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Carotenoids and Retinoids in Human Nutrition

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ABSTRACT: Since the discovery of vitamin A as a fat-soluble growth factor in the early part of the century, research into carotenoids and retinoids has attracted the attention of many scientists. These two groups of compounds are still being actively studied all over the world since many gaps in knowledge exist and new frontiers are being pursued. Recent developments in studies into the possible roles of carotenoids and retinoids beyond their classical functions in vision have created a great deal of excitement in the biomedical community. This review covers a wide range of topics pertaining to these two closely related compounds. Particular emphasis is given to the functions of these compounds and their roles in human nutrition. Various aspects of vitamin A deficiency and studies on carotenoids and retinoids in cancer development and prevention are reviewed in some detail.

KEY WORDS: carotenoids, retinoids, human nutrition, vitamin A deficiency, cancer development and prevention.

I. INTRODUCTION

Vitamin A, or retinol, is an essential nutrient for man and all mammalian species since it cannot be synthesized within the body. Deficiency of the vitamin results in adverse effects on growth, reproduction, and resistance to infections. Various mild to severe biochemical and clinical signs develop, depending on the degree of severity of the insufficiency. The most important manifestation of severe vitamin A deficiency is xerophthalmia, and irreversible blindness may eventually occur in one or both eyes.

Xerophthalmia is not a condition of recent recognition. The ancient Papyrus Ebers, an Egyptian medical treatise of about 1500 B.C., and the Chinese at about the same time, had already documented the occurrence of night blindness.[1,2] For the treatment of this early stage of xerophthalmia, liver or liver extract was recommended. However, the identification, isolation, synthesis, and production of vitamin A by chemical synthesis are all more recent achievements, accomplished during the 20th century.

Some 75 years ago, vitamin A was discovered as a fat-soluble growth factor present in liver and recognized as an essential biochemical for normal vision in man and animals. It is, in fact, the first vitamin to be discovered. Today, after several decades, very little is known about the vitamin's biochemical mechanisms of action in the different functions except for the process of vision.[1] Vitamin A deficiency remains one of the major public health nutritional problems in many developing countries and is an important cause of preventable blindness. Afflicting large numbers of pre-school children in southeast Asia, vitamin A deficiency is often associated with protein-energy malnutrition, parasitic infestation, and diarrheal disease. The interaction between vitamin A and infection has recently been given a great deal of attention. The synergistic effect between vitamin A deficiency and infection may be responsible for excessive childhood morbidity and mortality in many developing regions of the world. Recent studies on vitamin A supplemen-

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tation and child survival and mortality have created a great deal of interest and debate in the international health and nutrition community.

The major source of vitamin A in the diet of most communities in developing countries is carotenoids. A review of vitamin A in human nutrition would thus be incomplete without due consideration to these pigments of provitamin A activity.

Carotenoids are synthesized exclusively by photosynthetic microorganisms and by members of the plant kingdom where they play a fundamental role in metabolism. Aside from providing aesthetic qualities as colorants in the plant and the animal kingdoms, these pigments also take part in the photosynthetic process. Most importantly, the carotenoids serve the animal kingdom as sources of vitamin A activity.

A large number of pigments in living organisms — carotenoids, chlorophylls, anthocyanins, and porphyrins — provide the rich variety of color in nature. The carotenoids, believed to have derived their name from the fact that they constitute the major pigment in the carrot root, *Daucus carota*, are undoubtedly among the most widespread and important. This group of pigments is found throughout the plant kingdom (although their presence is often masked by chlorophyll) and in insects, fish, birds, and other animals. These pigments provide a whole range of light yellow to dark red colorings, and when complexed with proteins, green and blue colorations are achieved. Thus, a wide variety of foods and feeds — yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobsters, salmon, trout, yellow corn, etc. — owe their color principally to carotenoids, as do certain food color extracts from natural sources such as palm oil, paprika, annatto, and saffron.[3-5]

This review discusses these two large groups of interrelated compounds, which are still being actively studied all over the world. Many gaps in knowledge exist, and new frontiers are being pursued. Recent developments in studies into the possible roles of carotenoids and retinoids beyond their classical functions have created a great deal of excitement in the biomedical community. This is one of the few reviews on the subject that deals with the carotenoids and retinoids together. A wide range of topics is discussed, with particular attention to their importance to human nutrition. A detailed discussion of analytical methods for retinoids and carotenoids is treated in a separate review by the author.[6]

II. HISTORICAL PERSPECTIVE

Carotenoids and retinoids have had a long and interesting history. Biologists, organic chemists, and biochemists were fascinated by the brightly colored carotenoid pigments more than a century ago. Studies on these pigments have been traced to the beginning of the 19th century, when the crystalline yellow pigment *carotene* was first isolated in 1831 and the naming in 1837 of the yellow pigments of autumn leaves as *xanthophylls*.[7-9] In the early part of the 20th century, it was clearly shown that there existed a whole family of carotenoids, and that these were isoprenoid derivatives.

In 1913 McCollum and Davis[10] reported the existence in certain foods of an essential lipid-soluble substance capable of promoting growth in rats. They called this substance "fat-soluble A" to distinguish it from essential water-soluble nutrients, which they called "water-soluble B".[11] During the subsequent decade, further information about the existence and properties of this soluble essential nutrient was obtained from the work of a number of investigators, including McCollum and associates Osborne and Mendel, Stepp, Steenbock, Moore, and others. It was shown that "fat-soluble A" (now known as vitamin A) not only maintained growth in rats but was capable of preventing night blindness and xerophthalmia. Subsequently, the relationship between vitamin A in animals and the provitamin carotene in plants was also clarified, particularly after Karrer and associates[12] determined the chemical structure of β-carotene in 1930 and of retinol in 1931 using a highly purified vitamin A extract that they had obtained from shark liver oil.[13,14] Using such retinol preparations, the first oily retinol esters (e.g., retinyl acetate) were prepared.[13]

The structure of β-carotene had been determined as early as 1930.[12] These two results made it possible to see from a chemical standpoint why β-carotene is a natural precursor of vitamin A

compounds. This relationship was discovered between 1928 and 1930 in a large number of animal experiments.[15,16] The elucidation of the vitamin A precursor function of these pigments has proved to have great scientific and economic importance.

In 1937, Holmes and Corbet[17] were able to crystallize pure retinol from fish liver. Various crystalline esters of retinol were obtained from fish liver oils by Baxter and Robeson.[18,19] These pure compounds made possible the accurate determination of a number of physicochemical characteristics of carotenoids. Several years later, Arens and Van Dorp[20] and Isler and associates[21] succeeded in achieving the chemical synthesis of pure retinoic acid and retinol. Shortly thereafter, the total synthesis of β-carotene was also reported.[22]

Many studies were conducted in the first half of this century dealing with various aspects of the physiology and metabolism of vitamin A. Particularly noteworthy was the identification by Wald,[23] Morton,[24] and Morton and Goodwin[25] of the chromophore of the visual pigment as retinaldehyde. These various studies provided considerable information about the role of vitamin A in vision, and about the pathology and pathophysiology of vitamin A deficiency.

In the later half of the century, major advances in carotenoid and retinoid research have taken place. Since the middle of the century, rapid development in spectroscopic techniques, particularly nuclear magnetic resonance (NMR) and mass spectrometry (MS), have revolutionized structural studies of these compounds. In the area of separation and analysis, various new methods have been developed.[6,26-28] The development of high-performance liquid chromatography (HPLC) is said to have revolutionized the problem of isolation and characterization of carotenoids and retinoids, and their metabolites. A whole array of new *in vitro* bioassay systems for retinoids have been developed, some of which are said to be even more sensitive than the chemical detection methods.[29]

Rapid progress has taken place in the area of chemical synthesis, particularly for retinoids. Advances in synthetic organic chemistry have resulted in the synthesis of well over a thousand new retinoids. Some of these compounds superficially bear little resemblance to retinol or retinoic acid, but nevertheless retain many of the biological activities of these substances. Advances in tracer chemistry now permit the synthesis of almost any desired retinoid in radioactively labeled form.[30] Remarkable progress has been made in the synthesis of carotenoids, particularly to meet the needs of a wide range of commercial uses.[31]

Major advances have occurred in understanding the intermediary metabolism as well as the transport systems of retinoids, both extracellular and intracellular. The development of cell culture techniques has facilitated study of the effects of retinoids on cell differentiation and proliferation, one of the most basic problems of biology.[32] Fundamental to these new studies is the realization that retinoids are highly potent agents for control of these processes. In this regard, the retinoids have had a particularly striking role in helping to illuminate the problem of malignant cell differentiation. There has thus been a thrust in the development of new retinoids for prevention or treatment of disease, particularly in the areas of oncology and dermatology.[33-35] The domain of retinoid research is said to have moved well beyond its classical roots in the study of nutrition and vision.

Similarly, in addition to their role as vitamin A precursors, the carotenoids are now thought to play specific roles in mammalian tissues related to their function in plants.[9,36] Carotenoids, with their highly reactive conjugated double bonds, act as free radical traps or antioxidants. Based on the ability of carotenoids to protect plants and bacteria against photosensitivity, trials have been carried out in the use of these pigments for the treatment of erythropoietic porphyria, a condition in which patients suffer from an extreme degree of photosensitivity.[37] These led to studies in the use of β-carotene and other carotenoids in the treatment of various skin tumors.

III. NOMENCLATURE AND STRUCTURE

A. Nomenclature

The International Union of Pure and Applied Chemistry (IUPAC) Commission on Nomenclature of Organic Chemistry and the International

Union of Biochemistry (IUB) Commission on Biochemical Nomenclature jointly recommended revised rules for the nomenclature of carotenoids.[38] Known as the IUPAC-IUB 1974 Recommendations, these rules are adhered to in this review.

Relatively recent reviews on the nomenclature and structure of carotenoids include those of Weedon,[4] Isler,[7] Davies,[39] and Goodwin.[8] These, and the IUPAC-IUB document,[38] have been particularly useful in the preparation of this section.

The term "carotenoids" embraces a large number of naturally occurring fat-soluble compounds that are colored red and yellow. The characteristic chromophore is due to the presence of a series of conjugated double bonds. This large family of pigments has been grouped as hydrocarbons (lycopene and α-, β-, and γ-carotenes) and their oxygenated derivatives (oxycarotenoids or xanthophylls).

With regard to retinoids, the IUPAC-IUB Joint Commission on Biochemical Nomenclature[40] has defined them as:

a class of compounds consisting of four isoprenoid units joined in a head-to-tail manner. All retinoids may be formally derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the terminus of the acyclic portion. To avoid confusion with previously used names in this field no parent hydrocarbon is named.

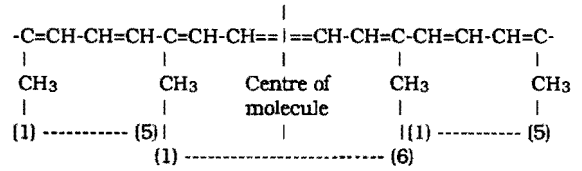
In recent years, the term, "retinoid" has been taken to include both the naturally occurring compounds with vitamin A activity and synthetic analogs, with or without biological activity, of retinol. Carotenoids are not included in this group of compounds.[41]

The Commission further recommended that the term vitamin A should be used as the generic descriptor for retinoids that exhibit qualitatively the biological activities of retinol. This term should be used in derived terms such as *vitamin A activity, vitamin A deficiency,* and *vitamin A antagonist.*[40]

B. Structure

1. Basic Structure

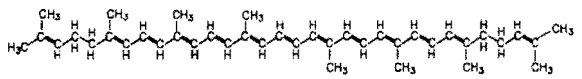
The basic structure of carotenoids consists of eight five-carbon isoprenoid units (ip), joined in the normal head-to-tail manner, except at the center of the molecule where the order is reversed so that the C_{40} skeleton, viewed as a whole, is symmetrical. Thus, the two central methyl groups are in a 1,6 position relative to each other while the remaining nonterminal methyl groups are in a 1,5 relationship,[8,38] as shown in Structure I:



(I)

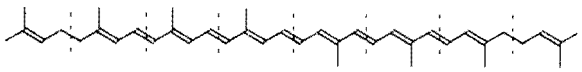
Using the abbreviation of *ip* for the isoprenoid residue, the C_{40} carotenoids can be represented as: *ipipipip-pipipipi*.

The acyclic $C_{40}H_{56}$ conjugated polyenelycopene (II) can be taken as the prototype of the carotenoids.



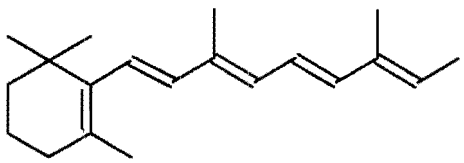
(II)

The shorthand form of the carotenoid formula is also often used (IIa), the broken lines indicating formal division into isoprenoid units:



(IIa)

In the case of retinoids, the basic hydrocarbon (III), also known as axerophthene, has been designated as deoxyretinol:



Deoxyretinol (III)

2. Modifications to the Parent Compound

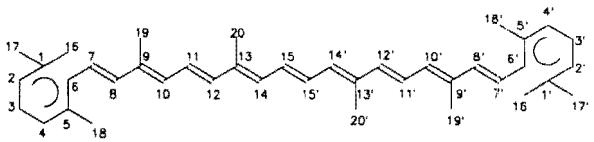
Based on the basic structure of the carotenoids (I and II above), other carotenoids can then be considered as related to it by means of structural changes in one or both halves of the molecule. These changes may be brought about by reactions involving (1) hydrogenation, (2) dehydrogenation, (3) cyclization, (4) insertion of oxygen in various forms, (5) double bond migration, (6) methyl migration, (7) chain elongation, and (8) chain shortening.[8] Retinol (vitamin A) and related C_{20} compounds are not included in this class of compounds.

Though the number of basic structural modifications are comparatively few, their occurrence in different combinations accounts for the remarkable variety of natural carotenoids. About 400 are now known and the number is increasing rapidly. The number of hydrocarbons (carotenes) is relatively small, most being oxygenated carotenoids (xanthophylls).[8]

Similarly, for retinoids, changes to the basic hydrocarbon give rise to the various retinoids. These include changes to the functional group at the 15 position of the basic hydrocarbon (III), the hydrogenation level, and chain length.

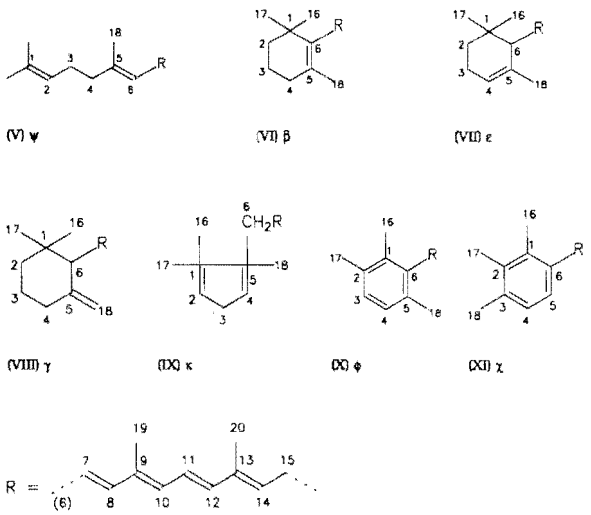
a. Carotenoid Hydrocarbons

The carotenoid hydrocarbons are known as carotenes. Specific names of compounds in this group are based on the stem name "carotene". The structure and numbering of the parent carotene is given below (IV).

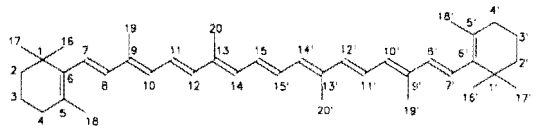


(IV)

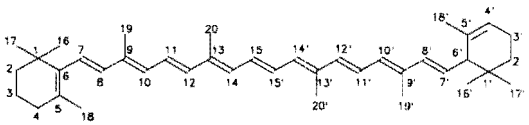
The semi-circles at the two terminations represent two double-bond equivalents. The systematic name of a specific carotenoid hydrocarbon is constructed by adding two Greek letters as prefixes to the stem name carotene, depending on the two C_9 end groups which it contains. Some of these end groups, and their corresponding Greek letter prefixes, have been compiled from Goodwin[8] and IUPAC-IUB[38] and are given in Table 1.



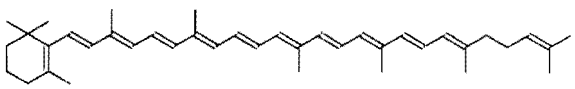
Examples of carotenes are



β-Carotene [β,β-carotene] (XII);



α-Carotene [β,ε-carotene] (XIII);



γ-Carotene [β,ψ-carotene] (XIV).

In the above examples, as well as others discussed in this section, their systematic names are given in brackets behind their trivial names. The first Greek-letter prefix in the systematic name is separated from the second by a comma, and the second is connected to the stem name by a hyphen. These prefixes are cited in alphabetical order (β [beta], ε [epsilon], κ [kappa], ϕ [phi], χ [chi], ψ [psi]).

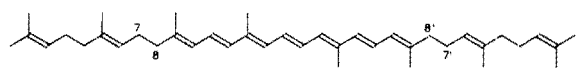
TABLE 1
End Group Designation of Carotenes

Type	Prefix	Formula	Structure
Acyclic	ψ (psi)	C_9H_{15}	V
Cyclohexane	β, ε (beta, epsilon)	C_9H_{15}	VI, VII
Methylene-cyclohexane	γ (gamma)	C_9H_{15}	VIII
Cyclopentene	κ (kappa)	C_9H_{17}	IX
Aryl	φ, χ (phi, chi)	C_9H_{11}	X, XI

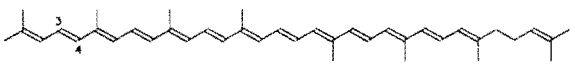
Adapted from References 8 and 38.

In numbering a carotenoid formula which is asymmetrical (the two end groups are dissimilar), unprimed numbers are allotted to the end of the molecule associated with the Greek letter prefix cited first in the systematic name, as indicated in the numbering of α-carotene (XIII). Carotenoid formulas are usually drawn so that the unprimed numbers are on the left hand side.

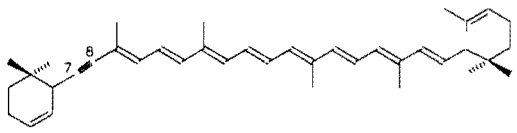
Changes in hydrogenation levels of the parent carotenes are indicated by prefixing "hydro" or "dehydro" to the basic name, together with the locants specifying the carbon atoms at which the hydrogen atoms have been added or removed, e.g.,



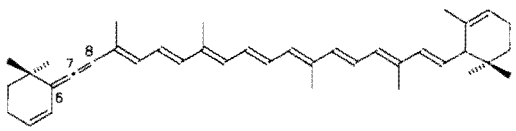
Zeta-Carotene [7,8,7',8'-tetrahydrolycopene] (XV)



3,4-Didehydro-ψ,ψ-carotene (XVI)



7,8-Didehydro-ε,ε-carotene (XVII)



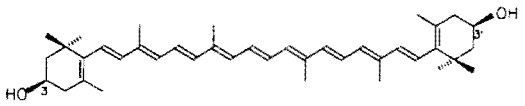
6,7-Didehydro-ε,ε-carotene (XVIII)

However, almost all naturally occurring acetylenic and allenic carotenoids contain oxygen and are considered in the next section.

b. Oxygenated Carotenoids

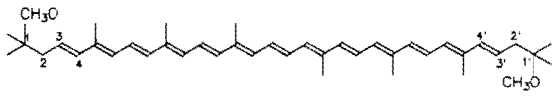
Oxygenated carotenoids are known collectively as xanthophylls. Examples of some of the oxygen-containing characteristic groups present are[8]

(i) ***hydroxy,*** as in zeaxanthin [β,β-carotene-3,3'-diol] (XIX):



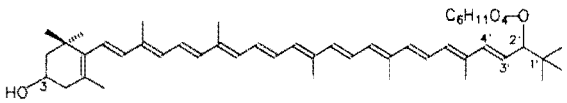
(XIX)

(ii) ***methoxy,*** as in spirilloxanthin [1,1'-dimethoxy-3,4,3'4'-tetrahydro-1,2,1'2'-tetrahydro-ψ,ψ-carotene] (XX):



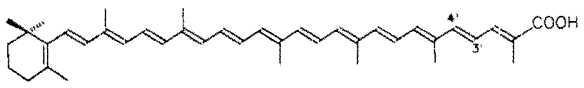
(XX)

(iii) ***glycosyloxy,*** as in myxoxanthophyll [2'-(β-L-rhamno-pyranosyloxy)-3',4'-didehydro-1',2'-dihydro-β,ε-carotene-3,1'-diol] (XXI):



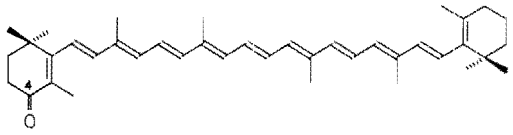
(XXI)

(iv) ***carboxy***, as in torularhodin [3′,4′-didehydro-β,ψ-carotene-16′-oic acid] (XXII):



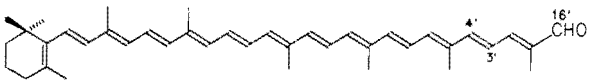
(XXII)

(v) ***oxo***, as in echinenone [β,β-caroten-4-one] (XXIII):



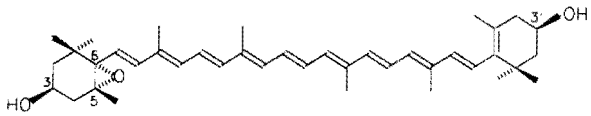
(XXIII)

(vi) ***aldehyde***, as in torularhodin aldehyde [3′,4′-didehydro-β,ψ-carotene-16′-al] (XXIV):



(XXIV)

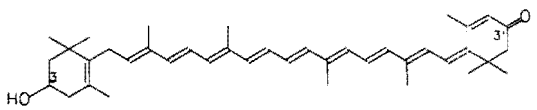
(vii) ***epoxy***, as in antheraxanthin [5,6-epoxy-5,6-dihydro-β,β-carotene,3,3′-diol] (XXV):



(XXV)

c. Retro-carotenoids

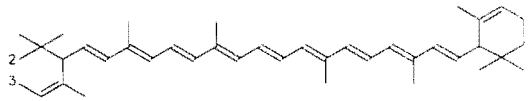
Retro-carotenoids are those in which all single and double bonds of the conjugated polyene system have shifted by one position. The carbon atoms delineating the conjugated systems are indicated in the systematic numbering. The prefix *retro* is preceded by the pair of locants, e.g.,



3-Hydroxy-6′,7-*retro*-β,ε-carotene-3′-one (XXVI)

d. Seco-carotenoids

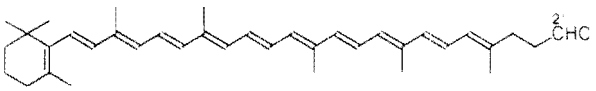
Seco-carotenoids is a carotenoid which undergoes fission of the bond between two adjacent carbon atoms (other than carbon atoms 1 and 6) of a cyclic end group, with retention of the C_{40}-carbon skeleton, e.g.,



2,3-Seco-ε,ε-carotene (XXVII)

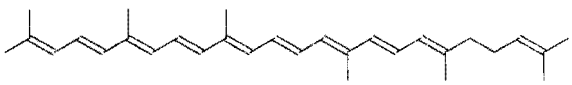
e. Apo-carotenoids

A carotenoid in which the carbon skeleton has been shortened by the formal removal of fragments from one or both ends is named apo-carotenoid, e.g.,



2′-Apo-β,ψ-caroten-2′-al (XXVIII)

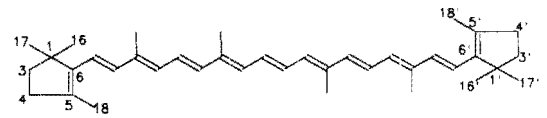
For purposes of nomenclature, the C_{30} carotenoids are treated as apo-carotenoids, e.g.,



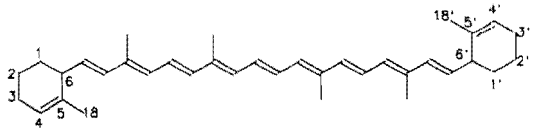
4,4′-Diaponeurosporene (XXIX)

f. Nor-carotenoids

In *nor*-carotenoids, carbon atoms have been formally removed from the carotenoid by processes other than cleavage of carbon-carbon double bonds. Examples of these are



2,2′-Dinor-β,β-carotene (XXX)



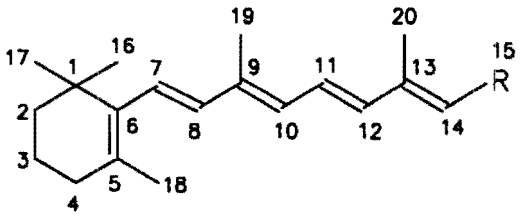
16,17,16',17'-Tetranor-ε,ε-carotene (XXXI)

g. Higher Carotenoids

Until recently, no natural carotenoid was known in which the carbon skeleton contained more than 40 carbon atoms. Over the last few years, however, studies on the pigments of various nonphotosynthetic bacteria have revealed the occurrence of a number of C_{45}- and C_{50}-carotenoids, the higher carotenoids.[4] These are a class of hydrocarbons and their oxygenated derivatives consisting of more than eight isoprenoid units joined in a manner similar to that of the C_{40}-carotenoids. They are named as mono- or di-substituted C_{40}-carotenoids.

h. Retinoids

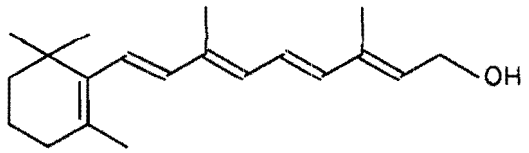
For numbering the carbon atoms of the retinoids, the Commission has recommended that the system described for carotenoids[38] is to be adopted. The suggested representation of the skeletal formula is as given in (XXXII):



Skeletal formula of retinoid (XXXII).

