotenoids in man. The distribution of epiphasic carotenoids in atherosclerotic lesions. *J. Clin. Invest.* 35:1243–47
18. Blattná, J., Krcová, H., Blattny, C. 1976. Study of the composition of some carrot cultivars from different areas. 1. Content of β-carotene. *Sborník Vysoké Skoly Chem. Technol. Praze* 45:87–96
19. Bolin, H. R. 1977. Effect of processing on nutrient composition and texture of prunes. *J. Food Quality* 1:125–33
20. Bolin, H. R., Stafford, A. E. 1974. Effect of processing and storage on provitamin A and vitamin C in apricots. *J. Food Sci.* 39:1034–36
21. Boonjawat, J., Olson, J. A. 1975. The metabolism of radioactive crustaxanthin (3,3',4,4'-Tetrahydroxy-β-carotene). *Comp. Biochem. Physiol. B* 50:363–68
22. Britton, G. 1976. Biosynthesis of carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, ed. T. W. Goodwin, pp. 262–327. London: Academic
23. Britton, G. 1976. Later reactions of carotenoid biosynthesis. *Pure Appl. Chem.* 47:223–36
24. Britton, G., Goodwin, T. W. 1969. The occurrence of phytoene 1,2-oxide and related carotenoids in tomatoes. *Phytochemistry* 8:2257–58
25. Buchecker, R., Eugster, C. H. 1979. Search for 3'-epilutein and 3'0-didehydrolutein in egg yolk, in flowers of *Caltha palustris* and in autumn leaves. *Helv. Chem. Acta* 62:2817–24
26. Buma, T. J., van der Veen, H. K., C., Weerstra, R. 1977. Seasonal variation of the colour of milk. *Neth. Milk Dairy J.* 31:31–39
27. Carnevale, J., Cole, E. R., Crank, G. 1979. Fluorescent light catalyzed autoxidation of β-carotene. *J. Agric. Food Chem.* 27:462–63
28. Ceneno Martinex, C. 1977. Variations in the quantitative composition of carotenoids in lucerne and its effect on pigmentation in chickens. *Rev. Nutr. Anim.* 15:87–103
29. Cerdá-Olmeda, E., Torres-Martínez, S. 1979. Genetics and regulation of carotene biosynthesis. *Pure Appl. Chem.* 51:631–37
30. Chan, H. T. Jr., Kuo, M. T. H., Cavaletto, C. G., Nakayama, T. O. M., Brekke, J. E. 1975. Papaya puree and concentrates; changes in ascorbic acid, carotenoids and sensory quality during processing. *J. Food Sci.* 40:701–3
31. Chang, Y.-H., Raymundo, L. C., Glass, R. W., Simpson, K. L. 1977. Effect of higher temperature on CPTA-induced carotenoid biosynthesis in ripening tomato fruits. *J. Agric. Food Chem.* 25:1249–51
32. Chichester, C. O., Nakayama, T. O. M. 1965. Pigment changes in senescent and stored tissue. In *Chemistry and Biochemistry of Plant Pigments*, ed. T. W. Goodwin. London: Academic
33. Csorba, I., Buzas, Z., Polyak, B., Boross, L. 1979. High-speed video-densitometric determination of chlorophylls and carotenes separated by thin-layer chromatography. *J. Chromatogr.* 172:287–93
34. Cyronak, M. J., Osianu, D., Britton, G., Simpson, K. L. 1978. Transformation of 13,14 and 11,12-Monoepoxycanthaxanthin on magnesia. *J. Agric. Food Chem.* 26:712–15
35. Davies, B. H. 1979. Solved and unsolved problems of carotenoid formation. *Pure Appl. Chem.* 51:623–30
36. Davies, B. H., Taylor, R. F. 1976. Carotenoid biosynthesis—the early steps. *Pure Appl. Chem.* 47:211–21