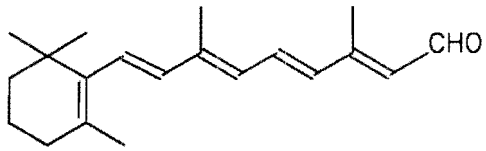
Structures of the three naturally occurring retinoids are

(i) ***retinol***, also known as vitamin A, vitamin A alcohol, vitamin A_1, vitamin A_1 alcohol, axerophthol, or axerol.



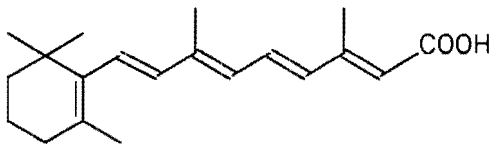
Retinol (XXXIII)

(ii) ***retinaldehyde,*** also known as retinal, vitamin A_1 aldehyde, retinene, or retinene$_1$. The name *retinal,* however, is to be avoided so as not to be confused with the adjective retinal (pertaining to the retina).



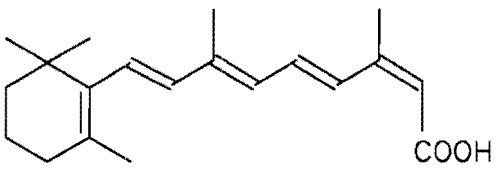
Retinaldehyde (XXXIV)

(iii) ***retinoic acid,*** also known as vitamin A acid, vitamin A_1 acid, or tretinoin,

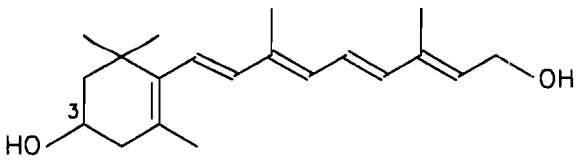


Retinoic acid (XXXV)

On the basis of this numbering scheme, geometric isomers or substituted compounds can be named unambiguously, e.g., 13-*cis*-retinoic acid or isotretinoin (XXXVI), and 3-hydroxy-retinol (XXXVII).



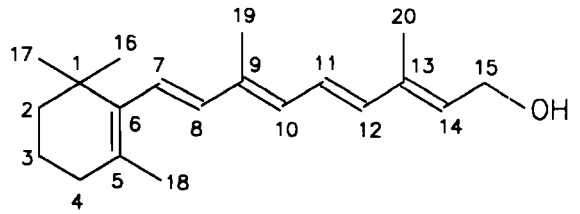
13-*cis*-Retinoic acid (XXXVI)



3-Hydroxyretinol (XXXVII)

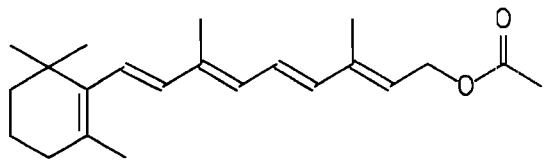
To name the retinoids systematically, however, a different numbering scheme has to be used.[40,42] The carbon atom bonded to the functional group is given the number 1.

The systematic name for retinol is thus all-*trans*-3,7-dimethyl-9-(2,6,6-trimethylcyclo-hex-1-en-1-yl)-nona-2,4,6,8-tetraen-1-ol.

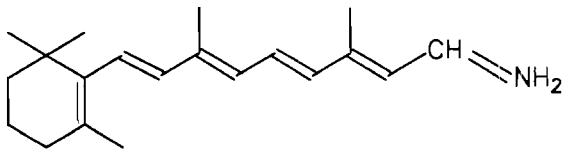


Retinol (XXXIIIA)

Functional substitution at the C-15 position (see [XXXII] above) of the basic hydrocarbon is denoted by the use of the group names retinyl (R is CH_2-) or retinylidene (R is CH=), with retention of the original numbering of the basic hydrocarbon. Examples of these are

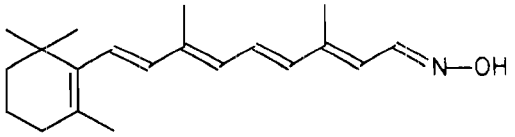


Retinyl acetate (XXXVIII)

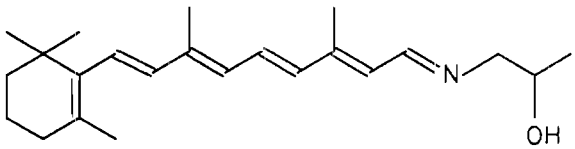


Retinylamine (XXXIX)

Compounds derived from retinaldehyde are named either as aldehyde derivatives or as compounds substituted by the bivalent retinylidene moiety, e.g.,

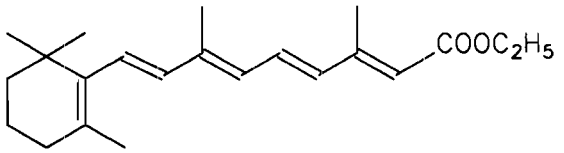


Retinaldehyde oxime (XXXX)

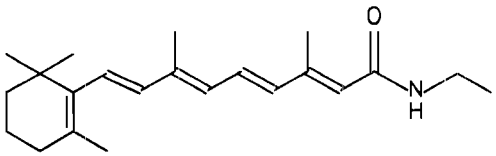


N-Retinylidene-1-aminopropan-2-ol (XXXXI)

Similarly, derivatives of retinoic acids are named as carboxylic acid derivatives, e.g.,



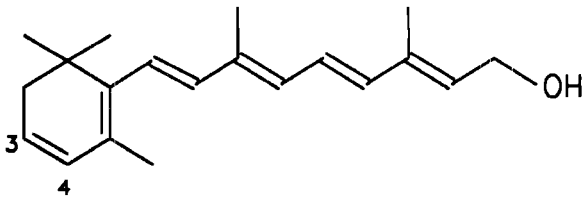
Ethyl retinoate or retinoic acid ethyl ester (XXXXII)



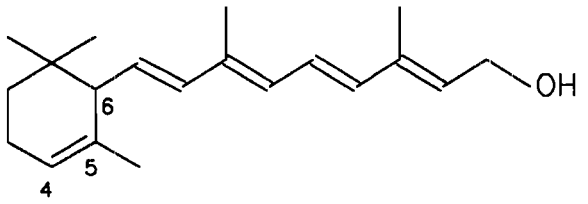
N-Ethylretinamide or retinoic acid ethylamide (XXXXIII)

i. "Hydro" and "Dehydro" Retinoids

Retinoids that differ in hydrogenation level from the corresponding parent compound are named by use of the prefixes "hydro" and "dehydro" together with locants specifying the carbon atoms at which hydrogen atoms have been added or removed. These prefixes are nondetachable and, if both occur in one name, are cited in the order dehydro before hydro. Example of these are



3,4-Didehydroretinol (also known as dehydroretinol or vitamin A_2) (XXXXIV)



4,5-Didehydro-5,6-dihydroretinol, also known as α-vitamin A (XXXXV)

j. Other Retinoids

Other modifications covered by the IUPAC-IUB report[40] include the substituted retinoids, *seco*-retinoids, *nor*-retinoids, and *retro*-retinoids. These are generally similar to those described for carotenoids.

C. *Cis* and *Trans* Isomerization

It is now known that most, if not all, carotenoids can be converted into mixtures of geometrical isomers *in vitro* under appropriate conditions. The principal methods are refluxing in an organic solvent, photochemical, contact with active surfaces, and irradiation in the presence of iodine.[39,43]

A corollary to these methods is that inadvertent isomerization may be induced by prolonged heating of carotenoid solutions (e.g., refluxing in light petroleum, hexane, or benzene for 30 min can isomerize up to 40% of a carotenoid), or by exposure of carotenoid solutions at room temperature to sunlight or artificial light (50% of all-*trans* zeaxanthin can be lost in this way from a benzene solution in 15 min).[39] Isomerization can also result from prolonged contact with active surfaces, for example, when the carotenoid is adsorbed on alumina. The most commonly used method of stereomutation is to expose a solution of the carotenoid, containing catalytic amounts of iodine, to light (iodine-catalyzed photoisomerization).

The number of theoretically possible isomers of a carotenoid is determined by the number of sterically effective double bonds, and by the symmetry of the molecule (more isomers being possible in the asymmetrical molecule). For a conjugated system with n noncyclic double bonds, the number of stereoisomers N is given by the expressions:[43]

$N = 2^n$ for unsymmetrical systems

$N = 2^{(n-1)/2} \cdot (2^{(n-1)/2} + 1)$ for symmetrical systems, n odd

$N = 2^{(n/2)-1} \cdot (2^{n/2} + 1)$ for symmetrical systems, n even

Thus, β-carotene (9 sterically effective double bonds, symmetrical) has 272 possible isomers, and lycopene (11 effective double bonds, symmetrical) should be capable of existing in 1056 isomers. However, because of steric hindrance between lateral methyl groups and olefinic protons, some of the double bonds are "hindered", leaving four "unhindered" (methyl-substituted) bonds in the case of β-carotene and six in the case of lycopene. Hence, the numbers of sterically unhindered isomers are much fewer than

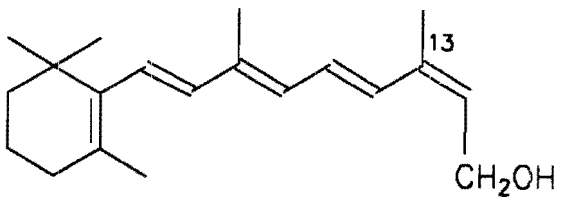
those theoretically possible, 20 being expected for β-carotene and 76 for lycopene.[39]

The stereomutation products of all-*trans* carotenoids exhibit certain characteristic features.[39] *Cis* carotenoids are generally less stable than their all-*trans* isomers. They are also more soluble than the corresponding all-*trans* compounds. The melting points of *cis* isomers are lower (by 20 to 120°C) than those of their all-*trans* counterparts. Because of stereomutation on fusion, some *cis* isomers exhibit double melting points. The most important of these features, from the analytical point of view, is the relationship between *cis-trans* isomerism and UV-vis light absorption and the different adsorption affinities.

Cis carotenoids exhibit light absorption of lower intensity than their all-*trans* isomers and, as a general rule, the principal light absorption maxima are shifted to shorter wavelengths (approximately 2 to 5 nm for one and 10 nm for two sterically "unhindered" *cis* double bonds). One of the most noticeable features in the spectra of mono-"unhindered"-*cis* carotenoids is the appearance of a subsidiary peak at about 340 nm, in the near-UV region. The wavelength difference between this *cis* peak and the longest wavelength maximum of the all-*trans* compound is said to be rather constant, 142 ± 2 nm for C_{40}-carotenoids possessing 10 or 11 conjugated double bonds. When compared on a molar basis, *cis* carotenoids show less intense absorption than their all-*trans* isomers.[43] These changes are illustrated in Figure 1. Other spectroscopic changes (infrared, proton magnetic resonance) in relation to *cis-trans* isomerization have also been described by Weedon.[43]

The geometrical configuration of a carotenoid has a considerable effect on its adsorption affinity. Thus, mixtures of carotenoid isomers can be resolved by the various techniques of adsorption chromatography. The isomerization of an all-*trans* carotenoid may either increase or decrease the adsorption affinity with the result that, in a column chromatogram, some *cis* zones appear above the all-*trans* carotenoid while others run below. In the absence of a precise configurational assignment, *cis* isomers may be named according to their positions on such a chromatogram. Those isomers that are less polar than the all-*trans* carotenoid are neo A, neo B, etc. in order of their decreasing polarity, while those more polar than the all-*trans* form are named neo U, neo V, etc. in order of their increasing adsorption affinities.[39]

For the retinoids, the polyene chain in the parent compound has the *trans* configuration about all the double bonds, unless the contrary is indicated.[40] The stereochemical prefixes E or Z may be used, and should always be used where *cis* or *trans* might be ambiguous. For example, 13-*cis*-retinol (XXXXVI), also known as neovitamin A, is (7E,9E,11E,13Z)-retinol.



(XXXXVI)

Similarly, 13-*cis*-retinoic acid, also known as isotretinoin, is designated as (7E,9E,11E,13Z)-retinoic acid (see [XXXVI] above).

The six known isomers of retinol are all-*trans*, 11-*cis* (neo b), 13-*cis* (neo a), 9,13-*cis* (iso b), 9-*cis* (iso a), and 11,13-*cis* (neo c). The older designations for these isomers are given in brackets. Both the biological activity (Table 7, Section VI.B) and physical properties of retinol, retinaldehyde, and retinoic acid are highly dependent on their isomeric forms.[44]

The isomerization of all-*trans* retinol is stimulated by temperature, light, and iodine. The isomerization of retinaldehyde is particularly well studied due to its involvement as a visual pigment. Details of this are given in Frickel[42] and Olson.[44]

IV. PHYSICAL AND CHEMICAL PROPERTIES

Before 1950, a main tool for the analysis of chromatographic fractions was various color reactions, the most important being the Carr-Price reaction. In this reaction, a polyene dissolved in chloroform reacts with antimony trichloride and

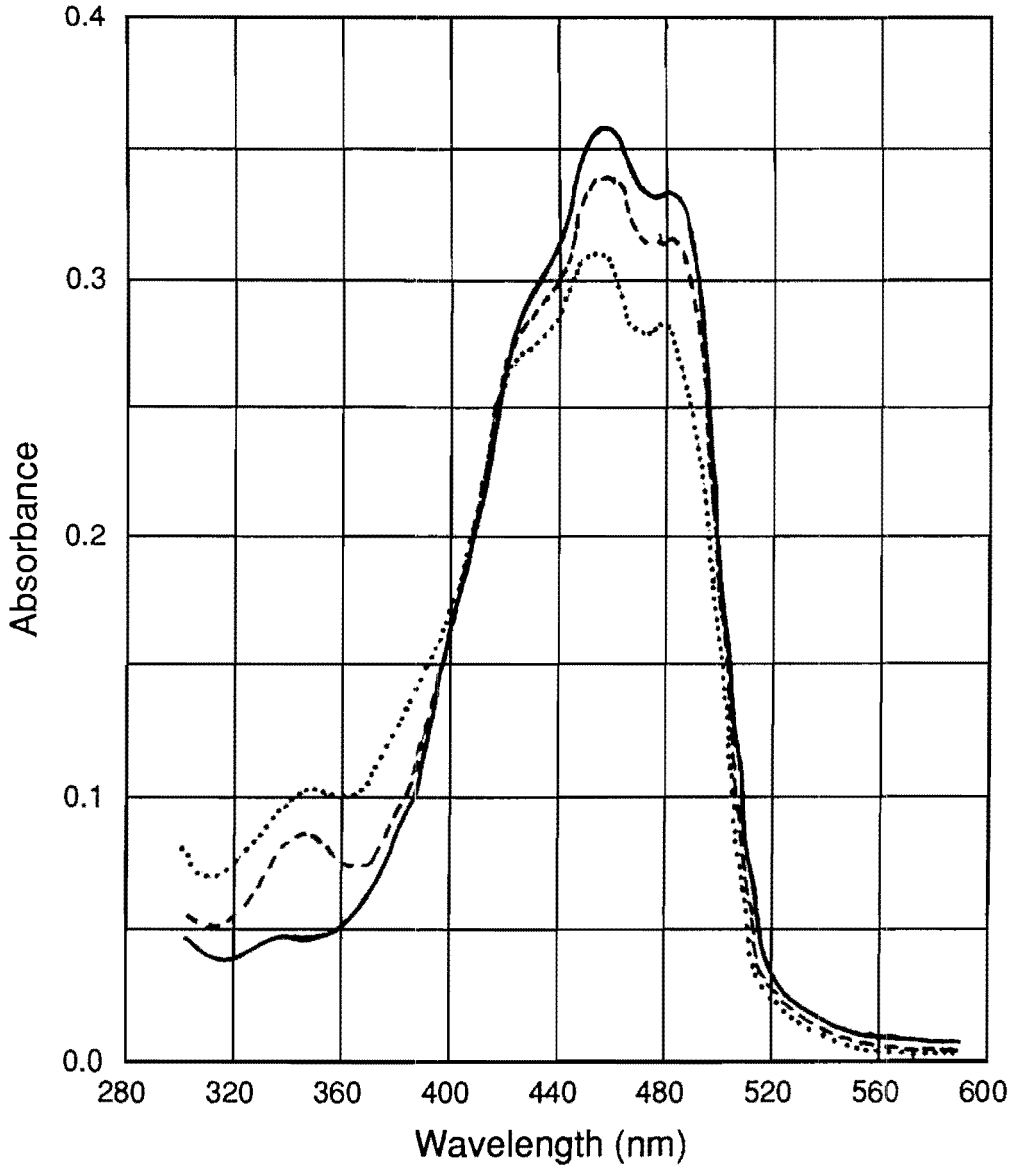


FIGURE 1. Light absorption spectra of all-*trans* (——) β-carotene, and cis isomers after treatment with 30 μg (- - - -) and 50 μg (····) iodine per 100 μg carotene.

the transient deep blue coloration measured at 620 nm. This reaction is still used for vitamin A determination. Besides these reactions, C and H analysis were the only methods readily available for characterizing new structures. The availability of a number of physical methods has greatly influenced carotenoid research and the pace of progress has quickened. With the ever-increasing number of synthetic and naturally occurring materials available for comparison, the physical methods led in turn to valuable data and empirical rules. It was possible to develop new methods for the synthesis of polyenes and elucidate new structures of minor or complicated carotenoids.[45]

A. Physical Characteristics

Being a group of very diverse compounds, the carotenoids can be expected to have very different physical characteristics. However, some generalizations in these properties have been given by Borenstein and Bunnell.[3] Carotenoids are said to crystallize in a variety of forms, the color of

the crystals varying from deep red through violet to almost black. Their melting points are usually fairly high and tend to increase with increasing molecular weight and functional groups. For example, β-apo-8′-carotenal, β-carotene, and canthaxanthin have melting points of 136 to 140°C, 170 to 182°C, and 208 to 210°C respectively. The conjugated double bond system of the carotenoids renders the crystalline materials very sensitive to oxidative decomposition when exposed to air. The carotenoids are insoluble in water, slightly soluble in vegetable oils, moderately soluble in aliphatic and aromatic hydrocarbons, and very soluble in chlorinated hydrocarbons (e.g., chloroform).

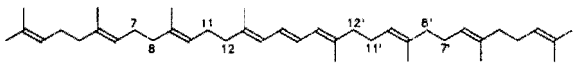
Some physicochemical data of vitamin A and esters are summarized in Table 2. Physicochemical characteristics of several *cis*-isomers of the vitamin have been tabulated by Schwieter and Isler.[46]

B. Absorption Spectroscopy

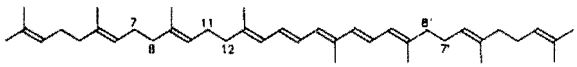
This technique has been the cornerstone for the characterization of carotenoids for more than 50 years. It is still the diagnostic tool most easily available to many laboratories.

The characteristic absorption spectrum of a carotenoid is determined by the number of double bonds and of various additional structural features, e.g., the substituents and *cis-trans* isomerism, and by the solvent.[45]

First, with regard to an increase in a chain double bond, it has been shown that this results in an increase of the absorption maximum varying from 7 to 35 nm.[45,48] An example of this is seen in going from *trans*-phytoene (7,8,11,12, 7′,8′,11′,12′-octahydrolycopene) (XXXXVII) to *trans*-phytofluene (7,8,11,12,7′,8′-hexahydrolycopene) (XXXXVIII), which involves the extension of the chromophore by two double bonds. The increase in absorption maximum is 61 nm, i.e., from 286 to 347 nm (Table 3).



trans-phytoene (7,8,11,12,7′,8′,11′,12′-octahydrolycopene) (XXXXVII)



trans-phytofluene (7,8,11,12,7′,8′-hexahydrolycopene) (XXXXVIII)

When a ψ (psi)-end group of lycopene cyclizes to a β (beta)-end group, as in γ-carotene, there is a hypsochromic shift (shift to higher frequencies, i.e., lower wavelength), a loss of fine structure, and a reduced intensity (hypochromic effect). These effects are even more pronounced upon formation of a second ring as in β-carotene. These changes are illustrated in Figure 2.

TABLE 2A
Physicochemical Data of Some Vitamin A Compounds

Physical characteristics	Vitamin A alcohol	Vitamin A acetate	Vitamin A palmitate
Appearance	Pale yellow prism crystals	Yellow prismatic crystals	Yellow amorphous or crystalline
Melting point (°C)	62–64	57–60	27–29
Molecular formula	$C_{20}H_{30}O$	$C_{22}H_{32}O_2$	$C_{36}H_{60}O_2$
Molecular weight	286.44	328.5	524.8
Solubility (g/100 ml)	Soluble in most organic solvents; soluble in fats and oils; insoluble in water, glycerol		
Spectrophotometric data			
λ_{max}	325 nm (in ethanol)	326 nm (in ethanol)	325–328 nm (in ethanol)
$E^{1\%}_{1\ cm}$	1835	1550	940–975

Adapted from Reference 47.

TABLE 2B
Physicochemical Data of Some Vitamin A Compounds

Physical characteristics	Vitamin A aldehyde	Vitamin A acid	Vitamin A_2 (dehydroretinol)
Appearance	Orange crystals	Crystals	Yellow crystals or oil
Melting point (°C)	61–64	180–182	17–19
Molecular formula	$C_{20}H_{28}O$	$C_{20}H_{28}O_2$	$C_{20}H_{28}O$
Molecular weight	284.42	300.44	284.42
Solubility (g/100 ml)	Soluble in most organic solvents; solution in fats and oils; practically insoluble in water		
Spectrophotometric data			
λ_{max}	373 nm (cyclohexane)	350 nm (ethanol)	288, 352 nm (ethanol)
$E^{1\%}_{1\ cm}$	1548	1510	820; 1450

Adapted from Reference 47.

TABLE 3
Visible Light Absorption Properties of Some Isoprenoid Polyene Hydrocarbons[a]

Polyene	Number of conjugated double bonds	Principal light absorption maxima (nm)		
Phytoene	3	298	286	276
Phytofluene	5	366	347	331
ζ-Carotene	7	425	401	380
α-Carotene	8	449	421	398
Neurosporene	9	470	440	416
ε-Carotene	9	471	440	418
δ-Carotene	10	487	456	431
Lycopene	11	504	472	443
1,2-Dihydro-3,4-dehydrolycopene	12	518	483	457
3,4-Dehydrolycopene	13	535	500	468
3,4,3′,4′-*bis*Dehydrolycopene	15	540	510	480

[a] Determined on dilute solutions of the all-*trans* isomers in light petroleum or hexane. From Moss, G. P. and Weedon, B. C. L., in *Chemistry and Biochemistry of Plant Pigments*, Vol. 1, 2nd ed., Goodwin, T. W., Ed., Academic Press, London, 1976, chap. 3. With permission.

The effect of *trans* → *cis* isomerization (stereomutation) is the appearance of a subsidiary *cis* peak about 140 nm below the absorption maximum of the longest wavelength of the corresponding all-*trans*-carotene. There is also a lower extinction coefficient. These effects were discussed in greater detail in Section III.C.

The solvent in which the absorption spectrum of a carotenoid is measured has a marked effect on the position of the maxima and on the molecular absorbance of the compound. These variations, with β-carotene as an example, are given in Table 4. Figure 3 shows the absorption spectra of β-carotene in several solvents. A more complete tabulation of the absorption maxima of some well-known carotenoids in various solvents has been given in Davies.[39]

From the beginning of research into reti-

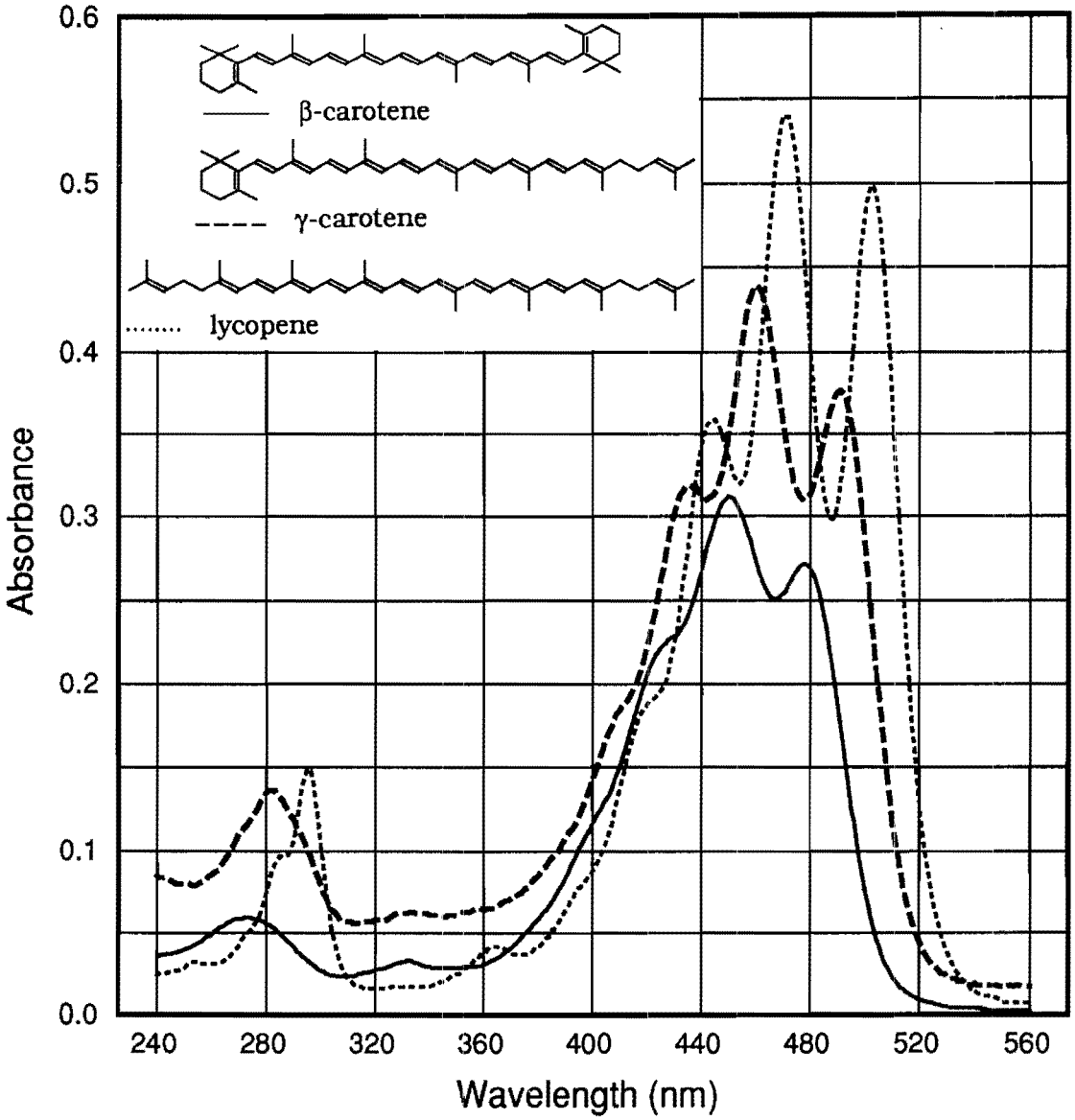


FIGURE 2. Ultraviolet and visible light absorption spectra of β-carotene (——), γ-carotene (- - - -), and lycopene (····) in hexane.

noids, absorption spectroscopy has been employed as an aid in the characterization of structure.[42] UV absorption characteristics of some vitamin A compounds have been included in Table 2. Similar data for the *cis*-forms of retinol, retinaldehyde, and retinoic acid have been tabulated by Morton.[50]

Retinol and its esters fluoresce in UV light. This fluorescence property of retinol has been extensively studied and has been used in the assay of the vitamin. Retinol emits a strong yellow-green fluorescence, with excitation at 327 nm and emission at 510 nm.

C. Infrared Spectroscopy

This technique has not been used routinely for identifying carotenoids, mainly because the conjugated polyene system gives rise to only very weak bands. With the advances in other spectroscopic techniques, IR spectroscopy has become

TABLE 4
Effect of Solvent on Absorption Maxima and Absorbance of β-Carotene

Solvent	Position of main absorption maximum (nm)	$E^{1\%}_{1\ cm}$
Hexane	453	2592
Cyclohexane	457	2505
Benzene	465	2337
Chloroform	465	2396
Methylene chloride	463	2448
Carbon disulfide	484	2008
Dioxan	461	2471
Ethanol	453	2620

Adapted from Reference 49.

even less widely used. It has, however, proved to be of value for detecting certain special structural features such as acetylenic, allenic, hydroxy, and unreactive keto groups, e.g., in fucoxanthin and capsanthin. Vetter et al.[45] and Moss and Weedon[48] have tabulated some infrared absorption bands of carotenoids.

Similarly, neither the retinol nor the retinaldehyde series show characteristic differences for the various isomers in IR spectroscopy.[46] Frickel[42] made only a brief mention of IR absorption spectra in his comprehensive account of the physical properties of retinoids.

D. Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy, particularly proton magnetic resonance (PMR), has been described as a very important and effective tool for the elucidation of carotenoid structures.[45] New developments in ^{13}C-magnetic resonance spectroscopy are said to suggest that this technique will prove to be an even more powerful method for structural studies.[48] Detailed discussions on the use of NMR spectroscopy in the study of carotenoids have been given by Vetter et al.[45] and Moss and Weedon.[48]

NMR spectroscopy has also proved useful in studies of retinoids. A large number of ^{1}H-NMR and ^{13}C-NMR spectra of geometric isomers of the naturally occurring retinoids and of their synthetic derivatives have been published. This data bank makes it possible in most cases to determine the configuration of a novel retinoid or carotenoid structure rapidly and reliably. Moreover, the efficiency of commercial instruments has been improved greatly in the past few years, so that very small amounts of the material are required to obtain the NMR spectra. In combination with a chromatographic method such as HPLC, it is possible to achieve a reliable structure assignment, even in the case of very small amounts of a retinoid as obtained, for example, when a retinoid is isolated from biological material. Frickel[42] has given a detailed account of the use of NMR spectroscopy in structural studies of retinoids.

E. Mass Spectrometry

MS is said to have had tremendous impact on the elucidation of carotenoid structures, particularly when frequently not more than 100 μg of a pigment are required for analysis. Three different kinds of information useful for structural analysis can be obtained from a mass spectrum:[45]

1. The molecular weight and, if measured to an accuracy of a few parts per million, the elementary composition of a compound.
2. Certain structural features may be deducible from the fragmentation pattern, provided that spectra of a number of structurally related compounds are available for comparison.
3. Proof of the identity of the different samples; mass spectra usually contain numerous peaks and are easily reproducible, so that they are generally considered to be excellent for this purpose.

Vetter et al.[45] have also discussed some problems in using MS for investigating carotenoid structure. Because of the partial decomposition of samples during vaporization for MS, reproducibility of the mass spectra of carotenoids is said to be poor. This poor reproducibility does not, however, normally interfere with the use of MS for molecular weight determinations. It was

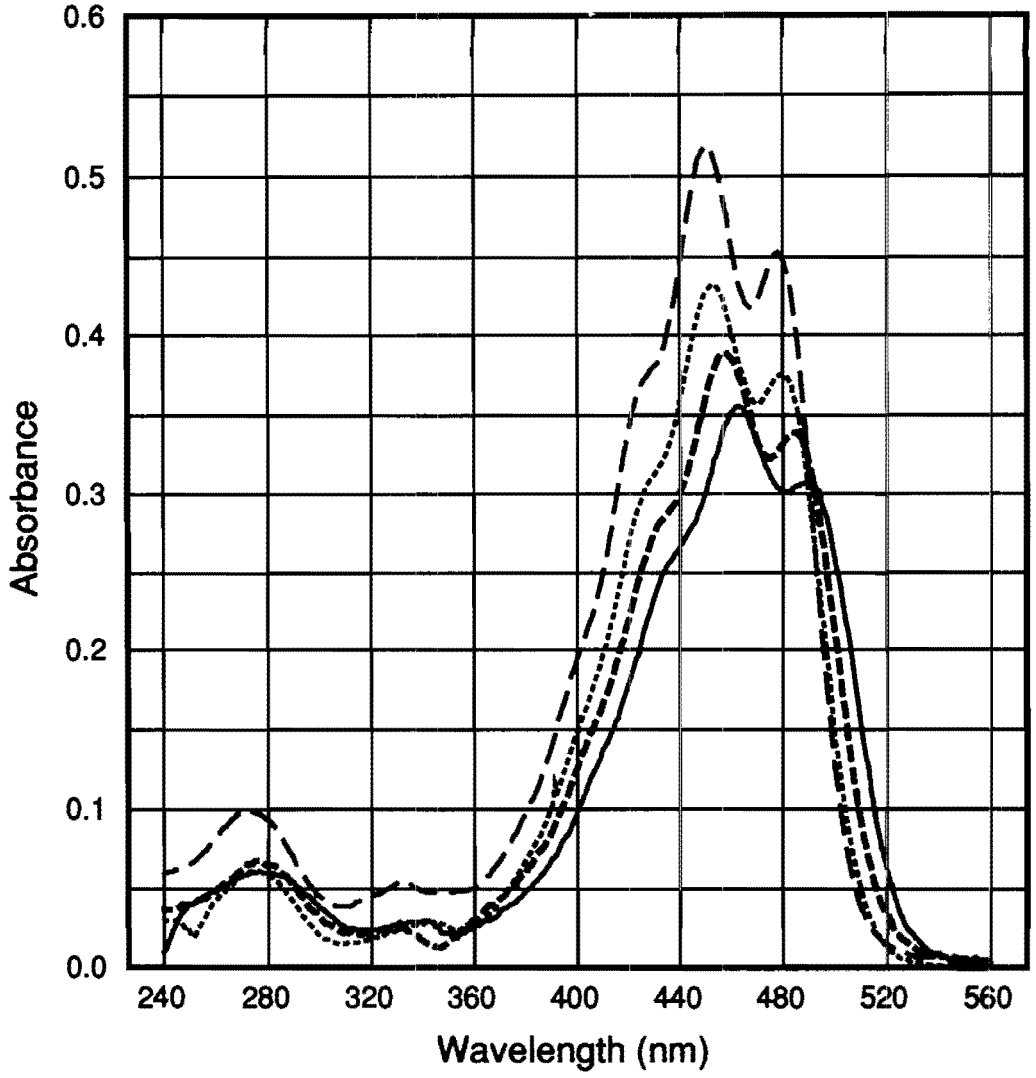


FIGURE 3. Absorption spectra of β-carotene in chloroform and dichloromethane (——), tetrahydrofuran (- - - -), ethyl acetate (····), and *n*-hexane (— — — —).

nevertheless felt that MS remains a very useful tool for the elucidation of carotenoid structure.

A detailed discussion of the use of MS in carotenoid study has been given by Vetter et al.[45] and Moss and Weedon.[48] Both publications also include a compilation of the mass spectra data of various carotenoids which should serve as good reference materials.

F. Chemical Properties

1. Reactions of Carotenoids

Most of the discussions in the literature on the chemical properties of carotenoids refer to the use of these reactions in assisting the structural elucidation of these compounds. Carefully chosen chemical tests are said to be useful in confirming suggestions of structure arising from spectroscopic evidence and are often crucial in the recognition and differentiation of the number and type of the functional groups. Comprehensive reviews on the subject include those of Goodwin,[8] Davies,[39] moss and Weedon,[48] and Liaaen-Jensen.[51] Some of these reactions are discussed below.

Oxidative degradation of carotenoids had been carried out classically by ozonolysis. Oxidative cleavage of carbon-carbon double bonds could

be achieved, and from the carboxylic acids produced, conclusions were drawn concerning the end groups of carotenoids. In the early structural studies on carotenoids, much valuable information was obtained by partial degradation using chromic acid and permanganate. Stepwise degradation of carotenoids allowed early workers to isolate large degradation products such as apocarotenals and ketonic products, which permitted conclusions to be drawn concerning the structure of the natural carotenoids.[51]

Most carotenoids are stable to alkalis, hence the common use of saponification as an early step in the purification process.[8]

Treatment of a carotenoid containing a conjugated carbonyl group with sodium borohydride in ethanol or tetrahydrofuran will result in a dramatic change in absorption spectrum.[8] There is a hyposochromic shift of 20 to 30 nm and an enhancement of the fine structure of the spectrum.

2. Reactions of Retinoids

In the absence of antioxidants, retinol and its derivatives are very unstable toward oxygen. In practical terms, oxidation is the most important cause of destruction. Traces of metallic ions, notably copper and iron, catalyze the oxidation.[2] The degradation process can be monitored by the rapid decrease of the extinction at 328 nm. The oxidation products are said to be ill defined, usually exhibiting a rather broad band at 270 to 280 nm.[46]

Other chemical oxidizing agents are also a threat to unprotected vitamin A structures. Selective mild oxidizing agents, such as manganese dioxide, can transform retinol into retinal. On the other hand, reducing agents such as lithium aluminium hydride or borohydrides can reduce retinoic acid or retinal to retinol.[1]

Vitamin A is extremely sensitive toward acids, which can cause rearrangement of the double bonds and dehydration, followed eventually by the addition of the solvent and by *cis-trans* isomerization.[46] Ethanolic hydrochloric acid and hydrobromic acid are particularly reactive in this respect.[2] In the absence of oxygen, retinol and its derivatives are stable toward alkali.

Light, together with iodine, catalyzes bond isomerization to the unstable 11-*cis* and other more stable isomers. In light of higher intensity, other more drastic photochemical reactions take place, leading to dimerization to form kitol and other polymers.[1]

Acidic reagents give transient — mainly blue — color reactions with vitamin A. The brilliant blue color obtained with the Carr-Price reagent (antimony trichloride in chloroform), with an absorption maximum at 620 nm, has become a widely used color reaction for vitamin A determination. Other reagents that have been used include trifluoroacetic acid,[52] trichloroacetic acid,[53,54] and, 1,3-dichloropropan-2-ol.[55] The fading of the color in these tests is rapid and makes quantitative measurement difficult. They are, however, especially useful for qualitative or comparative measurements. The use of the reaction for the biochemical assessment of nutritional status is discussed in Section IX.B.2.b.

Chemically, dehydroretinol or vitamin A_2 is much more sensitive to the effects of oxygen than retinol and decomposition occurs rapidly. In other aspects, it resembles retinol in many of its chemical properties. There is some evidence that, in addition to the all-*trans* isomer, the 13-*cis* isomer occurs naturally. It is however relatively unimportant in mammals and has been shown to have only 30 to 40% of the biological activity of retinol.[2]

V. METABOLISM

A. Biosynthesis of Carotenoids

Comprehensive accounts of the biosynthesis of carotenoids are given in Goodwin[56] and Britton.[57] The following summary has been condensed from the above, and particularly from Goodman and Blaner[58] and Simpson and Chichester.[59]

Although C_{30}, C_{45}, and C_{50} carotenoids are produced by some nonphotosynthetic bacteria, most naturally occurring carotenoids (in particular those of higher plants) are C_{40} tetraterpenes, biosynthesized by the well-established terpenoid pathway. The basic feature of these compounds is the repeating isoprenoid 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.

The ubiquitous acetyl coenzyme A is the basic carbon source in the synthesis of carotenoids, three molecules of which result in the formation of 3-hydroxy-3-methyl-glutaryl coenzyme A (Figure 4). This is then converted to mevalonic acid, a pivotal compound in the synthesis pathway. Phosphorylation and decarboxylation of this give rise to isopentenyl pyrophosphate, which is also a key compound, serving as the building block for successive C-5 units. Condensation of two molecules of isopentenyl pyrophosphate (after isomerization of one of them to dimethylallyl pyrophosphate) yields the 10-carbon compound geranyl pyrophosphate. Subsequent addition of 5-carbon isoprene units (via isopentenyl pyrophosphate) leads to the formation of the 15-carbon and 20-carbon intermediates, namely, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, respectively. In the pathway of sterol biosynthesis, two molecules of farnesyl pyrophosphate condense to form one molecule of squalene, which is subsequently cyclized to the sterol ring structure. In carotenoid biosynthesis, in contrast, two molecules of geranylgeranyl pyrophosphate condense to form the first common carotenoid precursor, the 40-C hydrocarbon phytoene. This is then converted to progressively more unsaturated compounds, leading to the formation of lycopene. Lycopene is then cyclized to the monocyclic δ-and γ-carotenes, which in turn are further cyclized to yield α- and β-carotene, respectively. The introduction of oxygen results in the formation of the xanthophylls.

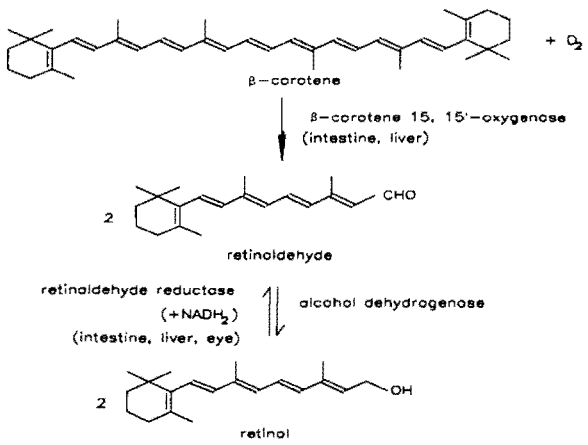


FIGURE 4. Biosynthetic formation of carotenoids. (From Simpson, K. L. and Chichester, C. O., *Annu. Rev. Nutr.*, 1, 351, 1981. With permission.)

B. Conversion of Carotenoids to Vitamin A

After the existence of fat-soluble vitamin A was well recognized and accepted, considerable confusion arose during the 1920s regarding two quite different types of substances showing vitamin A activity in animals.[60] One group was the highly colored hydrocarbon compounds found extensively in the plant kingdom and collectively called carotenoids. The other compound is almost colorless and is concentrated in the liver. This confusion was ultimately resolved following two important studies in the early 1930s. Moore[16] demonstrated that rats fed massive amounts of carotenoids extracted from carrots deposited large amounts of vitamin A in their livers. At about the same time, the classical studies of Karrer et al.[12,13] decisively established the chemical relationship between β-carotene and vitamin A. It became clear that carotene acts as a precursor for vitamin A and is converted to the vitamin in the animal body.