37. De Luca, L. M. 1978. Vitamin A. 2. The fat-soluble vitamins. In *Handbook of Lipid Research*, ed. H. F. De Luca, pp. 1–67. New York: Plenum
38. De Luca, L. M., Adamo, S., Bhat, P. V., Sasak, W., Silverman-Jones, C. S., Akalovsky, I., Frot-Cortaz, J. P., Fletcher, T. R., Chader, G. J. 1979. Recent developments in studies on biological functions of vitamin A in normal and trans-

formed tissues. *Pure Appl. Chem.* 51:581–91
39. DeRitter, E., Purcell, A. E. 1981. Carotenoids as colorants and vitamin A precursors. In *Carotenoid Analytical Methods,* ed. J. C. Bauernfeind, In press. New York: Academic
40. Deshmukh, D. S., Ganguly, J. 1964. Effect of dietary protein contents on the intestinal conversion of β-carotene to vitamin A in rats. *Ind. J. Biochem.* 1:204–7
41. Devel, H. J. Jr. 1957. *The Lipids, Their Chemistry and Biochemistry,* Vol. III. New York: Interscience
42. Donchev, K., Lichev, P. 1979. Carotenoid content in the skin and fruit flesh of some peach cultivars. *Gradinar. Lozar Nauka* 16:31–37
43. El-Gorab, M. I., Underwood, B. A., Loerch, J. D. 1975. The roles of bile salts in the uptake of β-carotene and retinol by rat everted gut sacs. *Biochem. Biophys. Acta* 401:265–77
44. El-Tinay, A. H., Chichester, C. O. 1970. Oxidation of β-carotene. Site of initial attack. *J. Org. Chem.* 35:2290–93
45. Erdman, J. W. Jr., Lachance, P. A. 1974. Failure of the nonvitamin A active carotenoid lycopene to act as an antihypercholesterolemic agent in rats. *Nutr. Rep. Int.* 10:227–84
46. Eugster, C. H. 1979. Characterization, chemistry and stereochemistry of carotenoids. *Pure Appl. Chem.* 51:463–506
47. Fletcher, D. L., Harms, R. H., Janky, D. M. 1978. Yolk color characteristics, xanthophyll availability and a model system for predicting egg yolk color using β-apo-8'-carotenal and canthaxanthin. *Poultry Sci.* 57:624–29
48. Franca, M. H., deC., Zucas, S. M. 1977. Mixtures or rice and beans supplemented with retinol, methionine and calcium and values for retinol and carotene in liver. *Rev. Farmácia Bioquim. Univ. Sáo Paulo* 15:131–47
49. Garbin, G., Horgas, D. 1977. Effect of drying temperature on β-carotene stereoisomers in maize grain. *Pol. Znanstvena Smotra* 40:131–41
50. Gawecki, K., Potkanski, A., Lipinska, H. 1977. Effect of carophyll yellow and carophyll red added to commercial feeds for laying hens on yolk colour and its stability during short-term refrigeration. *Roczniki Akad. Rol. Poz.* 94:85–93
51. Gebhardt, S. E., Elkins, E. R., Humphrey, J. 1977. Comparison of two methods of determining the vitamin A value of clingstone peaches. *J. Agric. Food Chem.* 25:628–31
52. Georgiev, G. S. 1971. Carotenoids and vitamin A content in *salmo irideius* eggs and their significance in the initial periods of the embryogenesis. *Folia Balcanica* 2:1–10
53. Ghosal, A. K., Dwaraknath, P. K. 1976. Plasma carotene and vitamin A levels in cows, sheep and camels of the Thar desert. *Indian Vet. J.* 53:640–42
54. Glover, J. 1960. The conversion of β-carotene into vitamin A. *Vitam. Horm.* 18:371–86
55. Glover, J., Redfern, E. R. 1954. The mechanism of the transformation of β-carotene into vitamin A in vivo. *Proc. Biochem. Soc. Biochem. J.* 58:15