The conversion of β-carotene to retinol is an important biological process since, throughout the course of evolution, most, if not all, of the vitamin A utilized by animals was ultimately derived from this process. Although retinol has been synthesized chemically by several procedures in recent years, human requirements of vitamin A depend largely on dietary sources of the provitamins.

After absorption, the carotenoid is cleaved in the mucosal cell of the intestine to form vitamin A. The enzymatic cleavage of β-carotene into retinaldehyde with soluble enzyme preparations from rat intestinal mucosa and liver was reported by Goodman and Huang.[61] Subsequent studies have shown the mechanism of the reaction to be that of a dioxygenase reaction, in which molecular oxygen reacts with the central two carbon atoms of β-carotene, followed by the cleav-

age of the central double bond of carotene to yield two molecules of retinaldehyde[62,63] (Figure 5). The enzyme has been designated as β-carotene 15,15'-dioxygenase. Partially purified forms of the enzyme have been obtained from rat, hog, and rabbit intestines. However, because of lability during purification, the pure enzyme has not yet been isolated.[58]

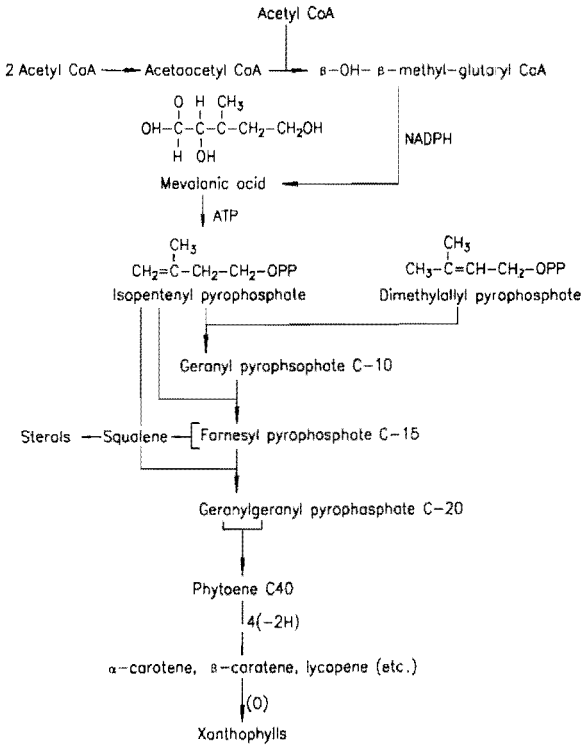


FIGURE 5. Conversion of β-carotene to retinol. (From Frolik, C. A., in *The Retinoids*, Vol. 2, Sporn, M. B., Roberts, A. B., and Goodman, D. S., Eds., Academic Press, Orlando, FL, 1984, chap. 11. With permission.)

According to Ganguly and Sastry,[60] the biological conversion of carotene into vitamin A may not be as straightforward as by a cleavage of the central double bond. He pointed out that, aside from this central cleavage theory, another theory has proposed a random cleavage of the carotene molecule. This discussion will not delve into these two different theories, which have been dealt with in some detail in the reviews of Ganguly and Sastry[60] and Glover.[65]

C. Absorption and Utilization

1. Absorption and Factors Affecting

After the ingestion of food, the provitamin carotenoids of vegetables and other plant sources, and pre-formed vitamin A of animal tissues are released from associated proteins by the proteolytic action of pepsin in the stomach, and of chymotrypsin and trypsin in the small intestine. The liberated carotenoids and vitamin A are dissolved in fatty globules which then pass into the lumen of the duodenum. Here, the globules encounter the bile salts and the pancreatic enzymes, which release various products of lipid digestion. Vitamin A esters are also hydrolyzed at this stage. These products of digestion then interact with bile salts and cholesterol to form soapy aggregates, termed mixed micelles, which solubilize the vitamin A and carotenes. These micelles diffuse into the glycoprotein layer surrounding the microvilli or brushborder of the mucosal cells, where they come into contact with the cell membranes. The components of the micelles, except the bile salts, then individually penetrate the lipid phase of the mucosal cell membranes to reach the cytoplasm.

The above summary outlines the processes involving the absorption of vitamin A and carotenes. Further details, especially the experimental evidence for the various processes, are given in Wolf,[1] Goodman and Blaner,[58] and Moore.[66] A discussion on the factors affecting the absorption of the vitamin and its precursor is given below, as these have an important bearing on human nutrition.

The efficiency of dispersion of vitamin A and carotenes is known to be affected by the presence or absence of other components in the diet, as well as by the general nutritional status of the subject. First, soluble protein and the peptides derived therefrom are able to assist in the dispersion of the vitamin. Increased protein level in the diet is also known to aid in the intracellular formation of retinaldehyde from carotene,[67] discussed below.

Second, fat in the diet provides the vehicle for transporting the vitamin A and carotenoids

from the stomach into the intestinal lumen and is the source of some of the digestion products which take part in micelle formation, as outlined earlier. In addition, some dietary lipids, such as seed oils, may also contain α-tocopherol, which has a protective antioxidant effect on vitamin A.[67] Studies by Hollander[68] show that the presence of fatty acids of varying chain lengths and the degree of saturation also affect absorption rates.

Third, from the summary of the absorption of vitamin A and carotenoid above, it is clear that an ample supply of bile salts is necessary for efficient absorption.

Carotenes in foods are usually less well absorbed than pre-formed vitamin A from the intestine. Under normal conditions, over 90% of ingested vitamin A is absorbed, and the efficiency of absorption decreases only slowly as the dose increases. On the other hand, about 70% of the ingested β-carotene is absorbed, and the efficiency of absorption decreases rapidly as the dose increases.[69] A wide range of absorption rates has been reported, depending on the vegetable sources. In some vegetables, as low as 5% of the carotene may be absorbed. It has been estimated that carotene in green leafy vegetables is two or three times better absorbed than that from red or yellow root vegetables such as carrrot.[2] Zhao[70] reported absorption rates ranging from 20 to 45% for various leafy vegetables, roots, and tubers. Children and infants are also known to absorb carotene inefficiently.[2] The absorption rate for carotenoids is lower probably because the solubility of carotene and other carotenoids in fat is limited, whereas vitamin A is more or less miscible with fats. In most rich sources, with the notable exception of red palm oil, carotene is present in the tissues of the vegetable in much greater concentration than can be dissolved in the accompanying fat.[66] Being less polar than vitamin A, β-carotene is also solubilized less well by surface-active agents, including the bile salts.[69]

Thus, vitamin A deficiency in human subjects can still arise when the diet supplies carotenes, instead of pre-formed vitamin A, but insufficient fat to facilitate the extraction of the provitamin and its carriage into the intestinal wall.[71] Absorption of carotenoids is also reduced in conditions that upset intestinal function, such as reduced secretion of pancreatic juice or bile, or impaired cell activity.

After absorption, the carotenoid is cleaved in the mucosal cell of the intestine to form vitamin A in the form of retinaldehyde. Current understanding of this conversion process was outlined previously in Section V.B. The retinaldehyde so formed is reduced promptly to retinol. During its passage through the intestinal epithelium, up to 75% of the retinol, irrespective of source, becomes esterified with long-chain fatty acids. These esters, mainly in the form of the palmitate, are then incorporated together with other lipids, and with apolipoproteins, into chylomicron particles that are secreted from the cell into the lymph.[58,67]

Some unconverted carotenoids are directly absorbed and pass into the blood where their composition reflects the diet. They may be deposited in the liver or elsewhere, such as the fat depots and and various organs.[59]

2. Uptake and Storage in Liver

After leaving the intestinal cells, retinyl esters in low density lipoproteins of the plasma are probably hydrolyzed by esterases on liver cell membranes. Intracellularly, unesterified retinol is bound by a specific cellular retinol-binding protein (cRBP) and transported to the endoplasmic reticulum, where it is again esterified, largely to the palmitate ester. This is then transferred and stored in a soluble complex macromolecule made up of various lipids, various polypeptide chains, and carbohydrates linked covalently to the protein.[67]

Vitamin A is remarkable for its preference of the liver as its site of storage and for its stability when stored.[66] Hepatic vitamin A (95% as long-chain retinyl esters, mostly retinyl palmitate) normally represents over 90% of the total body reserves of vitamin A.[58] In any individual, the magnitude of the store will depend not only upon the dietary intake of vitamin A and its provitamins, but also on the efficiency of absorption of the vitamin and its rate of expenditure. These factors may, in turn, be influenced by sex, rate of growth, state of health, etc.[1,66]

3. Transport in Plasma

As outlined above, the liver rapidly immobilizes and stores vitamin A which has reached the bloodstream after absorption from the diet. In the reverse direction, the liver can release vitamin A into the bloodstream to maintain a constant level in the blood even when the diet is deficient in the vitamin.[66]

Whereas absorbed dietary retinol is carried to the liver mainly as its ester in complex with lipoproteins, delivery of the vitamin to the target tissues is in the alcohol form. This is achieved by the hydrolytic action of retinyl ester hydrolase, which is also present in the storage complex. Upon hydrolysis, retinol is directly transferred from its binding site in the storage complex onto a protein, a retinol-binding protein (RBP) for transport to other tissues.[67] The RBP is the primary carrier for retinol in the plasma, and can exist as holo-RBP, i.e., a 1:1 molar complex with retinol, and as apo-RBP, which is free of retinol. Various detailed reviews of RBP, including its chemical structure, metabolism, and synthesis, have been given by Wolf,[1] Glover,[72] Chytil and Ong,[73] and Goodman.[74]

VI. BIOLOGICAL ACTIVITY

A. Vitamin A Activity of Carotenoids

Over 60 years ago, Steenbock[75] first conceived that yellow color might be associated with biological activity; and in 1930, Moore showed by biological criteria that β-carotene is converted to vitamin A. There is now considerable literature on the vitamin A activity of carotenoids, and recent reviews include those of Bauernfeind,[5] Bauernfeind et al.,[76] and Underwood.[77]

For vitamin A activity in mammals, it is now known that a carotenoid provitamin A compound must have at least one unsubstituted β(beta)-ionone ring with an attached polyene side. The other end of the molecule may vary in cyclic or acylic structure and can be lengthened but not shortened to less than an 11-carbon polyene fragment. β-Carotene possesses two β-ionone rings, one at either end of a long polyene chain, and is a provitamin A with high activity. α- and γ-carotene, each with one β-ionone ring, are biologically active at approximately half the β-carotene value.[65]

Because of the importance of carotenes (particularly β-carotene) and other carotenoids as a main source of vitamin A supply in human nutrition, many efforts have been made to evaluate the exact vitamin A potencies of individual carotenes and carotenoids (reviewed in Reference 78). The activity of the carotenoid vitamin A precursors can be illustrated in a number of ways: (1) by feeding the carotenoid to an intact animal under prescribed bioassay conditions and noting tissue changes, blood, liver, etc., or effect on growth; and (2) by using a purified or partially purified carotenoid cleavage enzyme in contact with the carotenoid under *in vitro* conditions and measuring retinaldehyde formation.[5]

Bauernfeind et al.[76] tabulated the vitamin A activities of some pure carotenoids measured in biological trials under well-defined laboratory conditions. WHO[78] has used the relationship of 1 μg of β-carotene as equivalent to 0.167 μg of retinol or 0.56 IU of vitamin A. This has gained acceptance (see References 77 and 79) and is also used in the Malaysian Food Composition Table.[80]

The earlier publication of Borenstein and Bunnell[3] estimated that only 6 or 7 of the more than 100 carotenoids could have provitamin A activity. More recently, Simpson and Chichester[59] estimated that 50 to 60 carotenoids and apocarotenoid compounds could have provitamin A activity. However, only a few of the identified carotenoids have both vitamin A activity and occur in significant amounts in natural foods commonly eaten by vertebrate animals.[5]

Table 5, summarized from a more comprehensive tabulation from Bauernfeind,[5] tabulates vitamin A activity of some carotenoids present in foods, expressed as a percentage of the β-carotene activity, the latter taken as 100%. It includes various carotenoid hydrocarbons. Some compounds, such as 5,6-monoepoxide and 5,8-monofuranoxide, are believed to possess biological activity despite the alteration of the β-ionone ring. This is presumably due to *in vivo* conversion back to the unaltered ring structure.[5] Oxygenated carotenoids are also biologically active and include cryptoxanthin, isocryptoxanthin, 5,6-dihydroxy-β-carotene, citranaxanthin, and torula-

TABLE 5
Provitamin A Activity of Some Common Carotenoids

Carotenoid	Activity (%)
β-Carotene	100
α-Carotene	50–54
3,4-Dehydro-β-carotene	75
γ-Carotene	42–45
7′,8′-Dihydro-γ-carotene (β-zeacarotene)	20–40
β-Carotene 5′,6′-monoepoxide	21
β-Carotene 5′,8′-monofuranoxide (mutatochrome, citroxanthin, flavacin)	50
3-Hydroxy-β-carotene (cryptoxanthin)	50–60
4-Hydroxy-β-carotene (isocryptoxanthin)	48
5,6-Dihydroxy-β-carotene	Active
β-Apo-2′-carotenal	Active
β-Apo-8′-carotenal	72
β-Apo-10′-carotenal	Active
β-Apo-12′-carotenal	120
β-Apo-8′-carotenoic acid	Active
Torularhodin	Active <50
Citranaxanthin	44

Adapted from Reference 5.

rhodin. Cryptoxanthin, present in maize, contributes significantly to vitamin A intake when high levels of this grain are present in animal feed.

As discussed in Section III.C, a great number of possible geometrical isomers can exist in the case of carotenoids. An all-*trans* configuration in the side chain provides higher biological activity than any of the *cis* isomers. However, because of the relative ease of *trans-cis* isomerization in solution, it is sometimes difficult to decide whether a *cis* isomer occurs in nature or whether it is formed during its isolation. One view of the biological activity of the *cis* isomers is that during digestion and/or absorption they are rotated, at least partially, on their asymmetric C atoms to the linear all-*trans* form.[5] It was, however, also pointed out that it is not known for certain whether such transformation must first take place before it can be converted to vitamin A.

The mechanism of cleavage of carotenoids to form vitamin A was discussed in Section V.B.

B. Activity of Vitamin A Congeners

The biological activity of the vitamin A molecule may be affected by (1) *cis-trans* isomerism; (2) other changes in the general structure of the molecule; (3) the substitution of the terminal hydroxyl radical by other radicals. Many laborious biological tests, mainly with rats and chicks, have been made to compare the activities of vitamin A and its numerous congeners. In general, minor changes from the structure of all-*trans* vitamin A leads to a reduction in biological activity. Gross changes, such as saturation of double bonds, usually cause complete loss of activity.[66]

Esterification of the hydroxyl group has no fundamental effect on activity, although minor differences may result if the efficiency of absorption is affected. Oxidation of retinol to the aldehyde also has little effect on activity. *Cis*-isomers of retinol have reduced activity, ranging from 23% for 11-*cis* and 9-*cis* isomers to about 75% for the 13-*cis* isomer.[50]

The vitamin A_2 analogs of all the above substances, which differ only in having an additional double bond between C-3 and C-4 in their aromatic rings, are generally considerably less active than the corresponding vitamin A congeners.[66] All-*trans* dehydroretinol (compound number XXXXIV in Section III.B.2.i), for example, possesses approximately half the activity of all-*trans* retinol.[50]

Retinoic acid is not converted to retinol, but lies on the pathway of the metabolism of retinol, possibly to an active metabolite.[1] It has high activity in the general system, but not in the visual system.[47,66] Its activity appears to depend greatly on the method of dosing. According to van Dorp and Arens,[81] it has 10% of the activity of retinol if given orally dissolved in arachis oil, 50% if given as the sodium salt by injection, and 100% if given as the sodium salt orally.

The interconversion of the various congeners of vitamin A after digestion by animals is a rather complicated problem, and many areas appear to be unclear. Conversions appear to occur readily between vitamin A and its esters, and between retinol and retinaldehyde. Some degree of *cis-trans* isomerism presumably occurs since the retina seems to be able to obtain from dietary supplies of the all-*trans* retinol the particular isomer of retinaldehyde which it requires for rhodopsin formation. On the whole, however, changes in the animal body between *cis* and *trans* isomers are much less extensive than might be expected. There seems to be no clear evidence of conversions between vitamin A_1 and vitamin A_2 derivatives.[66]

Wolf[1] has summarized the requirements for biological activity as: provided the substance reaches its target tissue (e.g., the tracheal epithelium), a hydrocarbon ring (five- or six-membered or aromatic) linked to a hydrocarbon chain of four conjugated double bonds with two methyl groups attached in the mode of two isoprene units, and ending with almost any functional group (polar terminal group), can have vitamin A activity in maintaining epithelial differentiation.

C. Units and Equivalency

In the early years of the study of vitamin A, there was considerable confusion concerning the exact activity of the vitamin since few pure preparations of either vitamin A or of the provitamin A were available. Various criteria have been used for the determination of vitamin A activity in rat assays, such as increase in body weight, occurrence of xerophthalmia, and changes in vaginal epithelium determined by vaginal smears.[82] Biological tests to determine the activity of the vitamins were tedious and did not produce reproducible results when comparing data obtained in different laboratories employing different types of animals, cages, temperature conditions, and even basic diets.

In order to arrive at some degree of uniformity in comparing results, early workers then decided on reference standards. These were single preparations of a compound either isolated from natural sources or synthesized in the laboratory, and maintained and distributed by an international agency such as the Health Organization of the League of Nations. These international standards were of varying degrees of purity and were modified from time to time as purity improved through technique or synthesis. These original standard preparations are no longer in existence, as each of the individual vitamins is now readily available in high purity.[83]

Because of the variation in the food sources of vitamin A activity in diets, i.e., pre-formed and precursor, a common mode of expressing their vitamin A value was needed. Vitamin A activity of foods was expressed in IU where 1 IU of vitamin A is equivalent to 0.3 μg of retinol, 0.344 μg of retinyl acetate, or 0.55 μg of retinyl palmitate; and 1 IU of provitamin A is equivalent to 0.6 μg of β-carotene or 1.2 μg of provitamin A carotenoids other than β-carotene.[69,78,84] Because of the considerably poorer utilization of dietary provitamins as compared with retinol, the expression of the total vitamin A activity of a diet has to be qualified by indicating the percentages of the activity coming from retinol and from provitamins.[69]

The use of IUs caused some confusion among scientists in estimating dietary vitamin A activity.[85] In the 1967 WHO report, it was recommended that the practice of expressing vitamin A values in terms of IU be discontinued in view of the availability of crystalline retinol as a ref-

erence standard. It was suggested that units of weight should be used instead, i.e., micrograms of retinol. The report also discussed the relative biological activities of the various vitamin (and provitamin) A compounds, and the calculation of vitamin A value of diets. These are outlined in the following paragraphs.

The major source of vitamin A in the diet of most communities is β-carotene. As has been discussed in the previous section on some aspects of metabolism, β-carotene is not utilized as efficiently as retinol by the human body. Based on the structure of β-carotene and retinol, one molecule of the provitamin can be expected to be enzymatically converted to two molecules of vitamin A. However, the overall biological efficiency of conversion, when absorbed by the intestine, is at best only about 50%, and occurs at lower levels of carotene intake, declining as intake rises.[77,78]

The variable efficiency of conversion is said to be more a function of the efficiency of absorption into mucosal cells, rather than in the efficiency of conversion to retinol from absorbed β-carotene.[77] Increasing levels of carotenoid intake are inversely related to the efficiency of absorption, and this, in turn, is influenced by various factors, including the character of the mixed diet, e.g., the amount of fat, the antioxidant content, and the digestibility of the carrier food. The WHO[78] report gave an extensive tabulation of studies of human carotene absorption, which showed wide variations. It was suggested that, for practical purposes, the best approximation for absorbability of β-carotene, in man, should be assumed to be one third (33%) of the amount ingested.[78]

Hence, on a weight basis, one sixth of the β-carotene found in foods is estimated to be converted to vitamin A in the body. Since dietary retinol is assumed to be 100% utilized by humans, 1 μg of β-carotene in the diet is taken to have the same biological activity as 0.167 μg of retinol.[78] Since the activity of other carotenoids varies considerably, some having no activity and others about 50% of that of β-carotene, this Committee of the WHO also accepted that the activity of other total mixed carotenoids (with vitamin A activity) be taken as one half of that of β-carotene.[78]

Subsequently, the United States National Academy of Sciences (NAS)[79] described the use of the term "retinol equivalent":

1 retinol equivalent
= 1 μg of retinol
= 6 μg of β-carotene
= 12 μg of other provitamin A carotenoids
= 3.33 IU of vitamin activity from retinol
= 10 IU of vitamin A activity from β-carotene

The total vitamin A value, in micrograms, of a food or composite diet would thus be calculated as the amount of retinol plus the equivalent amount of retinol that can be derived from the provitamins:[83]

$$\mu g \text{ retinol} + \frac{\mu g \text{ β-carotene}}{6} + \frac{\mu g \text{ provitamins}}{12}$$

designated as micrograms of retinol equivalents (RE).

The formula may also be rewritten:[77]

$$RE = [\mu g \text{ retinol}] + [0.167 \times \mu g \text{ of β-carotene}] + [0.083 \times \mu g \text{ of other provitamin carotenoids}] \quad (1)$$

The NAS[79] report has given some examples of calculating the retinol equivalents in a diet or foodstuff, based on the above-mentioned relationships.

Bieri and McKenna[83] have emphasized that the term "retinol equivalent" is a dietary concept for approximating the vitamin A activity in foods. It is not an equivalency in the usual chemical sense. It was further noted that the vitamin A value for many foods will be a rough approximation. When the form of vitamin A in the food is predominantly retinol, the analysis for retinol will be a reasonably accurate estimation of the retinol equivalents. On the other hand, when the provitamins are the predominant or only form of vitamin A, then the calculation of RE may contain a considerable error because of the approx-

imations made regarding the activity of the carotenoids.

D. Units Used in Reported Values

The food composition tables commonly used in the U.S. have expressed vitamin A values in IU, or both as IU and as RE.[77] The equivalency values of the IU and RE were discussed in Section VI.C. These values have been used for calculating the vitamin A values reported in food tables. Since analytical methods used for determination of many food tables are not able to provide values for individual provitamin A carotenoids, the conversion has usually been made based on the assumption that most of the carotenoids are β-carotene. For foods in which the provitamin A carotenoid consists wholly or almost completely of β-carotene, e.g., green leaves, the values for vitamin A activity obtained from such calculations are good. On the other hand, the values are poor if other partially active carotenoids are present in significant quantities.[86]

Food composition tables all over the world have adopted different formats for reporting vitamin A values of foods. Beecher and Khachik[87] have also highlighted this lack of uniformity in the presentation of these data. Food tables for use in England[88] have provided separate values on a weight basis (microgram) for retinol and carotene. Total vitamin A values were not tabulated. The authors pointed out that carotene values in most foods are for β-carotene; thus, the total vitamin A activity calculated from these values could be slightly overestimated.