56. Goerz, G., Ippen, H. 1977. Treatment of photodermatosis with carotenoids. *Deut. Med. Wochenschr.* 102:1051–55
57. Goodwin, T. W. 1949. *The Comparative Biochemistry of the Carotenoids,* p. 229. London: Chapman & Hall
58. Goodwin, T. W. 1971. Biosynthesis. In *Carotenoids,* ed. O. Isler. Basel: Birkhäuser Verlag
59. Goodwin, T. W. 1979. Thirty years of biosynthesis. *Pure Appl. Chem.* 51: 593–96
60. Goodwin, T. W. 1979. Biosynthesis of terpenoids. *Ann. Rev. Plant. Physiol.* 30:369–404
61. Goodwin, T. W. 1979. *Biochemistry of Carotenoids,* Vol. 1. London: Chapman & Hall. 2nd ed.
62. Goodwin, T. W. 1980. Nature and distribution of carotenoids. *Food Chem.* 5:3–13
63. Goodwin, T. W., Goad, L. J. 1970. Carotenoids and triterpenoids. In *The Biochemistry of Fruits and their Products,* ed. A. C. Holme, 1:305–68. London: Academic
64. Gronowska-Senger, A., Wolf, G. 1960. Effect of dietary protein on the enzyme from rat and human intestine which converts β-carotene to retinol. *J. Nutr.* 100:300–8
65. Grosch, W., Laskawy, G., Kaiser, K. P. 1977. Co-oxidation of β-carotene and canthaxanthin by purified lipoxygenase from soybeans. *Z. Leben. Unt. Forsch.* 165:77–81
66. Gross, J. 1979. Carotenoid changes in the mesocarp of the redhaven peach during ripening. *Z. Pflanzenphysiol.* 94:461–68
67. Gross, J., Budowski, P. 1965. Conversion of carotenoids to 3-dehydro-retinol (vitamin A_2) in the mouse. *Nature* 206:1254–55

68. Gross, J., Budowski, P. 1966. Conversion of carotenoids into vitamin A_1 and A_2 in two species of fresh water fish. *Biochem. J.* 101:747–54
69. Hinton, C. F., Fry, J. L., Harms, R. H. 1974. Influences of a xanthophyll-free pullet grower diet on subsequent egg yolk pigmentation. *Poultry Sci.* 53:223–26
70. Hirota, S. 1977. Carotenoid constitution of various tomato strains. II. Carotenoid contents of various hybrid strains separated from the cross of Beta orange and red strains. *Nip. Shok. Kogyo Gak.* 24:151–58
71. Hirota, S. 1977. Carotenoid constitution of various tomato strains. III. Carotenoid contents of double recessive hybrid strains separated from the cross of the β-orange strain with the yellow, tangerine and apricot strains. *Nip. Shok. Kogyo Gak.* 24:159–65
72. Hoerer, E., Dreyfuss, F., Herzberg, M. 1974. Carotenemia, skin colour and diabetes mellitus. *Acta Diabetol. Latina* 12:202–7
73. Honory, K. 1978. The effect of zinc on the assimilation of β-carotene in poultry. *Bul. Vet. Inst. Pulawy* 22:77–80
74. Hsieh, Y. C., El-Magoli, S., Karel, M. 1980. Determination of β-carotene in fruits and vegetables using HPLC. *Abstr. Papers, Inst. Food. Tech. Ann. Meet. New Orleans, La.,* June 8–11
75. Hsu, W.-J., Rodriquez, D. B., Chichester, C. O. 1972. Biosynthesis of astaxanthin. VI. Conversion of [^{14}C]lutein and [^{14}C] β-carotene in goldfish. *Int. J. Biochem.* 3:333–38
76. Indonesia Nutritional Blindness Prevention Project: *Characterization of vitamin A deficiency and xerophthalmia and the design of effective intervention program.* 1980. Jakarta, Indonesia: Dir. Nutr., Ministry Health, Rep. Indonesia & Helen Keller Inst.