A recent edition of the Japanese food tables[89] have also tabulated carotene and retinol values separately in micrograms. However, total vitamin A activity was expressed as "retinol potency" and given in IUs.

The FAO food tables for use in East Asia[90] have tabulated, in micrograms, the retinol content and the "β-carotene equivalent" of the foods. No total vitamin A values were given. Other tables using this format are the Australian[91] and the Philippines[92] food tables.

The food tables for Taiwan[93] and Thailand[94] have tabulated all foods in IUs of vitamin A. In the food tables compiled by China National Centre for Preventive Medicine,[95] carotenes are tabulated for plant foods, in micrograms, whereas vitamin A values in IU are given for foods of animal origin.

The Malaysian Food Composition Table[80] has also tabulated the retinol and carotene values separately on a weight basis (micrograms). Total vitamin A activity, expressed as RE, has been calculated based on the formula discussed in the previous section, namely:

$$\mu g \text{ retinol} + \frac{\mu g \text{ } \beta\text{-carotene}}{6}$$

As discussed above, the limitation of vitamin A value so calculated is that it has been assumed that the carotenoids determined are β-carotene. Such an assumption is, of course, not necessarily valid and depends a great deal on the foodstuff. More accurate vitamin A values can become available only when the various carotenoids can be separately determined.

VII. OCCURRENCE

A. Occurrence of Carotenoids in Plant Materials

The ability to produce carotenoids seems to have been developed at an early stage in evolution. Photosynthetic bacteria, the algae, spore-bearing vascular plants, and the higher plants preserve this capability. Animals, certainly the higher orders, are not capable of *de novo* carotenogenesis and are dependent for their carotenoids on those present in their diet.[4] This discussion is concerned mainly with those carotenoids present in higher plants, which are the more important food sources.

Between 1933 and 1948, the number of known naturally occurring carotenoids increased from 15 to about 80, and in the early 1970s,[7] rose sharply to about 300. A decade and a half later, Goodwin[96] reported that over 500 were known. Most of these, however, are xanthophylls which, as discussed previously, are oxygenated carotenoids. The common name and the semi-systematic equivalent, together with the structure of about 300 natural carotenoids, have been listed

in the 800-reference publication of Straub.[97] In another publication 5 years later, Straub[98] updated the list to 400 compounds in a 1600-reference monograph. Bauernfeind[5] has tabulated the occurrence of some 40 common carotenoids in various foodstuffs.

It has been estimated that nature produces about 100 million tons of carotenoid pigments per year.[99] most of this output is in the form of four major carotenoids,[4] viz., fucoxanthin, the characteristic pigment of many marine algae and undoubtedly the most abundant natural carotenoid, and the three main carotenoids in green leaves, i.e., lutein, violaxanthin, and neoxanthin. All other carotenoids are produced in relatively small amounts. However, some, like β-carotene and zeaxanthin, occur very widely; and others, such as lycopene, capsanthin, bixin, and spirilloxanthin, constitute the principal pigments in a particular organism.

How, and in which chemical and physical form, carotenoids occur in nature is of importance, particularly to the food technologist involved in the processing and development of new food products. These aspects are also important to the nutritionist because carotenoids are sources of vitamin A. The overall carotenoid pattern may vary from relatively simple mixtures to extremely complex ones. The simplest mixtures may be found in foods of animal origin due to the limited ability of the animal to absorb, modify, and deposit carotenoids. The other extreme is the formidable array of carotenoids encountered in, for example, citrus products, dehydrated alfalfa meal, or paprika.[3,5]

The concentrations in which carotenoids occur vary enormously from one source to another. The highest concentration of carotenes has been found in the red fringe of the corona of the pheasant's-eye narcissus, *Narcissus majalis*. Here β-carotene can constitute up to 16% of the dry weight. Furthermore, the daily rate of β-carotene formation reaches 70 μg/mg dry weight, which is over 10,000 times the rate observed in carrot roots.[100]

The following is a summary of the distribution of carotenoids in various plant materials, emphasizing particularly those products that are of importance to human nutrition. Data presented include those reported some years ago (generally prior to 1970) using column chromatography and thin-layer chromatography, as well as those obtained more recently (after the 1970s) using HPLC. HPLC procedures have become more widely used for the analysis of carotenoids in foods, mainly because of the ability of the technique to effect rapid separation, its nondestructiveness, and (more importantly) the better resolution that is achieved.[6] Since older analytical methods have been known to be insufficiently specific for the quantitation of individual carotenoids, it is probable that some of the data quoted using these methods could be inaccurate.

1. Vegetables

Various studies (summarized by Goodwin[101]) have shown that leaves of higher plants usually contain the same carotenoids: β-carotene, lutein, violaxanthin, and neoxanthin. Pigments which appear frequently, but not constantly, and in smaller amounts are α-carotene, mutatochrome (β-carotene-5,8-epoxide), β-cryptoxanthin, lutein 5,6-epoxide, eloxanthin, taraxanthin, zeaxanthin, and antheraxanthin. The colorless polyenes, phytoene and phytofluene, are known to be rather widely distributed, but only at levels about one two-hundredth that of β-carotene. *Cis* isomers of β-carotene were also reported earlier, but it is now thought that they rarely, if ever, occur naturally, and are more likely to be artifacts of isolation.

The constancy of the qualitative distribution in leaves of high plants is remarkable and is said to be applicable to the most diverse plants from varying habitats.[101] In 35 green leafy and nonleafy tropical vegetables studied by Tee and Lim[86] using HPLC, the composition of the carotenoids was strikingly similar, with lutein and β-carotene invariably present as the major carotenoids. The root and fruit vegetables (generally colored) studied showed slightly greater variations in carotenoid composition. There are, however, considerable quantitative variations of these carotenoids. β-Carotene in the vegetables was found to vary from 20 to 85% of the total carotenoids. Similarly, total carotenoid content of these vegetables varies widely, ranging from <0.2 mg/100 g edi-

ble portion for lettuce to >35 mg/100 g for several of these vegetables.[86]

2. Seeds

In mature cereal seeds used in human nutrition, the carotenoid level is about 100 μg/100 g fresh weight, of which 10% is carotene.[102] β-Carotene is usually the only carotene present, but α-carotene is said to be the major pigment in millet. Total carotenoid in varieties of sorghum with yellow endosperm falls within the range 8.4 to 118 μg per 100 g, and the percentage of β-carotene varies from "trace" to 26%. The xanthophyll mixture in cereal seeds is complex and generally similar to that in green leaves, with lutein as the major component.

Maize (yellow corn) is widely used as food in the fresh, frozen, and canned forms, and is also processed in the form of corn flour, meal, and grits. The pigments of maize have been studied extensively by various investigators. Indeed, zeaxanthin and β-cryptoxanthin were first isolated from this source.[102]

Total carotenoid content in maize has been reported to range from 1 to 5.5 mg/100 g.[5] There are, however, considerable qualitative and quantitative variations in the carotenoids of maize varieties and hybrids grown in different countries. Quackenbush et al.[103] studied 125 strains of maize and reported the presence of 11 pigments. The major pigments are phytoene, lutein, and zeaxanthin. Others are phytofluene, β-carotene, β-zeacarotene, ζ-carotene, α-cryptoxanthin, β-cryptoxanthin, zeaxanthin esters, and polyhydroxy pigments.

Sweet corn is another commonly consumed food item. Lee et al.,[104] in one of the few recent studies available that determines individual carotenoids in several cultivars of processed sweet corn, reported monohydroxy carotenoids as the major carotenoids present, followed by hydrocarbon and polyhydroxy carotenoids. The first group consisted mainly of zeinoxanthin and β-cryptoxanthin. Zeinoxanthin was, in fact, the major carotenoid in all four cultivars studied. Hydrocarbon carotenoids consisted mainly of α-, β-, and γ-carotenes, and β-zeacarotene. Based on the detail composition available, vitamin A values of the sweet corn cultivars were calculated to range from 5.0 to 7.7 RE.

Green soya bean (*Soja glycine*), cow pea (*Vigna sinensis*), Lima bean (*Phaseolus lunatus*), pea (*Pisum sativum* var. Thomas Laxton), and French beans (*Phaseolus vulgaris*) have been found to contain 200 to 700 μg/100 g (fresh weight) of carotene.[102] The qualitative distribution in peas and beans is said to be probably very similar to that in leaves.

3. Fruits

There are relatively more data on the occurrence of carotenoids in fruit. In ripening fruit, the decrease in chlorophylls is frequently accompanied by an increasing concentration of carotenoids and by an increase in the ratio of carotenes to xanthophylls.[3] Goodwin[102] has tabulated the qualitative distribution of carotenoids in 150 types of fruit. The quantitative distribution of total carotenoids and β-carotene in 50 fruits are also tabulated in this publication. The changes in the distribution of carotenoids during ripening of fruits were also discussed. A discussion of the distribution of carotenoids in several individual fruits is given in Borenstein and Bunnell.[3]

Unlike the leafy vegetables, fruits tend to show greater variations in carotenoid composition. β-Carotene was invariably present, but its proportion varied considerably, ranging from 100% in a variety of Thai mango to less than 10% for watermelon. β-Cryptoxanthin, known to possess provitamin A activity, was found in significant proportions in most of the fruits studied. Vitamin A activity of the fruits was much lower than that of the leafy vegetables.[86]

A fruit that has particular significance in carotenoid content is oil palm (*Elaeis guineensis*). The pericarp of the fruit yields the deep-red colored palm oil, which has been well known for its unusually high content of carotenoids. Unrefined palm oil is one of the richest known natural sources of β-carotene.[105] The food composition table for use in Africa has tabulated the β-carotene equivalent content of palm oil as ranging from 37,300 to 128,700 μg/100 g.[106] A later study of 30 samples of palm oil bought from market places in Nsukka, Nigeria, Mudambi, and

Rajagopal[107] reported β-carotene equivalent contents ranging from 85,920 to 165,200 μg/100 g, with a mean value of 137,835 μg/100 g. The Malaysian food table has given an entry of 57,100 μg/100 g.[80]

Early studies on the fractionation of carotenoids in palm oil have been reported by Hunter and co-workers.[108,109] Recently, Tan et al.[110] reported the detailed separation and identification of carotenoids in several Malaysian palm oil processed fractions. Seven previously unreported hydrocarbon carotenoids were identified, namely, phytoene, phytofluene, ζ-carotene, α-zeacarotene, β-zeacarotene, neurosporene, and δ-carotene. In addition, the presence of a α-, β-, and γ-carotenes and lycopene was confirmed. Crude palm oil, crude palm olein, and filtered palm olein were found to have a total carotenoid concentration ranging from 70,000 to 80,000 μg/100 g, and most of this was β-carotene. On the other hand, refined, filtered, and deodorized palm olein was devoid of hydrocarbon carotenoids due to the rigorous refining process the fraction had undergone. The carotenoid profile of palm kernel oil was also rather different, containing only 30 μg/100 g of α-zeacarotene.

Crude palm oil, with its high carotene content, is of particular nutritional importance. It has been consumed for years in Africa. Latham[111] has urged the wider cultivation and consumption of palm oil in Africa for the prevention of vitamin A deficiency. Locally, the importance of palm oil in the treatment of vitamin A deficiency has long been emphasized.[112] However, the available palm oil currently marketed for cooking has been refined to the extent that carotene content has been reduced to a low level. Although a red-colored crude palm oil may not be acceptable to consumers, some effort could be made to accustom them to a semi-refined oil. Such an oil would retain some of its β-carotene content as well as other carotenoids which, although not important as vitamin A precursors, may provide beneficial health effects. In addition, a good proportion of the tocopherols, which are present in high concentrations in palm oil, could be retained. Chong[104] has reviewed the beneficial properties of palm oil, including effects on development of coronary heart disease and cancer.

4. Roots

The most important plants from the point of view of root carotenoids are the carrot and the sweet potato. In fact, the term carotenoids and/or carotene is believed to have been derived from the fact that they constitute the major pigment in the carrot root, *D. carota*.[5] The major pigments are α-, β-, and γ-carotenes; and traces of lycopene, δ-carotene, ζ-carotene, neurosporene, phytoene, and phytofluene have been reported. The relative amounts of α-carotene to β-carotene may vary from 5–10 to 51% depending on the strain; deep orange-red strains usually have relatively less α-carotene than β-carotene. The usual values for the carotene content of carrots are 6 to 12 mg/100 g fresh weight.[102] The xanthophyll fraction of commercial carrots is only 5 to 10% of the total pigments, but the percentage rises to 75 to 93% in yellow carrots, and to at least 95% in wild carrots.[102]

Carrot has been studied by various investigators in recent years using HPLC.[6] Using a combination of HPLC and thin-layer chromatography, Simon and Wolff[113] studied in detail the carotenoid composition of several genetically diverse collections of carrots. Total carotenoid was reported to range from 6.3 to 54.8 mg/100 g fresh weight. Six carotenes were identified, namely, α-, β-, γ-, ζ-carotene; β-zeacarotene; lycopene. The major carotenoids were, however, α- and β-carotene, together contributing over 90% of the total carotenoids. Of the two carotenes, the latter was found in higher concentrations, accounting for 44 to 79% of all carotenoids. Similarly, Heinonen[114] also reported α- and β-carotene as the major carotenes in a number of carrot cultivars studied. Lutein was reported as a minor carotenoid in the samples studied.

The carotenoids of sweet potatoes (*Ipomea batatas*) have received considerable attention,[3] and are of particular importance to this region since it is an important item in the diet. β-Car-

otene is also the main pigment present in sweet potatoes and only traces of xanthophylls are found. Singh and Bradbury[115] provided more recent data on carotenoids in several South Pacific root crops. Vitamin A value of the crops studied were, in decreasing order, yam > sweet potato > *Colocasia* taro > *Xanthosoma* taro > giant taro.

B. Distribution of Retinoids

Vitamin A is found exclusively in animals, where it occurs as the free alcohol and also in esterified form. It is widely found in mammals, birds, amphibia, reptiles, and marine fish. Lower marine invertebrates usually do not contain pre-formed vitamin A. Crustacea usually contain only carotenoids, commonly astaxanthin in crabs, lobsters, and shrimps.[116]

Livers of the vertebrate classes mentioned are the richest food sources of pre-formed vitamin A because of the special role of the liver in the uptake, storage, and controlled release of dietary pre-formed and biosynthesized vitamin A.[77] Before the availability of chemically synthesized vitamin A, the principal source of vitamin A concentrates was, in fact, the liver and/or body oils of marine fish.[117] The vitamin is believed to be synthesized by fishes from the large amounts of carotenoids they ingest with the plankton, algae, and smaller crustaceans that form their diet.[116] Animal milk and eggs contain pre-formed vitamin A in variable amounts, depending on the diet of the animals from which they are obtained. Among free-living herbivores, the level is said to fluctuate with the carotenoid level in the diet.[77]

Recent data on retinol and carotenoid content of foods using HPLC have been provided by various investigators. Retinol and carotenoids in various foods in the Netherlands were determined separately by two HPLC procedures, and the results used for calculation of vitamin A value of total diets.[118] Using a novel approach, Tee and Lim[119] reported the simultaneous determination of retinol and the content of major carotenoids of 40 foods of animal origin. β-Carotene content in the foods studied was generally low, and the contribution of other provitamin A carotenoids is probably insignificant. One of the few studies on ready-to-eat foods was reported by Heinonen et al.[120] for a number of Finnish foods. Retinol and several carotenoids (β-carotene, lutein, and lycopene) in the foods were determined by two HPLC procedures.

In addition to the more commonly found form (vitamin A_1), a second molecular variation known as A_2 (3′-dehydroretinol) exists in the tissues of fresh water fish.[77] The latter has approximately a third of the biological potency of the former. The contribution of this form to human vitamin A intake is significant only in areas where fresh-water fish is eaten. Small amounts of vitamin A_1 aldehyde may be derived from fish roes and from hen's eggs, but their practical importance is probably negligible.[66] Other substances with vitamin A activity are also said to have been found in fish oils. These are, however, usually of considerably lower biological activity than retinol. Examples of these are 13-*cis*-retinol, kitol, anhydroretinol, and rehydroretinol.[2,116] With regard to retinoic acid, Moore[66] pointed out that there is no evidence that it contributes significantly to the vitamin A content of human or animal diets, and that there is, indeed, little or no evidence that it is present at all in natural foods.

VIII. FUNCTIONS AND USES

A. Carotenoids as Precursors of Vitamin A

The varied functions of carotenoids include provitamin A activity, light yellow to dark red food colorants, absorbers of light energy, oxygen transporters, and probably other functions unknown at this time. Although it is incorrect to consider vitamin A activity as a general function of carotenoids, the provitamin A activity of the carotenoids is nevertheless given emphasis in this discussion because of the importance of vitamin A in human nutrition.

Animals are not capable of *de novo* synthesis of vitamin A-active substances, neither pre-formed retinol and its derivatives nor the carotenoid precursor forms.[77] Thus, apart from the pre-formed vitamin A contained in foods such as milk, eggs, liver, and fish liver oils, and their derivatives (and of course synthetic vitamin A), the main sources of supply of this vitamin for

man are the carotenes and related carotenoids. These pigments possess vitamin A activity and are the so-called provitamin A. Indeed, β-carotene is the most important vitamin A precursor in human nutrition since its concentration in food and feed ingredients, particularly of leaf origin, greatly exceeds that of the other vitamin A active compounds.[76]

The diets of population groups in the tropical world rarely contain milk, eggs, or liver, which are the rich sources of pre-formed vitamin A. Thus, there has been a great deal of emphasis on carotenoids, particularly from leafy vegetables, as the sources of vitamin A to these communities. Vitamin A deficiency is prevalent, and the tragic irony is that there is an abundance of vegetation in these countries.[121]

In the U.S., the average diet of the usually available food is estimated to provide about 7500 IU (2250 RE) of vitamin A per day. About 3500 IU (1050 RE) is derived from vegetables and fruits, 2000 IU (600 RE) from fats and oils, and dairy products, and 200 IU (600 RE) from meat, fish, and eggs.[5] Various estimates of percentage contribution of provitamin A to the human diet place the figure at around 50%.[79,122,123]

As discussed earlier, provitamin A activity may be derived from various carotenoids. The above estimates of contributions of carotenoids to total vitamin A activity of diets must be assumed as tentative, until food tables become more complete in listing both β-carotene and other provitamins A.

B. Carotenoids in Photosynthetic Tissues

The other established functions of carotenoids in plants are related to their ability to absorb visible light. In the case of photosynthetic tissues, they appear to have two well-defined functions: (1) in photosynthesis itself, and (2) in protection of the photosynthetic tissue against photosensitized oxidation. Comprehensive reviews of these functions are given by Krinsky,[124] Burnett,[125] and Goodwin.[126]

Carotenoids could act as accessory light-absorbing pigments in the photosynthesis process. By their absorption at wavelengths lower than that absorbed by chlorophyll, they extend the wavelength of light that can be used in photosynthesis.[37]

It has been observed that carotenoid pigments in all photosynthetic organisms, bacteria, algae, and higher plants, play an important role in protecting these organisms against the seriously damaging effects of photooxidation by their own endogenous photosensitizer, chlorophyll. Experiments made in photosynthetic organisms lacking colored carotenoid pigments have supported these observations. Many workers have confirmed these findings and have extended the observations to nonphotosynthetic bacteria, plants, and animals.[127] Extending these data to disease treatment in humans, carotenoids have been used for the treatment of patients with photosensitivity disease. In this disease, known as light-sensitive porphyria, the porphyrins produced resemble the porphyrin ring of chlorophyll and act as photosensitizers in the patient. Mathews-Roth[37,128] reported increased tolerance to sun exposure by erythropoietic protoporphyria patients after β-carotene administration. The use of carotene in the treatment of other photosensitive disorders has been reported.[129]

C. Antioxidant Functions of Carotenoids

Various proposals have been put forth to explain the protective function of carotenoids against harmful photosensitized oxidations discussed in the previous section. Krinsky[127] discussed the major mechanisms whereby these pigments exert this function: (1) quenching of triplet sensitizers; (2) quenching of singlet oxygen ($^1O_2$); (3) inhibition of free radical reactions. The photochemical reactions that can induce photodamage were reviewed, and the possible mechanism of action of carotenoids on the reactive chemical species produced was discussed. In photochemically induced oxidations, carotenoid pigments have been shown to have the capacity to quench the first potentially harmful intermediate, the triplet sensitizer, at a significant rate. The remaining triplet sensitizer species could then continue to initiate a series of reactions, depending on the availability of oxygen and the nature of other potentially reactive species in the environment, with

the production of singlet oxygen and free radicals. These three mechanisms of carotenoid protection against oxidation are summarized in Figure 6. In an updated review of the subject, Krinsky[130] described in some detail these biological actions of carotenoids.

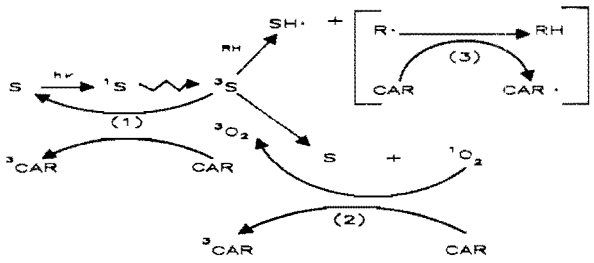


FIGURE 6. Mechanisms of carotenoid protection against oxidations: (1) quench triplet sensitizers; (2) quench singlet oxygen; (3) quench free radical intermediates. hν = light; S = sensitizer molecule; ^1S = singlet excited species; ^3S = triplet sensitizer; $^1O_2$ = singlet oxygen; $^3O_2$ = triplet oxygen; R· = free radical species; CAR = carotenoidies; ^3CAR = triplet state of carotenoid. (From Krinsky, N. I., *Pure Appl. Chem.*, 51, 649, 1979. With permission.)

The ability of carotenoids to deactivate reactive chemical species such as singlet oxygen, triplet photochemical sensitizers, and free radicals has been actively studied in recent years, with the main focus on β-carotene. Some insight into the antioxidant activities of other naturally occurring carotenoids has also been reported. Studying the antioxidant activity of β-carotene and other related carotenoids on the free radical oxidation of methyl linoleate, Terao[131] reported that canthanxanthin and astaxanthin were more effective antioxidants. It was postulated that the introduction of oxo groups at 4 and 4′ positions into these two carotenoids enhanced the antioxidant activity of these compounds. Di Mascio et al.[132] studied the singlet oxygen quenching capability of several carotenoids and reported that lycopene was the most efficient in this action. It would appear that opening of the β-ionone ring to an open chain, as in lycopene, increases the quenching ability.

It has been suggested that reactive oxygen species and free radicals may play an important role in cancer development. These species are continually being formed in human tissues and their safe sequestration is an important part of antioxidant defense. Thus, the protective effects of carotenoids against the harmful effects of oxidation would be expected to have a protective effect against cancer. The role of carotenoids and retinoids in cancer is discussed in Section IX.D.

D. Carotenoids as Food Colors

There is much to the saying: "Man eats with his eyes as well." Thus, color of food is a significant factor in determining its acceptability. Man associates a particular food with its specific "natural" color. He becomes cautious when a food shows an unexpected color, interpreting it as a possible sign of spoilage, poor processing, or adulteration. An important use of carotenoids (especially carotene) is in food coloring.

Natural extracts containing carotenoids have been used for coloring food for centuries: annatto with bixin as the main coloring component, saffron with derivatives of crocetin and other carotenoids, paprika containing the two pigments capsanthin and capsorubin, xanthophyll extracts from leaves, carrot extracts of varying purity, and red palm oil.[3,76]

Several synthetic carotenoids are presently available, making it possible for them to be used widely in coloring processed and fabricated foods.[5] β-Carotene was the first synthetic carotenoid to be marketed in 1954[76] and is now probably the most widely used carotenoid for coloring foods. With the different commercial forms of this carotene, it is technically and economically feasible to color a wide variety of fat- or water-based foods including butter, margarine, cheese, ice cream, wheat products, vegetable oils, cake mixes, candy, soups, desserts, fruit juices, and beverages.

Another commercially available carotenoid, β-apo-8′-carotenal, is used where an orange to reddish-orange shade is desired, such as in toppings, frostings, candies, confections, pastry fillings, cheese sauces, cheese spreads, cake mixes, soups, salad dressings, etc. Canthaxanthin has an unusually high tinctorial potency and is a useful food colorant in the red range. It is used, for example, in tomato products, soups, barbecue

sauces, fruit drinks, simulated meat, and shrimp and lobster products. Hathcock et al.[133] have cautioned against the excessive use of canthanxanthin. Persons consuming large number of tanning tablets (to produce a skin color similar to suntan) containing mainly canthaxanthin were found to have crystalline golden deposits on their retinas. The use of β-apo-8′-carotenal in combination with β-carotene, or either one in combination with canthaxanthin, expands the color range of the individual compounds.[5] A more comprehensive survey of the application of carotenoids in coloring foods and pharmaceuticals is given in Borenstein and Bunnell[3] and Bauernfeind et al.[76]

Bauernfeind[5] surveyed the worldwide legal status of β-carotene, β-apo-8′-carotenal, canthaxanthin, and β-apo-8′-carotenoic acid ethyl ester as food colors. β-Carotene is permitted in some 40 countries, whereas over 20 countries allow the use of the other three carotenoids. The Food Regulations currently enforced in Malaysia also permit the usage of these four pigments in foods.[134]

E. Role of Vitamin A in Vision

The different categories of function of vitamin A in mammals can be broadly grouped under five headings: (1) vision, (2) bone growth, (3) reproduction, (4) maintenance of epithelia, and (5) overall growth.[1] The following discussion concentrates on two of these functions: its role in vision and the maintenance of epithelia.

It has become evident from the work of Wald and Hubbard[135] that the active retinoid in the visual process is 11-*cis*-retinaldehyde, but the biochemical mechanisms of the other biological functions of retinoids, for example, maintenance of epithelial tissues (discussed below), and other functions listed above remain unclear.[1,64]

The only established function of retinol in the retina is to serve as the precursor of 11-*cis*-retinaldehyde, the chromophore of all known visual pigments. An outline of the involvement of the vitamin in the photosensitive system is given below. Comprehensive reviews of the subject have been given by Wald and Hubbard[135] and the more recent publications of Wolf[1] and Bridges.[136]

The retinas of most vertebrate eyes possess two kinds of light receptors: rods, for vision in dim light, and cones, for vision in bright light and color vision. Each of these organs contains a photosensitive pigment which bleaches on exposure to light. Some aspects of this process lead to a nervous excitation, which, transmitted from one neuron to another along the optic pathways to the brain, ends in exciting visual sensations.[135]

Four such visual pigments are widely distributed among vertebrate eyes: rhodopsin and porphyropsin in rods, and iodopsin and cyanopsin in cones. Each is composed of a polyene chromophore united with a specific type of protein, called an opsin. The visual pigments and their opsins are insoluble structural components of the receptor organelles, the photosensitive outer segments of the rods and cones.

Two chromophores are known to be involved, retinaldehyde and 3-dehydroretinaldehyde, together with two main types of opsin, one found in rod outer segments and the other in cone outer segments. The two retinaldehydes, both in the *cis* form, combine with the two kinds of opsins to yield the four main classes of visual pigments. The need for retinol to supply the chromophores of the visual pigments is the reason why, in vitamin A deficiency, the first symptom is a fall in sensitivity of both rod and cone vision, a condition known as "night blindness" or "nyctalopia".[135]

F. Vitamin A in Maintenance of Epithelia

The most striking and extensive lesions caused by vitamin A deficiency are those affecting epithelial growth and differentiation. They are all defects of the outer and inner linings of the body which, consequently, invite invasion by microorganisms. There is generally an increase in the proportion of squamous keratinizing cells (cornification), accompanied by a decrease in the proportion of columnar, mucus-secreting cells. It is, however, known that different epithelia are affected differently. In epithelial tissues such as intestinal mucosa, where there are normally no keratinizing cells, there is simply a decline in mucus-secreting cells. In other tissues such as the cornea and epidermis, where there are nor-

mally no mucus-secreting cells, hyperkeratosis results from the deficiency.[1,67]

In the eye, apart from the defects in the retina caused by disappearance of rhodopsin, many epithelial lesions occur in vitamin A deficiency. These lesions are manifest in both the conjunctiva and cornea as a result of lack of vitamin A for maintaining the normal differentiation of epithelial tissue. The earliest change is in the conjunctiva tissue, resulting in one or more patches of dry, nonwettable conjunctiva, a condition termed conjunctival xerosis. This is sometimes accompanied by the appearance of small plagues of a silvery grey hue, usually with a foamy surface, called bitot's spots. In more severe vitamin A deficiency, the structural changes go on further to involve the cornea. In the beginning, corneal xerosis takes place, giving the cornea a hazy appearance. This progresses to corneal ulceration, frequently referred to as keratomalacia, and may eventually end in impairment of vision in varying degree. These structural changes are described in Section IX.B.2.a.

IX. VITAMIN A IN HUMAN NUTRITION

A. Recommended Levels of Vitamin A Intake

Present knowledge of human vitamin A requirements has been obtained from field surveys and controlled dietary studies. Rodriguez and Irwin[137] have given a thorough review of the studies carried out to determine these requirements. Field surveys provide a general idea of the total vitamin A intake of population groups, with and without overt clinical and biochemical vitamin A deficiency symptoms. They are, however, of limited value in quantitating human vitamin A requirements. Most of the controlled studies have been based on dark-adaptation tests. The role of vitamin A in the visual cycle is the only metabolic function for which the mechanism has been elucidated. Serum vitamin A concentration has been the second most commonly used criterion. Some investigators have also measured growth, susceptibility to infections, and changes in the epithelial tissue. Human vitamin A requirements have also been estimated on the basis of data obtained from controlled animal experiments.

As with other nutrients, recommended levels of intake of vitamin A vary from country to country. This is clearly shown in a study of the recommended dietary allowances (RDA) of 17 countries for 9 nutrients by the Committee on Recommended Dietary Allowances of the International Union of Nutritional Sciences.[138] For vitamin A, the recommended level for adult men and women ranged from 600 to 1500 μg RE, with a mean value of 910 μg RE. The addition amount for pregnancy ranged from 0 to 1000 μg RE, and for lactation, from 200 to 1920 μg RE.

The safe level of vitamin A intake recommended by FAO/WHO[139] is tabulated in Table 6. These levels are considerably different from the 1967 recommendations.[78] The recent recommendations are appreciably lower for adults and older children, but higher for young children.

The recommendations by the U.S. NAS[79] for vitamin A are generally higher than the abovementioned FAO/WHO recommendations. The recommended allowance for adult men is 1000 RE per day, and for women, 800 RE. For pregnancy, an additional 200 RE is recommended,

TABLE 6
Safe Level of Vitamin A Intake

Group	Age (years)	RE per day (μg)
Infants and children (both sexes)	0–1	350
	1–6	400
	6–10	400
	10–12	500
	12 15	600
Boys	15–18	600
Girls	15–18	500
Men	18+	600
Women	18+	500
Pregnant women		600
Lactating women		850

From FAO/WHO, *Requirements of Vitamin A, Iron, Folate and Vitamin B_{12}*, Report of a joint FAO/WHO Expert Consultation, FAO Food and Nutrition Series No. 23, Food and Agriculture Organization, Rome, 1988. With permission.

and for lactation, an extra 400 RE. For children 1 to 3 years of age, however, the recommended allowance of 400 RE per day is the same as the FAO/WHO recommended safe intake.

B. Vitamin A Deficiency

Vitamin A malnutrition, as in other nutrient malnutrition, can be of two kinds, namely, overnutrition and undernutrition. In the former, there is acute or chronic hypervitaminosis A. At the other extreme is hypovitaminosis A or vitamin A deficiency. Hypervitaminosis A is relatively rare and could be caused by self-prescription of large pharmacological doses of vitamin A. In contrast, vitamin A deficiency is fairly common, particularly among poor children in developing countries. It has been described as among the most widespread and serious nutritional disorders to afflict mankind.[140] The problem has been said to have remained largely unchecked and continued to be the cause of a high toll in blindness and death among young children.

The term "xerophthalmia" literally means "dry eye", and in a restricted sense is a term used by ophthalmologists to describe the changes in the eye that occur when the secretions of the paraocular glands or of the goblet cells of the conjunctiva dry up, leading to discontinuity of the fluid films usually present over the surface of the conjunctiva and cornea. However, in a broader sense, and in a public health context, the term has been applied to the syndrome of severe vitamin A deficiency.[69] The term has been taken to cover all the ocular manifestations of vitamin A deficiency (including night blindness), and would thus be taken to denote an advanced degree of vitamin A deficiency with a potential threat to sight.[140] Particular emphasis has been given to the term because of its direct relation to the most tragic sequel of the deficiency — blindness.

"Vitamin A deficiency" refers to any state in which the vitamin A status is subnormal. It can be presumed to occur when the habitual intake of the total vitamin A is markedly below the recommended dietary intake.[69] It therefore includes xerophthalmia.

1. Epidemiology of Vitamin A Deficiency

The basic underlying cause of vitamin A deficiency is a chronic inadequate dietary intake. The epidemiology of the problem is, however, rather complex. The factors involved may be considered in terms of a host-environment-agent interrelationship, as in other diseases or disorders.

a. Host Factors

Age is considered predominant among all of the host factors involved.[69] Young children constitute the most vulnerable age group, and the most serious eye lesions commonly occur in them. This is related to their relatively high vitamin A requirements for growth, increased needs due to the frequent occurrence of infections, low intake from milk of undernourished mothers, and failure to supplement carotene-poor staples with dark green leaves and other rich sources.[141]

There appears to be evidence that males are more susceptible to xerophthalmia. This is said to be true for all stages of ocular lesions, all ages, and in many countries.[69] However, reasons for this sex difference are unclear. It is felt that the reasons could be more cultural than biologic.[141]

b. Agent Factors: The Diet

Vitamin A deficiency is highly prevalent in communities where the dietary staple is rice, with little or no consumption of animal foods, dark-green leafy vegetables, or of yellow/orange fruits. This is also true for communities dependent on cassava, white potato, or other carbohydrate-dense foods that are virtually devoid of vitamin A and carotenoids. Vitamin A deficiency is essentially a condition of a poor socioeconomic environment.[142] In these communities where foods from animal sources are too expensive, carotenes from plant sources are of paramount importance. However, due to ignorance and/or neglect, even these cheaper sources of vitamin A are very often not given to the children. Thus, it is common to find "poverty in the midst of plenty", and de-

struction of eyes by xerophthalmia in environments where carotene-rich green leaves are abundant.[69] It is therefore ironic that vitamin A deficiency should be prevalent in southeast Asia, amidst plenty of greens and a variety of colored fruits.

As discussed in Section V.C.1, fats are important in the absorption and metabolism of carotenoids and vitamin A. Where diets are unusually low in fat, inefficient absorption of dietary carotenoids has been implicated as contributing to development of xerophthalmia. Roels et al. reported improved absorption of carotenoids following improved fat intakes in Ruanda[71] and Indonesia.[143]

Unsatisfactory early childhood feeding practices have an important bearing on the development of vitamin A deficiency. Xerophthalmia is rarely reported among breast-fed infants and seldom reported in children who continue to be breast-fed in the second year.[77] However, this is not to be so in many communities since the mothers themselves tend to be undernourished, with a very low vitamin A status, and consequently the milk produced has a low concentration of the vitamin. Early weaning from the breast would worsen the situation. This is further aggravated when the child is weaned to an inadequate diet of rice and other cereals or tubers, somewhat devoid of vitamin A.

c. Environmental Factors

Where xerophthalmia endemicity is extremely high, as in parts of Indonesia, the disease tends to occur all year round, with less seasonal variations. On the other hand, where the incidence rate drops, vitamin A deficiency appears highly seasonal.[69] The deficiency is associated with, among other things, particular seasons in which precipitating factors occur, such as dry periods when the supply of fresh fruits and vegetables are scarce.

d. Vitamin A Deficiency, Infection, and Child Survival

There is an indisputable relationship between the occurrence of infectious diseases and xerophthalmia.[69,77,144] Any serious illness, whether acute or chronic, depresses the appetite, impairs absorption, increases vitamin A requirements, and has other effects on general metabolism that may lower the vitamin status, thus precipitating the occurrence of the disease.[69,141] Hence, there is a close association between xerophthalmia and respiratory tract infections, gastrointestinal diseases, and intestinal parasitic infestations, especially ascariasis. Measles appear to occupy a rather special position, and many cases of xerophthalmia reported from parts of Africa have been associated with measles.[141] It takes a severe form in the undernourished child, with marked impairment of the cell-mediated immune response, and affects the cornea even in well-nourished children.

The interactions between vitamin A and infection have recently been given a great deal of attention. Various pieces of evidence, including laboratory experiments, clinical and epidemiologic clues, have shown that vitamin A deficiency plays an important role in resistance to infection, most apparent for respiratory infection. The synergistic effect between vitamin A deficiency and infection may be responsible for excessive childhood morbidity and mortality in many developing regions of the world.[145] In a group of West Java Indonesian preschool-age rural children, Sommer et al.[146] reported that the mortality rate among children with mild xerophthalmia (night blindness and/or Bitot's spots) was on the average 4 times the rate, and in some age groups 8 to 12 times the rate, among children without xerophthalmia. These investigators further reported that children with mild xerophthalmia were more likely to develop respiratory disease and diarrhea than non-xerophthalmia children, and that this increased risk was more closely associated with their vitamin A status than with their general nutritional status.[147] Further studies carried out by Sommer et al.[148] in Sumatra showed that vitamin A supplementation (200,000 IU vitamin A twice at six-month intervals) was able to reduce mortality among the preschool children by as much as 34%. More recently, Rahmathullah et al.[149] also reported a drastic reduction (on average by 54%) in mortality among children in southern India supplemented with a small (8333 IU) weekly

dose of vitamin A. To avoid criticism of the study design, a randomized, placebo-controlled, masked clinical trial among a large number of children (over 15,000) was carried out.

These studies on child survival and mortality created a great deal of interest and debate in the international health and nutrition community. These findings also had wide policy implications.[150] Questions have been raised regarding the validity of the findings, including those pertaining to experimental designs and measurements, and data analysis and interpretation.[151,152] Keusch,[153] however, felt that the evidence available support the implementation of vitamin A programs in specific cases — wherever there is evidence that a population has a vitamin A deficiency, wherever protein-energy malnutrition is common, and wherever there is an excess of measles deaths. It was, however, pointed out that vitamin A supplementation should not be a substitute for immunization, primary health care, and improved sanitation and water supplies. The controversy continues, and the Subcommittee on Vitamin A Deficiency Prevention and Control of the U.S. NAS has considered studies into vitamin A and child mortality of significant research priority.[154] In the meantime, the International Vitamin A Consultative Group (IVACG) has issued an interim statement that evidence is accumulating that vitamin A does reduce mortality by a mechanism(s) which is still unclear.[155] The statement also made it clear that the impact of improved vitamin A nutrition will vary with the severity of vitamin A deficiency and the contributions of other ecological factors.

Studies into the mechanisms of the interaction between vitamin A and infection have been undertaken for many years. Recent findings indicate that vitamin A deficiency could adversely affect epithelial integrity and function, lymphoid mass, specific immunity (i.e., cell-mediated and humoral), and nonspecific mechanisms of host resistance.[144,145,156,157]

2. Assessment of Vitamin A Status

Regional or nationwide surveys should be carried out to determine the frequency (prevalence) and severity of vitamin A deficiency in the population. Before any intervention program is launched, a careful assessment of the situation should be made. Such surveys should be aimed at establishing:

1. Whether vitamin A deficiency exists in the vulnerable age group
2. The nature, magnitude, severity, and geographical distribution of this deficiency
3. Whether this deficiency constitutes a problem of public health magnitude
4. A baseline for evaluating the effectiveness of future intervention

Assessment of vitamin A status should include, whenever possible, clinical examination, biochemical determinations, and dietary assessment. Sommer et al.,[158] WHO,[140] and, more recently, Underwood[159] have provided some general guidelines for the conduct and analysis of such surveys. The following subsections outline the important aspects of each of the assessment methodologies mentioned.

a. Clinical Assessment

As discussed in Section IX.B, vitamin A deficiency is a systemic disease affecting epithelial structures in a variety of organs, the eye being the most obvious and dramatic example. Clinical signs and symptoms occurring in the eye are specific to vitamin A deficiency. Thus, these have been widely used in clinical assessment of vitamin A status.

The ocular signs of vitamin A deficiency have been grouped under the term xerophthalmia, which literally means "dry eye". The major xerophthalmia signs have been classified at the WHO/USAID meeting on Vitamin A Deficiency and Xerophthalmia.[69] This was subsequently modified in another WHO meeting.[140] The classification is reproduced in Table 7. Following this, a brief description of the signs is given. Details have been published in various monographs and reviews.[69,77,140,141,160-162] A field guide for the detection and control of xerophthalmia was also published by WHO.[163] Most of these publications include colored photographs of the ocular signs.

TABLE 7
Xerophthalmia Classification by Ocular Signs

Night blindness	XN
Conjunctival xerosis	X1A
Bitot's spot	X1B
Corneal xerosis	X2
Corneal ulceration/keratomalacia <1/3 corneal surface	X3A
Corneal ulceration/keratomalacia ≥1/3 corneal surface	X3B
Corneal scar	XS
Xerophthalmic fundus	XF

Reproduced from *Control of Vitamin A Deficiency and Xerophthalmia*, Report of a Joint WHO/UNICEF/USAID/ Helen Keller International/IVACG Meeting, World Health Organization, Geneva, 1982. With permission.

With the exception of corneal scar (XS) and xerophthalmic fundus (XF), the ocular signs in Table 7 are arranged in increasing order of severity of active vitamin A deficiency. However, this does not mean that all the earlier stages have necessarily occurred when a later stage is detected. The earliest ocular symptom is that of night blindness (XN), or impaired dark adaptation, and is the result of too little available retinol for the rapid regeneration of rhodopsin in the eye. The symptom is said to be quite specific for vitamin A deficiency among young children, but is less so in older children and adults.[77] WHO[140] considered it a useful screening tool in that it correlated closely with other evidence of vitamin A deficiency, such as serum retinol levels. However, its use requires a careful detailed history taking and is particularly useful in communities where the phenomenon of night blindness is recognized by the community.

The earliest structural change in xerophthalmia is conjunctival xerosis (X1A in Table 7) consisting of one or more patches of dry, nonwettable conjunctiva, with loss of transparency. X1A may be associated with various degrees of conjunctival thickening, wrinkling, and pigmentation. It has, however, been pointed out that these latter signs are poorly reproducible, highly variable, and nonspecific, and should not be used in isolation in making a diagnosis of X1A.[140,164]

The xerosis process in X1A extends to the formation of Bitot's spots (X1B in Table 7). These are small plaques of a silvery gray hue, usually with a foamy surface, consisting of keratinized, desquamated conjunctival epithelial cells. X1B usually appears on the temporal quadrant of the conjunctival surface and may occur on one or both eyes. The concurrent appearance of X1A and X1B is usually consistent with hypovitaminosis A.[77]

The more serious, blinding effects of severe vitamin A deficiency involving the cornea consist in the early stages of xerosis, loss of transparency, and nonwettability, as in the conjunctiva, giving the cornea a hazy appearance (X2 in Table 7). Subsequent to X2, there is loss of continuity of the epithelium with formation of inflammatory "ulcer". Further progression of this corneal ulceration, frequently referred to as keratomalacia (X3A and X3B in Table 7), may eventually end in impairment of vision to varying degrees.

XS may be indicative of past severe vitamin A deficiency, but they may be due to other etiologies as well. When observed, a careful history must be taken for evidence of trauma rather than xerophthalmia causative.[77] XF is a rare sign, usually accompanied by XN and/or X1A and X1B.

Patients with prolonged and severe vitamin A deficiency may have, in addition to the eye lesions, a widespread dryness, wrinkling, slate-gray discoloration, and hyperkeratosis of the skin. It is, however, difficult to separate the role of vitamin A deficiency in causing these signs from the part played by other nutrient deficiencies. Even more controversial is the etiology of follicular hyperkeratosis or phrynoderma, ascribed

by some workers to be due to vitamin A deficiency.[140,164]

b. Biochemical Examination

Measurement of the serum or plasma vitamin A level remains the most practical, available biochemical means of assessing the status of nutriture in spite of limitations in sensitivity and specificity.[77] Since vitamin A is stored in high concentrations in the body, almost entirely in the liver, the plasma level does not closely reflect the level in the body as a whole. The plasma concentration reflects the body status only under two very different circumstances:[69] when the body stores have been critically depleted, and when the liver has become saturated with vitamin A. The U.S. Interdepartmental Committee on Nutrition for National Defense (ICNND)[165] recommended the interpretation of plasma vitamin A levels summarized in Table 8.

TABLE 8
Interpretation of Plasma Vitamin A and Carotene Levels

Level	Vitamin A/100 ml (μg)	"Carotene"/100 ml (μg)
High	>50	>100
Acceptable	20–49	40–99
Low	10–19	20–39
Deficient	<10	<20

Adapted from Reference 164.