77. Jayarajan, P., Reddy, V., Mahanram, M. 1980. Effect of dietary-fat on absorption of beta-carotene from green leafy vegetables in children. *Indian J. Med. Res.* 71:53–56
78. Jen, J. J. 1974. Carotenoids of yellow and red lutescent tomatoes. *J. Agric. Food Chem.* 22:908–10
79. Jen, J. J., Thomas, R. L. 1978. Antagonistic effect of CPTA and far-red light on the carotenogenesis in lutescent tomatoes. *J. Food Biochem.* 2:23–27
80. Kamath, S. K., Arnrich, L. 1973. Effect of dietary protein on the intestinal biosynthesis of retinol from ^{14}C-β-carotene in rats. *J. Nutr.* 103:202–6
81. Kamdar, L. D., Shukla, P. C. 1974. Studies on the effect of green vs dry fodder feeding on blood plasma β-carotene and vitamin A levels of Kankrej bullocks. *Indian Vet. J.* 51:124–28
82. Kanner, J., Mendel, H., Budowski, P. 1978. Carotene oxidizing factors in red pepper fruits (*Capsicum annuum* L.): Oleoresin-cellulose solid model. *J. Food Sci.* 43:709–12
83. Karawya, M. S., Ghourab, M. G., El-Shami, I. M. 1975. A study of β-carotene in certain Egyptian vegetable organs. *Egyptian J. Pharm. Sci.* 16:339–44
84. Karunajeewa, H. 1978. Effect of saponin and synthetic oxycarotenoids on egg yolk colour. *J. Aust. Inst. Agric. Sci.* 44:206–7
85. Kaur, B., Manjrekar, S. P. 1975. Effect of dehydration on the stability of chlorophyll and β-carotene content of green leafy vegetables available in northern India. *J. Food Sci. Tech. India* 12:321–23
86. Khan, N. A., Khalil, J. K., Khan, I. 1975. Changes in carotene content of carrots during maturity, processing and storage under different temperature conditions. *Pakistan J. Sci. Res.* 27:112–14
87. Kim, K. S., Roh, S. M., Park, J. R. 1979. Effect of light quality (red, blue) on the major components of hot pepper fruit. *Korean J. Food Sci. Tech.* 11:162–65
88. Kobayashi, K., Iso, H., Nishiyama, K., Akuta, S. 1979. Carotenoid pigments and colour of fruits in Japan. VIII. The relationship between carotenoid pigments and colours of fresh and canned loquat fruits (*Eriobotrya japonica* Lindl). *Nip. Shok. Kogyo Gak.* 25:426–30
89. Krinsky, N. I. 1971. Function. In *Carotenoids,* ed. O. Isler. Basel: Birkhäuser Verlag.
90. Krinsky, N. I. 1979. Carotenoid protection against oxidation. *Pure Appl. Chem.* 51:649–60
91. Krinsky, N. I., Cornwall, D. G., Oncley, J. L. 1958. The transport of vitamin A and carotenoids in human plasma. *Arch. Biochem. Biophys.* 73:233–46
92. Lakshmanan, M. R., Pope, J. L., Olson, J. A. 1968. The specificity of a partially purified carotenoid cleavage enzyme of rabbit intestine. *Biochem. Biophys. Res. Commun.* 33:347–52
93. Lakshminarayana, S., Bazquez Salinas, C. 1978. Changes in chemical composition of mangoes fruit during ripening as

influenced by storage temperature. *Abstr. Pap. Am. Chem. Soc.* 176

94. Landen, W. O. Jr., Eitenmiller, R. R. 1979. Application of gel permeation chromatography and nonaqueous reverse phase chromatography to high pressure liquid chromatographic determination of retinyl palmitate and β-carotene in oil and margarine. *J. AOAC* 62:283–89
95. Lautner, V., Fiedler, J., Krejči, L. 1978. Contents of vitamin A and carotenoids in pheasant's eggs with regard to fortification of the feeds. *Biol. Chem. Živočišné Výroby Vet.* 14:141–46
96. Lazar, J., Jenčik, F., Mezencev, J., Pauerova, K. 1973. Utilization of some vitamins in dairy cows on mono diets and large amounts of urea. 1. Vitamin A and carotene in serum. *Folia Vet.* 17:41–48
97. Lee, R. G., Neamtu, G., Lee, T.-C., Simpson, K. L. 1978. Pigmentation of rainbow trout with extracts of floral parts from *Tagetes erecta* and *Curcubita maxima* marica. *Rev. Roum Biochim.* 15:287–93
98. Lee, T.-C., Chen, T., Alid, G., Chichester, C. O. 1978. Stability of vitamin A and provitamin A (carotenoids) in extrusion cooking processing. *AICHE Symp. Ser.* 74:192–95
99. Lee, Y. C. 1976. *Vitamin composition of tomato cultivars and computer simulation of ascorbic acid stability in canned tomato juice.* Dissertation. Michigan State Univ., East Lansing
100. Leung, W.-T. W., Busson, F., Jardin, C. 1968. *Food Composition Table for Use in Africa.* Washington DC: DHEW
101. Leung, W.-T. W., Butrum, R. R., Chang, F. H., Narayana Rao, M., Polacchi, W. 1972. *Food Composition Table for Use in East Asia.* Washington DC: DHEW
102. Liaaen-Jensen, S. 1978. Marine carotenoids. In *Natural Products—Chemical and Biological Perspectives,* ed. P. J. Scheuer, 2:1–73
103. Liu, L.-I., Parrish, D. B. 1979. Biopotency of vitamin A in fortified flour after accelerated storage. *J. Agric. Food Chem.* 27:1134–36
104. Liu, Y. K., Luh, B. S. 1977. Effect of harvest maturity on carotenoids in pastes made from VF-145-7879 tomatoes. *J. Food Sci.* 42:216–20
105. Livingston, A. L., Kohler, G. O., Kuzmicky, D. D. 1980. Comparison of carotenoid storage stability in alfalfa leaf protein (pro-xan) and dehydrated meals. *J. Agric. Food Chem.* 28:652–56
106. Lotthammer, K. H. 1979. Importance of β-carotene for the fertility of dairy cattle. *Feedstuffs* 51:16–50
107. Lotthammer, K. H., Ahlswede, L. 1977. A specific effect of β-carotene, independent of vitamin A on the fertility of cattle. 3. Blood s rum studies (β-carotene, vitamin A, SGOT, total cholesterol, glucose, inorganic phosphorus). *Dtsch. Tierarztl. Wochenschr.* 84:220–26
108. Lotthammer, K. H., Ahlswede, L., Meyer, H. 1976. A specific effect of β-carotene, independent of vitamin A on fertility in cattle. 2. Further clinical data and conception rates. *Dtsch. Tierarztl. Wochenschr.* 83:353–58
109. Mallia, A. K., John, J., John, K. V., Lakshmanan, M. R., Jungalwala, F.B., Cama, H. R. 1970. Biological activity of epoxides of vitamin A. *Indian J. Biochem.* 7:102–3
110. Maruyama, T., Ushigusa, T., Kanematsu, H., Nilya, I., Imamura, M. 1977. Concurrent determination of vitamin A and β-carotene in fat and oil by high speed liquid chromatography. *J. Food Hyg. Soc. Jpn.* 18:487–92
111. Mathews, R. F., Crill, P., Locascio, S. J. 1974. Beta-carotene and ascorbic acid contents of tomatoes as affected by maturity. *Proc. Fla. State Hort. Soc.* 87:214–16
112. Mathews-Roth, M. M. 1975. Presence of carotenoids in the erythrocyte membranes of carotenemic and noncarotenemic subjects. *Clin. Chem.* 21:258–59
113. Mathews-Roth, M. M., Gulbrandsen, C. L. 1974. Transport of β-carotene in serum of individuals with carotenemia. *Clin. Chem.* 20:1578–79
114. Mathews-Roth, M. M., Hummel, D., Crean, C. 1977. The carotenoid content of various organs of animals administered large amounts of β-carotene. *Nutr. Rep. Int.* 16:419–23
115. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B., Harber, L. C., Kass, E. H. 1970. Beta-carotene as a photoprotective agent in erythropoietic protoporphyria. *N. Engl. J. Med.* 282:1231–34
116. Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B., Harber, L. C., Kass, E. H. 1977. Beta-carotene therapy for erythropoietic photoporphyria and other photosensitivity diseases. *Arch. Dermatol.* 113:1229–32
117. Mathews-Roth, M. M., Pathak, M. A., Parrish, J., Fitzpatrick, T. B., Kass, E. H., Toda, K. Clemens, W. 1972. A clin-

ical trial of the effects of oral β-carotene on the responses of human skin to solar radiation. *J. Invest. Dermatol.* 59: 349–53
118. McAnally, J. S., Szymanski, C. D. 1966. Metabolism of alpha-carotene. *Nature* 210:1366
119. Meyer, H., Ahlswede, L., Lotthammer, K. H. 1975. A specific effect of β-carotene, independent of vitamin A on fertility of cattle. 1. Methods, body development and ovarian function. *Dtsch. Tierarztl. Wochenschr.* 82:444–49
120. Mikolasek, A., Mader, P., Kozeny, V. 1977. Effects of dietary maize meal or lucerne meal on the carotenoid content of egg yolk. *Sbornik Vysoke Skoly Zemedelski Praze, Fakulta Agronomicka B* 1:129–39
121. Mohammad, N., Shah, M. A., Mahmood-Ul-Hassan, Ehteshamuddin, A. F. M. 1976. Effect of some additives and packing materials on the shelf-life of drum-dried carrot powder. *Pakisan J. Sci. Ind. Res.* 19:178–81
122. Muhilal, H., Gover, J. 1975. The affinity of retinol and its analogues for retinol-binding protein. *Biochem. Soc. Trans.* 3:744–46
123. Müller, R. K., Bernhard, K., Kienzle, F., Mayer, H., Rüttiman, N. A. 1980. Some recent advances in the synthesis of natural carotenoids. *Food Chem.* 5:15–45
124. *National Academy of Science Recommended Dietary Allowances,* pp. 30–54. 1974. Washington DC: Natl. Acad. Sci., 8th ed.
125. Neamtu, G., Salajan, G., Bilaus, C., Laszlo, T., Bodea, C., Simpson, K. L. 1977. Research on the utilization of carotenoid pigments. II. The effect of meal from fruits of *Hyppophae ramnoides* on the pigmentation of egg yolk. *Rev. Roum. Biochim.* 14:109–15
126. Newstead, D. F. 1976. Carotene and immunoglobulin concentrations in the colostrum and milk of pasture-fed cows. *J. Dairy Res.* 43:229–37
127. Norman, G. M., Sykes, A. H., Bayley, H. S. 1973. The influence of dietary and parenteral β-apo-8'-carotenoic acid ethylester and zeaxanthin on tissues and blood pigment levels in the laying hen. *Br. Poult. Sci.* 14:521–30
128. Olson, J. A. 1979. A simple dual assay for vitamin A and carotenoids in human liver. *Nutr. Rep. Int.* 19:807–13
129. Olson, J. A., Lakshmanan, M. R. 1970. *Enzymatic Transformation of Vitamin A, with Particular Emphasis on Carotenoid Cleavage in the Fat Soluble Vitamins,* ed. H. F. DeLuca, J. W. Suttie, pp. 213–26. Madison: Univ. Wis. Press
130. Oomen, H. A. P. C. 1974. Vitamin A deficiency, xerophthalmia and blindness. *Nutr. Rev.* 32:161
131. Oomen, H. A. P. C., McLaren, D. S., Escapini, H. 1964. Epidemiology and public health aspects of hypovitaminosis A: A global survey on xerophthalmia. *Trop. Geogr. Med.* 16:271
132. Ortaliza, I. C., Del Rosario, I. F., Caedo, M. M., Alcara, A. O. 1971. The availability of carotene in some Philippine vegetables. *Phil. J. Sci.* 98:123–31
133. Ortaliza, I. C., Del Rosario, I. F., Santos, M. H., Agular, C. G., Dumada-Ug, L. M. 1971. The availability of carotene in some Philippine vegetables II. *Phil. J. Sci.* 100:95–102
134. Peiser, G. D., Yang, S. F. 1979. Sulfite-mediated destruction of β-carotene. *J. Agric. Food Chem.* 27:446–49
135. Pereira, S. M., Begum, A., Dumm, M. E. 1966. Vitamin A deficiency in Kwashiorkor. *Am. J. Clin. Nutr.* 19:182–86