Of the categories thus outlined, only the "deficiency" state, i.e., <10 μg/100 ml, is recognized to be universally associated with both low liver reserves of vitamin A and an increased prevalence of clinical signs of deficiency. The so-called "low" category (10 to 20 μg/100 ml) should be interpreted with caution since low plasma values may not be associated with vitamin A deficiency, but instead with other conditions such as inadequate protein intake, parasitic infestation, and liver diseases.[69] Lewis et al.[166] pointed out the need for different cutoff levels of serum vitamin A for young children as compared with older children and adults. Based on analysis of the HANES data, these investigators found that younger children (aged 4 to 5 years) have lower serum vitamin A levels than the older children (aged 9 to 11 years).

Plasma carotene concentration is generally considered not to be a reliable indicator of vitamin A status because it reflects the level of immediate dietary intake.[69] Furthermore, depending on the dietary composition, a considerable proportion of the plasma carotenoids may be non-vitamin A compounds. However, when considered with plasma retinol levels, carotenoid analysis can be useful.[77,158] The ICNND[165] used the levels given in Table 8 for the interpretation of serum "carotene" levels. It would be more appropriate to refer to these levels as "total carotenoid" since the colorimetric method used is not able to determine individual carotenes.[6]

A common method for the analysis of serum retinol is based on the transient blue-colored complex which retinol and its esters form under anhydrous conditions with antimony trichloride-chloroform reagent (Carr-Price reaction). After precipitation of protein with ethanol, vitamin A and carotene in serum are extracted into petroleum ether. Carotene in solution can be quantitated by reading at 450 nm. The petroleum ether is evaporated off and the residue is reacted with antimony trichloride-chloroform reagent. The blue color formed is read at 620 nm. Details of the procedure are described in various publications (see, for example, Underwood and Stekel[167]). A common problem encountered is that the reagent develops turbidity in the presence of trace amounts of moisture. Another difficulty encountered is that the characteristic blue color formed by the reagent with vitamin A is subject to rapid fading.

In view of these difficulties, various modifications have been made to the method. These include the substitution of trifluoroacetic or trichloroacetic acid for antimony trichloride reagent, and the development of a micro-method for small volume of blood.[168] Other methods that have been in use include the spectrophotometric method based on UV inactivation[169] (also described in detail in Underwood and Stekel[167]), and a fluorometric method.[170] Further examples of all other methods have been described in detail in the compilation by Arroyave et al.[171] of se-

lected methods for the analysis of vitamin A and carotenoids in nutrition surveys. More recently, several HPLC methods have also been proposed.[172-177] A more detailed discussion on developments in the analysis of retinol in serum is given in the review by Tee and Lim.[6]

c. Dietary Assessment

The difficulty of obtaining food consumption data is well documented. Many factors are difficult to control, making the results obtainable by even the most conscientious workers approximations only. Dedicated workers are definitely a must.

For the calculation of nutrient intake from the food consumption data collected, a good food composition data base is required. It would be quite pointless in taking great pains to carry out the data collection, but lack good data on nutrient composition of foods for calculations. For vitamin A, obtaining good nutrient intake data is particularly difficult. This is related to the nonavailability of good food composition data bases with accurate vitamin A and carotene values. As has been previously pointed out in Section VI.D, vitamin A activity of foods in many food tables may be unreliable due to the lack of precision of methodologies for the analysis of vitamin A and carotenoid content of foods. In recent years, there has been particular emphasis on obtaining more accurate data on the types and concentrations of various carotenoids and retinoids in foods.[6]

Various approaches have been used to assess dietary intake of nutrients. Each has strengths and weaknesses, and the method of choice would have to depend on the objectives of the study. Dietary history to quantitate the exact amounts of foods eaten, e.g., using the 3 days 24-h recall method, can provide fairly good data. The method is, however, time-consuming, especially when used for large population groups. In addition, memory recalls are difficult to administer to young children. Household food consumption data can be obtained using the frequency of food consumption and purchase method. Such data, however, cannot be relied upon to provide an indication of the intake of individual members of the household. A compromise would be to collect family-based qualitative data to determine the frequency with which different foods are eaten. A comparison of three dietary methods for estimating vitamin A intake was recently reported by Russell-Briefel et al.[178]

The IVACG recently introduced guidelines for the development of a simplified dietary assessment to identify groups "at risk" for inadequate intake of vitamin A.[179] The semiquantitative method is particularly suitable for communities where almost all the vitamin A intake is from carotenoids contained in a limited number of food groups. Based on food composition tables, these foods are classified into groups of high, moderate, or low levels of vitamin A activity. It has been emphasized that the simplified method will not provide precise quantitative information on levels of intake and, alone, will not be adequate for a quantitative assessment of vitamin A status of either communities or individuals.

d. Other Assessment Procedures

Functional biochemical measures have been proposed as more dynamic means of identifying marginal vitamin A status, either among individuals or population groups.[159] Two such functional tests recently advocated are the relative dose-response (RDR) test and conjunctival impression cytology (CIC).

In the RDR test, two representative serum levels obtained before and after supplementation with vitamin A form the basis of the test. After an initial fasting blood sample is obtained, a liquid solution containing about 450 to 1000 μg retinyl palmitate is given orally. A small meal containing foods with some fat but minimal vitamin A is given after the dosing. After 5 h, a second blood sample is taken. Vitamin A in the blood samples is determined and RDR calculated. An RDR $> 20\%$ is considered to be positive and indicative of inadequate hepatic stores of vitamin A.[159] It is clear that the test would be very difficult to administer under field conditions.

CIC has been proposed to be a sensitive and specific indicator for the detection of marginal vitamin A deficiency among young children. Specimens of conjunctival epithelium are ob-

tained atraumatically by applying strips of cellulose acetate paper to the lower, outer (temporal) portion of the conjunctiva for 3 to 5 s, then gently peeled away. The paper with the adherent epithelial cells is then transferred to a fixative solution, stained, and examined under a microscope. The principle underlying the technique is that, since vitamin A is needed for maintenance of normal mucous epithelium, deficiency states brings about changes to the morphological characteristics of the epithelial cells. The number of goblet cells decreases and may even be absent in vitamin A deficiency. At the same time, metaplasia of the epithelial cells occurs.[180] It is clear that some training and experience is required to obtain a good impression of cells from the conjunctiva. The criteria for normal and abnormal cytology should be clearly defined. The procedure has been tried out by various investigators (e.g., Natadisastra et al.,[181] Kjolhede et al.,[182] and Reddy et al.[183]). Reports on the validity of the procedure have not been consistent and need confirmation. Natadisastra et al.[181] reported CIC to be highly sensitive and specific for the detection of physiologically significant vitamin A deficiency. On the other hand, when compared with fasting serum vitamin A levels and RDR, Gadomski et al.[184] reported low sensitivity and positive predictive value of CIC.

3. Prevalence of Vitamin A Deficiency

In most reviews on the prevalence of vitamin A deficiency, the global survey by Oomen et al.[185] has been quoted. Drs. Oomen, McLaren, and Escapini visited 36 countries and compiled data based on hospital records, government statistics, questionnaire studies, and personal observations. Based on this WHO-sponsored global survey and a detailed notification system in Jordan, the annual incidence of xerophthalmia in the world prior to 1970 was estimated to be 100,000.[140] The areas found to be most affected were the overpopulated, rice-dependent countries of Asia. Other regions that were affected to a lesser extent include Africa, Latin America, and the Middle East.

Subsequently, surveys have been conducted in several countries, most notably the nationwide prevalence survey in populous Indonesia. The incidence (appearance of new cases) of active corneal disease in Indonesia was estimated to be around 2.7 per 1000 preschool children per year.[162] Given an incidence of this order, the Administrative Committee on Coordination-Subcommittee on Nutrition of the United Nations (ACC-SCN)[186] has estimated that 400,000 to 500,000 preschool children in Bangladesh, India, Indonesia, and the Philippines combined will develop active corneal lesions resulting in partial or total blindness. It has been further pointed out that these are conversative estimates since the assumption that identical incidence rates prevail for all four countries is not correct; higher rates are known to occur in Bangladesh and India. The incidence of non-corneal xerophthalmia (mild, and generally reversible forms of the disease) in these countries has been estimated to be in the order of 5 million preschool children per year.

Applying these estimates from the four Asian countries mentioned previously, a worldwide projection has been made of 700,000 cases annually of new active corneal lesions among preschool children. The total incidence of non-corneal xerophthalmia among preschool children has been similarly estimated to be 6 to 7 million new cases per year, and 20 to 40 million suffering from at least mild deficiency at any one time, of which nearly half are in India.[187]

WHO[69] had suggested that, with respect to prevalence of vitamin A deficiency, the world may be divided into three regions:

1. Technologically developed countries where there is no xerophthalmia problem but some hypovitaminosis A may exist
2. Some rice-dependent developing countries of Asia where there is a problem of public health magnitude
3. The rest of the world, including Africa, Latin America, and the Middle East, where the problem is not extensive but is intermittently intensive and highly sensitive to changing social and economic conditions

Some detailed descriptions of the problem as encountered in each of these three regions are given in several reviews (e.g., see Reference 69, 77, 140, and 188). A map showing the world geo-

graphical distribution of vitamin A deficiency and xerophthalmia in 1984 has been provided by reports of the ACC-SCN.[186-187] A summary of the vitamin A deficiency problem of over 30 countries is also given in the 1985 report.

A review of the vitamin A deficiency problem in Malaysia was carried out, based mainly on the annotated bibliography of nutrition research in the country by Tee[189] for the period 1900 to 1979 and Tee[190] for the 1980 to 1984 period, and various recently published reports. Various studies carried out in different parts of the country have shown vitamin A deficiency to be an important sight-threatening disorder, affecting mainly young children on imbalanced diets. It was found to be the major single cause of blindness. The disease was most prevalent among the lower socioeconomic segments of the population. Vitamin A intake was generally low, with little or no retinol, and most of it from the provitamins. Even then, the amount of vegetables and fruits consumed was generally low.

No exact estimates of the magnitude of the problem were available. However, as seen from reports in the literature, the problem appeared to be confined to certain groups, mainly in the rural areas, and did not pose a major health hazard nationwide. The problem appeared to have lessened over the years, judging from reports up to the late 1970s and the early 1980s. There are probably very few cases of children with eye signs past X1A, and serum vitamin A <10 μg/ml. It is, however, recognized that there are many remote areas in the country where the vitamin A status is not known, including parts of peninsular Malaysia. Furthermore, the problem among urban squatters is little studied. Most of the studies relied on the less precise clinical signs and dietary inquires due to the lack of laboratory facilities to carry out biochemical investigations. Even for the latter, investigators have been faced with the difficulty of obtaining sufficient blood from subjects. A reliable micro-method would have to be established for local use. Extensive mapping of the vitamin A status of children in the country remains an important task.

4. Prevention and Control of Vitamin A Deficiency

Vitamin A deficiency has been considered one of the "big four" nutritional deficiencies in developing countries; the other three are protein-energy malnutrition, iron deficiency anemia, and endemic goiter due to iodine deficiency. Vitamin A deficiency is the most important cause of preventable childhood blindness in these countries. In recent years, there has been an increasing appreciation by governments and international agencies of the magnitude of the problem and of the means available for dealing with xerophthalmia.[191] Various intervention strategies have been implemented to combat the problem, and volumes have been written on these programs.[69,140,192-195] Intervention programs to prevent vitamin A deficiency are in operation nationally in at least 8 of the 34 countries with known vitamin A deficiency.[187] Coverage of the population at risk in some of these countries has reached sufficiently high levels, while in others coverage is still unsatisfactory. The Administrative Committee on Coordination of the United Nations has emphasized that, if intervention programs are energetically implemented, vitamin A deficiency could be reduced to a level that it no longer poses a public health problem.[187]

The objectives of an intervention program must necessarily depend on many factors, such as the severity of the problem. Inadequate vitamin A status varies from a marginal condition of inadequate body reserves of the vitamin without clinical signs, through the presence of early and reversible clinical signs, to a severely depleted state mainly characterized by advanced corneal changes and the high probability of blindness.[140] Control programs should therefore be directed toward the particular problem at hand.

Control of vitamin A deficiency may be approached through one of three major intervention strategies:

1. Improvement of dietary vitamin A intake
2. Vitamin A fortification of foods

3. Periodic administration of a massive dose of vitamin A

a. Improvement of Dietary Vitamin A Intake

Dietary improvement has been recognized as the major long-term solution to controlling vitamin A deficiency in a community. Through a combination of horticultural and related activities and educational and socioeconomic inputs the habitual intake of food sources of vitamin A is gradually increased and maintained at a level which minimizes the risk of developing vitamin A deficiency among the vulnerable segments of a population.[194] Even when other intervention strategies have been implemented, these measures should be carried out as on-going, long-term strategies.

In those parts of the world where vitamin A deficiency is prevalent, vegetable products are the main source of dietary vitamin A in the form of carotenes. Thus, horticultural and related activities to increase the availability of carotene-rich fruits and vegetables have been emphasized.[140] These would include not only matters related to production (varieties for promotion, pest and disease control), but also problems of transport, marketing, storage, and preservation. These activities should be coordinated with the aspects of nutrition education programs since availability does not necessarily mean consumption of these foods by the vulnerable segments of the population, especially young children. The reasons why these foods are not consumed in significant amounts, even in areas where they are abundant, should be understood.

b. Vitamin A Fortification of Foods

Fortification or nutrification of a widely consumed food with vitamin A offers a major technically feasible intervention which can be relatively inexpensive and effective.[191,194] Various considerations would have to be given before fortification can be considered a viable intervention. First, with respect to a suitable food vehicle for vitamin A fortification, it should have the following characteristics:

1. The food should be technically fortifiable.
2. It should be widely or universally consumed by the population of the target area, in quantities that will make a significant contribution to the diet.
3. There should be little variation in per capita daily consumption of the food.
4. The food item should show no appreciable changes in its organoleptic characteristics, so that its acceptability is not affected after addition of vitamin A.
5. It should be economically feasible to fortify the food on an industrial scale.

Other important factors in nutrient fortification of foods include:

1. The addition of the nutrient should not create an imbalance of essential nutrients.
2. The nutrient added should be stable under proper conditions of storage and use.
3. The nutrient should be physiologically available from the food.
4. There should be reasonable assurance against excessive intake to a level of nutrient toxicity.

Examples of fortification of vitamin A in sugar in Central America, monosodium glutamate in the Philippines, and dried skim milk in various food aid programs have been described by WHO.[140] The fortification of milk powder and margarine with vitamin A in this country has been practiced for some years. Bauernfeind[117] has discussed in detail the fortification of various foods and the technology of the process.

Depending upon local factors and program costs, fortification can offer both short- and long-term solutions in a chronically vitamin A-deficient population.[194] Since the amounts of vitamin A added are adjusted to permit the recipient to receive the approximate RDA for vitamin A, there is no risk of potential hypervitaminosis A. To date, there are no indications clinically or biochemically that hypervitaminosis A has resulted

from any of the nutrification projects currently in operation.[193]

c. Periodic Administration of a Massive Dose of Vitamin A

On the basis of available evidence from nearly 2 decades of experience, large-dose vitamin A distribution can generally be regarded as a safe and potentially effective intervention to prevent xerophthalmia.[195,196] The underlying objective is to maximize liver stores from a single dose with little, if any, risk of acute toxicity. A 200,000 IU dose of vitamin A (110 mg retinyl palmitate or 66 mg retinol acetate), with 40 IU vitamin E in an oil solution for oral administration, given every 3 to 6 months is used in most prevention programs described in the literature.[69,117,140,194,195] The program had been instituted in the early and mid-1970s in several countries with a severe xerophthalmia problem.[140] These included India, Bangladesh, Indonesia, Haiti, Sri Lanka, and the Philippines. The successful implementation of a massive vitamin A supplementation program covering 229 villages in Sumatra has been recently reported by Sommer et al.[148]

A large number of studies have been carried out on various aspects of the massive dosing of vitamin A. The IVACG monograph[194] and a recent WHO[196] booklet provide details of various aspects of the subject, including program implementation and effectiveness, estimates of the protective period, and efficiency of absorption and retention of the administered dose. Bauernfeind[193] has discussed in detail aspects related to safety of use of vitamin A in the program.

Although the approach appears conceptually simple, the adequacy and efficiency of programs pose major challenges and determine their success. Since the intervention involves a deliverer-recipient individual contact at specified times, a delivery system with a high requirement of personnel is needed to execute the program. However, the personnel involved need not have a high level of technical expertise. Distribution systems should be designed and implemented with emphasis on primary level conditions, existing infrastructures, and ongoing health activities, with the involvement of local leadership. This strategy has the advantage of immediate implementation, but suffers from the disadvantage that it applies only to the isolated nutrient vitamin A, and requires repetitive administration. In addition, complete coverage is seldom achieved. Of the total cost, the major percentage is in the mechanism for delivery and not of the nutrient per se. Education in health and nutrition should be an integral part of the periodic dosing program. Underwood[197] has emphasized that, although these programs have proven to be beneficial, the focus should be on approaches that foster practical solutions attainable through better utilization of available food and other resources. Although these preventive measures are more difficult to implement and take longer to bring about the needed behavioral changes, they are more permanent. More importantly, these measures address health and nutrition issues that commonly coexist with vitamin A deficiency.

Three models of delivery system have been suggested:[140] medical, universal, and targeted. The medical (or therapeutic) system is appropriate as an initial measure in any endemic area, and provides vitamin A prophylactically (as part of their treatment) to all preschool-age children who are ill or with deficiency signs and come to hospitals, clinics, and health centers. The targeted system focuses on specific high-risk groups, defined usually by age and/or location. In the universal (or prophylactic) system, all preschool-age children within a designated area receive a periodic dose of vitamin A at established intervals.

d. Evaluation of Vitamin A Deficiency Intervention Programs

A great deal of attention has been given to the development of strategies for the control and eradication of vitamin A deficiency all over the world. Many of these intervention programs have been carried out without appropriate evaluation. As with other intervention measures, lack of proper evaluation has resulted in the inability to determine whether the programs have produced the expected results. Even if changes are observed, it is not possible to estimate the extent to which those changes may be attributed to the programs. Recognizing this, the IVACG has put

forth a useful manual for monitoring and evaluating vitamin A deficiency intervention programs.[198] Details for evaluating the intervention programs discussed herein are given. Methodologies described include indicators for evaluation and sampling design.

C. Hypervitaminosis A

The intake of vitamin A above that which an individual can metabolize, either in a single excessively high intake (acute) or very high intakes for prolonged periods (chronic), causes hypervitaminosis A. It is essentially a result of abuse of this essential nutrient. Acute hypervitaminosis A has been reported to occur with ingestion of a single massive dose of 100,000 μg vitamin A in infants and young children, and 600,000 μg in adults. Chronic hypervitaminosis A may occur in infants after 4 months of receiving 10,000 μg daily, and in children and adults taking between 20,000 and 50,000 μg daily for several years.[199] Reviewing the evidence, Hathcock et al.[133] concluded that exposure to high doses of vitamin A (≥30,000 μg/day) for relatively short periods (days or a few weeks) or lower doses (7500 to 15,000 μg/day) for periods of several months or more can produce multiple adverse effects. Several predisposing conditions, such as viral hepatitis, cirrhosis, and other liver diseases, may greatly increase susceptibility and thus lower the amount of vitamin A necessary to produce adverse effects.[133] Blood levels of vitamin A become very high in hypervitaminosis A, well in excess of 100 μg/dl.[77] Various clinical symptoms have been recorded, including nausea, headaches, vomiting, diarrhea, irritability, drowsiness, fatigue, and abnormal responses of the blood, skin, hair, and bone.[2,193,200,201] These adverse symptoms are expected to vary with the dose and the duration of exposure, as well as with the age of the individual exposed. A rapid recovery usually results when excessive intakes are discontinued, and no permanent health effects are known to occur.[77] Bendich and Langseth[200] and Olson[201] have, however, emphasized the danger of possible teratogenic and embryotoxic effects of excess vitamin A intake in women of child-bearing age. Hathcock et al.[133] have given a detailed review of the animal studies carried out on toxicity and overdose of vitamin A.

Bauernfeind,[193] who has given a detailed account of the subject, estimated that worldwide several hundred instances of hypervitaminosis A probably occur annually, 90% of which cause fleeting side effects which are self-correcting within hours or a day after a single high level dosage. The remaining cases may cause sufficiently severe symptoms to require weeks or months to reverse. No deaths have been attributed solely to hypervitaminosis A. Vitamin A toxicity therefore remains a very minor clinical or nutritional problem. When hypervitaminosis A is suspected, a history of vitamin A consumption should be taken from the patient and serum retinol level determined. If overdosage is ascertained, the simple remedy is to stop the excessive vitamin A intake.

However, there is growing concern, especially in developed countries, that prevalence of chronic hypervitaminosis A may increase in future years.[77,201] There is fear that excessive intake could result from misuse or overconsumption, either out of ignorance or carelessness or out of the misconception among food faddists that continued excessively high intakes will provide some unusual health benefits. Vitamin A preparations are available commercially without prescription in concentrations of up to 7500 RE, and there are those who advocate routine megavitamin ingestion. Furthermore, for young children who are given concentrated vitamin supplements as well as fortified foods, they could be on the verge of toxicity. Such fears are real, and even developing countries should be wary of the situation. Underwood[77] had also pointed out that in view of the linkage of retinoids in animal studies to cancer prevention, and the epidemiologic associations in humans of dietary and serum levels of vitamin A to cancer risk, there is the danger that this will lead the uninformed public to increased consumption of megadoses. The Committee on Dietary Allowances of the U.S. National Research Council has recommended that regular ingestion of supplements of retinol exceeding 3000 RE by infants and children be undertaken only under the direction of a physician, and that for adults, regular ingestion of more than 7500 RE daily is not prudent.[79] Hathcock et al.[133]

have emphasized that educational efforts should point out clearly that there are no well-established therapeutic or prophylactic benefits to the ingestion of intakes above the usual RDA, and that toxicity is possible from excessive intakes.

Excessive consumption of carotenoids does not generate a correspondingly high vitamin A tissue content and does not cause hypervitaminosis A, although there is usually a rise in serum retinol level.[129] This has been attributed to the sharp decline in efficiency of intestinal absorption and rate of conversion of carotenoids to vitamin A in the body.[193] Individuals who consume high levels of carotenoids in pharmacological dosage forms, or as large quantities of carotene-rich fruits, will develop a yellow or orange pigmentation of skin, especially the palms of the hands and soles of the feet. Hypercarotenemia results, and serum levels of carotenoids may be in excess of 300 μg/dl.[77] These conditions have no effect on health and will slowly disappear following elimination of the excessive dietary carotenoids. Reviewing the evidence, Bendich[202] and Hathcock et al.[133] concluded that daily supplementation with high doses of β-carotene for extended periods of time is not associated with other side effects.