136. Pitt, G. A. J. 1978. Alpha-retinol. *World Rev. Nutr. Diet.* 31:65–70
137. Poston, H. A. 1969. Cortland Hatchery Report No. 37. *Fish. Res. Bul. NY* 32:41–43
138. Premachandra, B. R., Vasantharajan, V. N., Cama, H. R. 1976. Improvements in the nutritive value of tomatoes. *Curr. Sci.* 45:56–57
139. Premachandra, B. R., Vasantharajan, V. N., Cama, H. R. 1978. Availability of β-carotene from high β-tomato hybrids to vitamin A deficient rats and utilization of β-carotene in the presence of other carotenoids. *Indian J. Exp. Biol.* 16:473–77
140. Rajalakshmi, R., Chari, K. V., Advani, M., Chary, T. M., Patel, M. A., Mutalih, A. M., Bhat, T. K., Vyas, A. D. 1975. Comparative availability of β-carotene in different vegetables as compared to that of pure β-carotene and vitamin A. *Baroda J. Nutr.* 2:103–19
141. Ramachandran, K. 1973. Beta-carotene metabolism in diabetes. *Indian J. Med. Res.* 61:1831–34
142. Rao, S., Reddy, U. M. 1979. Effect of different methods of cooking on the carotene content of selected foods. *Proc. 1st Indian Conv. Food Sci. Tech. FSTA 11,* 12A871
143. Raymundo, L. C., Simpson, K. L. 1972. The isolation of a poly-cis-α-carotene from tangerine tomato. *Phytochemistry* 11:397–411
144. Reich, P., Shwachman, H., Craig, J. M.

1960. Lycopenemia. *N. Engl. J. Med.* 262:263–69
145. Renqvist, U. H., Devreeze, A. C., Even-Huis, B. 1978. The effect of traditional cooking methods on carotene content in tropical leafy vegetables. *Indian J. Nutr. Diet.* 15:1–158
146. Rodriguez, D. B., Simpson, K. L., Chichester, C. O. 1973. The biosynthesis of astaxanthin. XVII. Intermediates in the conversion of β-carotene. *Int. J. Biochem.* 4:213–22
147. Rodriguez, D. B., Simpson, K. L., Chichester, C. O. 1974. The biosynthesis of asthaxanthin. IVIII. The incorporation of carotenoids in the goldfish. *Int. J. Biochem.* 5:157–66
148. Roels, O. A., Trout, M., Lui, N. S. T., Anderson, O. R. 1972. VI. Vitamins and hormones; vitamin A and carotene. In *Standard Methods of Clinical Chemistry,* Vol. 7. New York: Academic
149. Savithry, K. N., Mallia, A. K., Cama, H. R. 1972. Metabolism and biological activity of anhydrolutein (3'-hydroxy-3,4-dehydro-β carotene) in rats. *Indian J. Biochem. Biophys.* 9:325–27
150. Schams, D., Hoffmann, B., Lotthammer, K. H. 1977. A specific effect of β-carotene, independent of vitamin A of fertility in cattle. 4. Effect on hormonal parameters during the cycle. *Dtsch. Tierarztl. Wochenschr.* 84:307–10
151. Seely, G. R., Meyer, T. H. 1971. The photosensitized oxidation of β-carotene. *Photochem. Photobiol.* 13:27–32
152. Sharman, I. M., Dick, A. P., Farthing, M. J. G., Bryant, J. R., Beevor, S. 1979. Carotenoids and retinol levels in the blood of ulcerative colitis patients and controls. *Abstr. Commun. Nutr. Soc.,* 54A
153. Sharma, R. V., Mathur, S. N., Dmitrovskii, A. A., Das, R. C., Ganguly, J. 1977. Studies on the metabolism of β-carotene and apo-β-carotenoids in rats and chickens. *Biochim. Biophys. Acta* 468:183–94
154. Simpson, D. J., Baqar, M. R., McGlassor, W. B., Lee, T. H. 1976. Changes in ultrastructure and pigment content during development and senescence of fruits of normal and rin and nor mutant tomatoes. *Aust. J. Plant Physiol.* 3:575–87
155. Simpson, K. L., Katayama, T., Chichester, C. O. 1981. Carotenoids in fish feed. In *Carotenoids as Colorants and Vitamin A Precursors,* ed. J. C. Bauernfeind, In press. New York: Academic
156. Simpson, K. L., Lee, T.-C., Rodriguez, D. B., Chichester, C. O. 1976. Metabolism in senescent and stored tissues. In *Chemistry and Biochemistry of Plant Pigments,* ed. T. W. Goodwin, 2:780–842. London: Academic
157. Singh, H., Cama, H. R. 1975. Metabolism of carotenoids. *J. Sci. Ind. Res.* 34:219–30
158. Smith, J. E., Borchers, R. 1972. Environmental temperature and the utilization of β-carotene by the rat. *J. Nutr.* 102:1017–24
159. Sneider, W. D., Rosso, G. C., Rogers, A. E., Wolf, G., Dowling, J. E., Callahan, M. J. 1974. *J. Nutr.* 104:1662–68
160. Sood, R., Bhat, C. M. 1974. Changes in ascorbic acid and carotene content of green leafy vegetables on cooking. *J. Food Sci. Tech.* 11:131–33
161. Sporn, M. B., Dunlop, N. M., Newton, D. L., Henderson, W. R. 1976. Relationships between structure and activity of retinoids. *Nature* 263:110–13
162. Stewart, I. 1977. High performance liquid chromatographic determination of provitamin A in orange juice. *J. AOAC* 60:132–36
163. Straub, O. 1976. *Key to Carotenoids-Lists of Natural Carotenoids.* Basel: Birkhäuser Verlag
164. Surmatis, J. D., Maricq, J., Ofner, A. 1958. Synthesis of carotene homologs. *J. Org. Chem.* 23:157–62
165. Sweeney, J. P., Marsh, A. C. 1973. Liver storage of vitamin A in rats fed carotene stereoisomers. *J. Nutr.* 103:20–25
166. Sweeney, J. P., Marsh, A. C. 1974. Liver storage of vitamin A by rats fed carrots in various forms. *J. Nutr.* 104:1115–20
167. Thomas, P. 1975. Effect of post harvest temperature on quality, carotenoids and ascorbic acid of alphonso mangoes on ripening. *J. Food Sci.* 40:704–6
168. Tsukuda, N. 1970. Studies on the discoloration of red fishes. VI. Partial purification and specificity of the lipoxidase-like enzyme responsible for carotenoid discoloration in fish skin. *Bull. Jpn. Soc. Sci. Fish* 36:725–33
169. Veloso-de Almeida, P. G., Brune, W., Kling deMoraes, G. H. 1977. Changes in levels of β-carotene and lycopene in stored tomatoes. *Rev. Ceres* 24:178–99
170. Watada, A. E., Aulenbach, B. B., Worthington, J. T. 1976. Vitamin A and C in ripe tomatoes as affected by stage of ripeness at harvest and by supplementary ethylene. *J. Food Sci.* 41:856–58

171. Weber, F., Laskawy, G., Grosch, W. 1974. Cooxidation of carotene and crocin by soybean lipoxygenase isoenzymes. *Z. Lebensm. Unters. Forsch.* 155:142–50
172. Weiss, R. R. 1975. *Influence of β-carotene and vitamin A on defined sexual functions of young bulls.* Dissertation. Tierarztliche Hochschule, Hannover
173. Yokoyama, H., White, M. J. 1966. Citrus carotenoids. VI. Carotenoid pigments in the flavedo of sinton citrangequat. *Phytochemistry* 5:1159–73
174. Yurdagel, Ü., Aktan, N., Ural, A. 1978. Changes in content of ascorbic acid and β-carotene in red peppers during canning. *Ege Üniv. Ziraat Faciiltesi Dergisi* 15:231–41
175. Zakaria, M., Simpson, K. L., Brown, P. R., Krstulovic, A. 1979. Useof reversed phase HPLC analysis for the determination of provitamin A carotenes in tomatoes. *J. Chromatogr.* 176:109–17
176. Zaynoun, S. T., Hunter, J. A. A., Darby, F. J., Zarembski, P., Johnson, B. E., Frain-Bell, W. 1977. The treatment of erythropoietic protoporphyria experience with β-carotene. *Br. J. Dermatol.* 97:663–68
177. Zile, M. H., Inhorn, R. C., De Luca, H. F. 1980. The biological activity of 5,6-epoxyretinoic acid. *J. Nutr.* 110:2225–30.