Hypercarotenemia, also known by other terms such as "carotenemia", "hyperlipochromia", "xanthemia", and "carotenosis cutis", may also be associated with various conditions other than excessive dietary intake.[129] These include diabetes mellitus, hypothyroidism, hypothalamic amenorrhea, anorexia nervosa, liver disease, and certain inborn errors of metabolism. Although the mechanisms of hypercarotenemia in these conditions have not been fully understood, it is known that the decreased rate of conversion of carotene to vitamin A is an important factor.

D. Carotenoids, Retinoids, and Cancer

Cancer is a condition of unrestrained cellular growth, commonly associated with poorly differentiated cells. The condition arises from a myriad of causes and may affect cells from essentially all tissues.[203] There appears to be three distinct stages in tumor development.[1] The first is the initiation stage, when a carcinogen causes a permanent change to a few cells of the affected tissue. In the preneoplastic or latent stage, the affected cells gradually develop into cancer cells. A characteristic feature of this phase is that the preneoplastic cells can sometimes remain transformed but not multiply for a long time. These cells can sometimes revert to normal or be prevented in their progression toward the tumor phase. Finally, in the third stage, the transformed cells are permitted or encouraged to multiply rapidly, transforming the tissue into a tumor. It is now known that cancer incidence is influenced by various environmental factors, aside from genetic factors. The environmental factors include the air, water, and food.[204] The food we eat daily can participate in tumor development in that certain food components can act as initiators (carcinogens) and/or promoters (cocarcinogens), thereby increasing the incidence or speed of development of particular tumors.

On the other hand, the foods we eat may contain components (anticarcinogens) that can act to block initiation, to enhance the immune system's ability to identify and destroy transformed cells, or to inhibit development to promotion stage, thereby lowering incidence or slowing tumor development. Epidemiologic studies, supported by experimental observations in laboratory animals have suggested that dietary practices are a promising area to explore in the search for preventive measures against cancer.[205] As Peto et al.[206] explained, it would be more attractive to discover anticancer substances in certain foods that can be prescribed rather than carcinogens that must be proscribed since people are more willing to accept prescription rather than proscription.

Thus, the role of diet in cancer development has been actively studied in recent years. Various epidemiologic and laboratory studies have been carried out. In the past 20 years or so, attention has been focused on carotenoids and pre-formed vitamin A. The possibility that carotenoids and retinoids may play a role in cancer development has added a whole new, exciting dimension to the studies of these compounds in human nutrition.

Several lines of evidence suggest that important relationships exist between carotenoids, retinoids, and cancer.[207-210] These are based on experimental studies of retinoids and carotenoids

in cancer, epidemiologic evidence suggesting associations between reduced risk of cancer and vitamin A status, and findings from clinical trials and studies in the field of oncology. Some recent findings from each of these three areas are highlighted herein.

1. Experimental Studies

Little is known, except in the visual process, about the mechanisms of vitamin A action at the molecular level. However, it has become increasingly clear that the "action" of vitamin A is shown most dramatically in its ability to control and direct differentiation of epithelial tissues. In all species studied thus far, including humans, vitamin A has been shown to exert a profound effect on differentiation.[33] There are numerous epithelial tissues throughout the body that are totally dependent on retinoids for their proper differentiation and growth. In some epithelia, such as those of the trachea and bronchi, a potentially premalignant lesion occurs in the absence of retinoids. Sporn et al.[211] observed a loss of normal columnar ciliated and mucus cells in tracheal epithelial organ cultures, as well as development of lesions of squamous metaplasia with heavy keratinization. Addition of retinoids to the cultures after development of such lesions caused reversal of the process of keratinization and replacement of the abnormal squamous cells by columnar ciliated and mucus cells. According to these investigators, epithelial tissues that depend on retinoids for normal cellular differentiation and growth account for well over half of the total primary cancer in both men and women. Besides bronchi and trachea, the affected organ and tissue sites include stomach, intestine uterus, kidney and bladder, testis, prostate, pancreatic ducts, and skin.

From the fact that vitamin A deficiency produces conditions at least morphologically similar to those found during the preneoplastic stages of carcinogenesis (e.g., squamous metaplasia in tracheal epithelium), it has been predicted that the vitamin may prevent the carcinogenic process from proceeding to the final stage by reversal or repair of epithelial, or by enhancement of the intrinsic capacity of the tissue to repair itself.[204,211] Since cancer is essentially a process of loss of cellular differentiation, while retinoids are involved in the induction or enhancement of cellular differentiation, the possibility that retinoids could arrest or reverse the carcinogenesis process seemed logical and worthy of exploration.[212]

Since the late 1970s, there have been numerous studies into the ability of retinoids to modify the processes of cell differentiation and proliferation. Studies have shown that retinoids can act directly on cells in culture to suppress the process of malignant transformation, whether caused by chemical carcinogens, radiation, or viral transforming factors.[212] They have been shown to be able to suppress the process of carcinogenesis *in vivo* in experimental animals. There is now extensive literature on the ability of retinoids to suppress the development of the malignant phenotype *in vitro*. More recently, it has been shown that retinoids can exert effects on certain fully transformed, invasive, neoplastic cells, leading in certain instances to a suppression of proliferation, and in other instances to terminal differentiation of these cells, resulting in a more benign, nonneoplastic phenotype.[213] There are, however, only a limited number of instances in which such profound effects of retinoids on differentiation and proliferation of tumor cells have been shown. Several detailed reviews of studies of effects of retinoids and possible mechanisms of action have been published.[32,213,214]

With the encouraging results obtained with retinoids, it was not unexpected that attention soon focused on carotenoids as well. However, compared with the retinoids, there are fewer experimental data on the effects of carotene or other carotenoids on cancer risks.[206] In the recent review by Goodwin,[96] it was pointed out that two carotenoids, β-carotene and canthaxanthin, were able to delay growth of tumors in mice induced by UV-A, UV-B, benzpyrene (BP), BP/UV–A, and 8-methoxypsoralen, irrespective of their inherent ability or otherwise to act as precursors of vitamin A. In addition, β-carotene was found to have a positive effect against 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced tumors. It was thought that, since canthaxanthin was not effective in this case, prior conversion into vitamin A may be required for activity. Phytoene was found

to be much less effective than the other two carotenoids in protecting against tumors induced by DMBA/croton oil or DMBA/UV-B. Temple and Basu[215] had also reported that β-carotene has a strong inhibitory action against 1,2-dimethyl-hydrazine (DMH)-induced colon tumors in mice. The level of carotene supplementation in the study was within the nutritionally relevant range for humans.

Recently, Schwartz and Shklar[216] reported the effectiveness of β-carotene and canthaxanthin in bringing about regression of DMBA-induced carcinomas of hamster buccal pouch. 13-*cis*-Retinoic acid was said to have no effect in the study. In a further communication, these investigators reported that algae extract rich in carotenoids, when administered by mouth, prevented tumor formation in the hamster buccal pouch experimental model.[217]

2. Vitamin A Status and Carcinogenesis: Epidemiological Studies

As has been discussed previously, studies in experimental animals have shown that retinol deficiency enhances susceptibility to chemical carcinogenesis. Turning now to epidemiologic studies, several have reported that vitamin A status in humans may be related to cancer development. With respect to vitamin A status, two groups of studies may be differentiated. The first deals with serum retinol or carotene levels and their association with cancer risk. Investigations into the relationship between dietary retinol or carotene intake and cancer risk are in the second group.

For both groups of studies, two approaches to the investigation have been used. The first is the prospective cohort study, where a group of subjects is followed for a sufficient period of time. The second approach is the retrospective case-control study. More investigations based on retrospective serum studies or dietary questionnaires have been carried out. In the case of dietary studies, these involve asking people with cancer cases and people without cancer controls their dietary intake of carotene and pre-formed retinol in previous years. From these data, the relative risk of cancer in people with low vitamin A intake, as opposed to that in people with high intake, can be estimated. The advantage of such studies is that they yield results faster, without the need to wait for years to see who develops the disease. Furthermore, they involve much less work.

An often-quoted investigation into the relationship between serum retinol level and risk of cancer is the study of Wald et al.[218] In a prospective study of about 16,000 men, serum samples were collected and frozen. Retinol levels were later measured in the stored samples from the 86 men who were subsequently notified as having developed cancer and from 172 controls who did not develop cancer. It was found that low retinol levels were associated with an increased risk of cancer, particularly lung cancer and gastrointestinal tract cancer. Wald et al.[219] reported findings of a similar study a few years later. Plasma samples from 5004 women were collected and stored. The levels of retinol, β-carotene, and vitamin E in 39 women who subsequently developed breast cancer were measured and compared with levels from 78 controls who did not develop cancer. In this study, however, it was found that plasma retinol levels were not related to the risk of breast cancer. β-Carotene levels showed a tendency to be lower in women who developed cancer than in controls, but the difference was not statistically significant. On the other hand, low plasma vitamin E was found to be associated with a significantly higher risk of cancer.

A similar study was reported a few years later by Willett et al.[220] Blood specimens were collected from 4000 subjects who were free of cancer at the time. These subjects were enrolled for the Hypertension Detection and Follow-up program. During a 5-year follow-up period, 111 subjects were diagnosed as having cancer. Blood from these patients were analyzed for retinol, retinol-binding protein, vitamin E, and total carotenoids. These measurements were compared with those in 210 controls who were matched for age, sex, race, and time of blood collection, and who remained free of cancer. Levels of these parameters were found to be similar for cases and controls. Thus, the study was not able to confirm other findings that low levels of vitamin A are related to cancer incidence. Similarly, using the same approach, Friedman et al.[221] also reported

no difference in retinol and retinol-binding protein between 151 cases who developed cancer after a 7-year follow-up, and 302 controls. It was concluded that retinol and retinol-binding protein levels were not useful in predicting the subsequent development of lung cancer.

In another recent case-control study of 99 patients with lung cancer and 196 matched controls in Washington County, Maryland, the relation of serum retinol, β-carotene, vitamin E, and selenium to cancer risk was reported.[222] A strong inverse association between serum β-carotene and the risk of squamous-cell carcinoma of the lung was observed. An association between low levels of serum vitamin E and the risk of any type of lung cancer was also reported.

Other investigators had used the approach of retrospective case-control studies to study the association. Peto et al.[206] reviewed the findings of nine such studies carried out in India, Pakistan, East Africa, the U.K., and the U.S., with most of them reported in the late 1970s. Blood retinol levels in recently diagnosed cancer patients were compared with levels of control patients. All these studies reported lower retinol levels in cases than in controls. In six of these studies, blood β-carotene levels were also studied. In the Indian, Pakistani, and East African studies, blood carotene levels were found to be significantly lower in cases, compared with the controls. The review, however, pointed out that these studies have not sufficiently shown the inverse association between either retinol or carotene with cancer. It was felt that these were relatively small studies, and in some of them, the controls might not have been appropriately chosen.

As mentioned earlier, another group of epidemiologic studies investigated the relationship between dietary intake of retinol and carotene and cancer risks. According to Peto et al.,[206] many of these studies have investigated this relationship almost by accident because dietary questionnaires designed for more general purposes happened to ask about the main local carotene source. It was realized only later that the lower cancer risks found among users of a particular foodstuff might be due to its carotene content. Findings from some of these epidemiologic studies are summarized below to illustrate the design of the studies and the findings obtained.

One of the early prospective epidemiological investigations into dietary vitamin A intake and cancer risk was that of Bjelke.[223] A total of 8278 men were followed-up for 5 years through postal questionnaires regarding their smoking and dietary habits. "Vitamin A index" was computed for the respondents and correlated to occurrence of lung cancer within the group. It was observed that the index for vitamin A intake was inversely associated with lung cancer incidence at all levels of cigarette smoking, and most clearly expressed among those aged 45 to 64 years.

Results from another prospective epidemiological study of 1954 middle-aged men employed at the Western Electric Company's Hawthorne Works in the Chicago Metropolitan area was reported by Shekelle et al.[224] The study commenced at the end of 1957, and the men were followed up for 19 years. Dietary intake of retinol and carotene were assessed. It was found that only dietary carotene was inversely related to the incidence of lung cancer. Intake of pre-formed vitamin A and of other nutrients was not significantly related. In addition, it was observed that neither carotene nor retinol intake was significantly related to the risk of other carcinomas grouped together.

Positive results were reported by Colditz et al.[225] in a prospective cohort study of 1271 Massachusetts elderly residents (66 years and above). In this 5-year follow-up study, dietary information was obtained by food frequency questionnaire. After controlling for age and smoking behavior, it was found that those in the highest quintile of intake of carotene-rich vegetables had a lower risk of cancer mortality than that of those in the lowest quintile. The trend of decreased cancer risk with increasing intake of carotene-containing vegetables was statistically significant. The investigators emphasized that even in old age, higher intakes of green and yellow vegetables are still associated with lowered risks of cancer death. However, they pointed out that, from such observed associations, it is still not known whether the protective relationship with such vegetables is truly one of cause and effect, and still less is known which components of such vegetables are chiefly involved.

A more recent prospective study was reported by Paganini-Hill et al.[226] of a cohort of 10,473

residents in California who were initially free of cancer and followed from 1981 to 1986. A health survey questionnaire was completed by all members of the cohort, and information obtained included usual frequencies of consumption of certain food items including fruits and vegetables, dairy products, liver, and cereal, as well as vitamin supplements taken. Cancer was confirmed in 643 subjects and included occurrence in various sites. Findings of the study showed little indication that increased intake of vitamin A or β-carotene from the diet or supplements decreased the risk of development of cancer.

The study of Ziegler et al.[227] is an example of a retrospective case-control dietary investigation of the association. It was a study of lung cancer in white males, conducted between 1980 to 1981 in six high-risk areas of New Jersey. Dietary intake of carotene, pre-formed retinol, and total vitamin A were determined by interviewing 763 cases and 900 controls or their next of kin. Subjects were asked about their usual frequency of consumption, several years earlier, of 44 food items, which were thought to provide 83% of the vitamin A in the American diet, and about their use of vitamin supplements. A statistically significant association between increased risk of lung cancer and decreased carotene intake was obtained. No inverse association between retinol intake or total vitamin A intake with risk of lung cancer was observed.

Another case-control study was conducted among the multiethnic population of Hawaii between 1979 and 1982 by Hinds et al.[228] Detailed dietary interviews were carried out for 364 primary lung cancer patients and 627 general population matched controls. It was found that total vitamin A intake (food sources plus supplements), vitamin A intake from food sources only, and carotene intake were each inversely associated with lung cancer risk in males, but not in females. Dietary vitamin C intake was also investigated and shown to be not significantly associated with lung cancer risk.

A smaller, similar study was reported by Stehr et al.[229] for 111 cases and a pair-matched control group in southwestern Pennsylvania, during the years 1978 and 1979. Interviews and/or mail correspondence included questions on food frequency history. It was found that stomach cancer cases showed a significantly higher proportion with lowered total vitamin A intake levels.

Peto et al.[206] analyzed findings from 5 prospective and 15 retrospective studies on the relationship between dietary vitamin A intake and cancer risk carried out in various countries. Most of these dealt with intake of carotene-rich vegetables. Varied relative risks (lower:higher intake) were reported, with many around 1.5 to 2.

Many of the epidemiological studies carried out have indicated a slightly lower-than-average incidence of cancer among people with an above-average vitamin A status. The studies, however, have not produced consistent results and sufficient proof.[199,206,227,230] The negative results from some of these studies could be due to the limited number of high-risk individuals in the study populations. Willett et al.[220] felt that, if indeed an inverse association between serum retinol and overall cancer incidence exists, it is probably complex and limited to certain population subgroups. Such subgroups may be defined by extreme values of serum retinol, certain levels of other nutrients, or specific types of cancers.

There appears to be greater emphasis on the possible protective effects of carotene. Inverse associations were noted more frequently with indices of carotene intake or carotene-containing foods than with indices of pre-formed retinol or retinol-containing foods.[203,207,209,227] Carotenoids as a whole, appear to be more promising as possible protective agents.[36,203,231] It appears that in the normal dietary range the intake of pre-formed vitamin A in individuals with an adequate vitamin A status, such as in developed countries, does not seem to be associated with protection against cancer. In other words, although some protection may be seen by increasing vitamin A intake in the lower consumption levels, intakes of retinol above RDAs would exhibit a threshold effect and would not reduce cancer risk further. In contrast, when total vitamin A for a population is marginal and carotenoids are the predominant source of vitamin A activity, such as in developing countries, both retinol and carotenoids, alone or in combination, would appear to reduce cancer risk.[36]

Although many observational studies of dietary factors and cancer incidence have already indicated a slightly lower than average incidence

of cancer among people with an above average intake of β-carotene, the association is probably rather complex. There are several possible interpretations for the inverse association observed:[203,204,206] (1) it may be just an artifact due merely to association of β-carotene ingestion with some truly protective dietary habits or components; (2) it may be due to association of β-carotene ingestion with avoidance of some truly harmful dietary habits or components; (3) it may be due to a genuine protective effect of β-carotene against the onset of cancer; and (4) it may be factors characteristic of a given life style, which by chance are related to dietary (carotene) pattern.

If carotenes do indeed offer some protection, it is thought that their anticarcinogenicity effect does not require their prior conversion to retinol. It is perhaps the β-carotene that is absorbed unchanged from the intestine which is chiefly responsible for this protective effect rather than any retinol or retinaldehyde generated in the intestine from β-carotene.[206,231]

Peto et al.[206] have suggested various mechanisms by which carotenoids may reduce cancer risks. These include (1) a direct retinoid-like effect of some carotenoids on cellular differentiation in the target tissues; (2) conversion in the target tissue of some carotenoids into molecules with some such retinoid-like effects; and (3) protection by carotenoids of the target tissues via mechanisms not related to control of cellular differentiation. In the last-named group, these mechanisms may include enhancing some immunological functions, or by quenching singlet oxygen, a highly reactive excited molecular species which may be generated as a toxic byproduct of many normal metabolic processes in both animals and plants. According to these authors, β-carotene is the most efficient quencher of singlet oxygen thus far discovered. In plants, the chief functions of carotenoids may be to quench the singlet oxygen produced by photosynthesis (Section VIII.B).

Almost all the studies encountered have mentioned the relationship of carotene or β-carotene to cancer risk. In the case of dietary studies, most of the investigators had also used these terms. This usage of these terms would not be quite correct since it is known that most food tables (used for calculating carotene intake in dietary studies) do not have entries specifically for β-carotene. It would be more accurate to speak in terms of carotenoids. Even for serum levels, if a more specific method was not used, such as using HPLC, it would not be correct to assume that β-carotene levels had been determined. In fact, very few reports of serum levels of other carotenoids exist in the literature. It is entirely possible that carotenoids other than β-carotene, which do not possess provitamin A activity, are the active agents in the associations observed. With further advancement in analytical methods, more accurate data on carotenoid types in foods as well as blood would become available.

3. Cancer Treatment and Prevention Trials

A considerable amount of literature has accumulated on the use of retinoids in the chemoprevention of experimentally induced cancer in animals. "Chemoprevention", according to Sporn et al.,[211] is a different approach from chemotherapy, which is used for treatment of invasive, malignant cells. Chemotherapy is a cytotoxic approach to invasive cancer in which a deliberate attempt is made to kill cancer cells by blocking key metabolic pathways. In contrast, chemoprevention is a new pharmacological approach to arrest or reverse premalignant cells during their progression to invasive malignancy, i.e., during the preneoplasia period, using physiological mechanisms that are not cytotoxic. It makes use of defined chemicals such as provitamins (β-carotene), vitamins (A, C, and E), synthetic analogs, or other substances (e.g., the trace element selenium) for reducing cancer incidence. Chemoprevention is being emphasized by the National Cancer Institute (NCI) as having the potential for making an important impact on nutrition-related cancer incidence.[205]

It is believed that synthetic retinoids would be more appropriate for the chemoprevention of cancer.[211,232] The naturally occurring forms of the vitamin A molecule have limited tissue distribution and are too ineffective. Furthermore, they are too toxic to be used practically for the prevention of cancer in man. Since the 1970s, many new synthetic analogs of vitamin A have been

prepared for this purpose. In the Roche laboratories alone, about 1000 retinoids were synthesized and tested biologically.[232] Molecular modifications were made to all the three components of the vitamin A molecule: the hydrocarbon ring, hydrocarbon side chain, and polar terminal group.[233] These synthetic retinoids have an entirely different pattern of specific biological activity, tissue distribution, and toxicity compared with natural forms of vitamin A.[211] Studies into the toxicity of retinol and the three synthetic retinoids tretinoin, isotretinoin, and etretinate in animals and man have been reviewed in detail by Kamm et al.[234]

Several detailed reviews of the use of retinoids in the chemoprevention of cancer in experimental animals have been published. Moon and Itri[34] have described the induction of various cancers and the successful use of retinoids in the chemoprevention of these cancers. They include cancers of the skin, lung, mammary glands, urinary bladder, and the digestive tract and associated organs. Ong and Chytil[33] have tabulated various studies carried out in this field.

Following successes obtained with laboratory animals, retinoids have been used in the treatment of various human carcinomas. Ong and Chytil[33] tabulated results from some 15 reports on the use of retinoids in man, which showed limited success. Moon and Itri,[34] in reviewing the use of several retinoids, felt that early clinical trials were largely uninterpretable for efficacy because of the small number of patients involved, the lack of control groups, variability in response definitions, and imprecise reporting of data. Nevertheless, evidence of activity is said to be apparent in several malignant conditions. Many of the studies have employed 13-*cis*-retinoic acid, etretinate, tretinoin, and isotretinoin. In some of the studies, a retinoid has been used with other agents in combination chemotherapy. Moon and Itri[34] also described the use of retinoids in cancer prevention, one of the most exciting potential uses for these compounds. Some of these studies had reported reversal or regression of the malignant condition or the prevention of recurrent lesions in the patients. However, the promising results achieved with certain premalignant and malignant changes were accompanied by a series of side effects in skin, mucus membranes, muscles, joints, and bones, as well as changes in serum lipids and hepatic function and teratogenicity.[235] Thus, retinoids for the use of the prevention and therapy of cancer conditions are still undergoing modifications to arrive at compounds satisfactory for clinical use.

Peck[35] has described the use of synthetic retinoids, particularly isotretinoin and etretinate, in the treatment of several dermatologic disorders. Extending from this success, current interest is to examine the use of retinoids in cancer prevention and therapy for cutaneous tumors. Results are still preliminary, requiring further investigation and clarification.

The clinical use of β-carotene and other carotenoids in the treatment of patients with a photosensitivity disease, known as light-sensitive porphyria, has been described by Mathews-Roth[37,128] (Section VIII.B). Work in this area soon led to trials of β-carotene for a variety of other conditions related to photosensitivity, with positive results in some.[36,128]

Since supplemental β-carotene can be ingested for long periods virtually without risk of toxicity, it was thought that long-term randomized cancer intervention studies with this compound may be carried out. Stich et al.[236] reported the supplementation of both β-carotene and retinol to a group of 40 rural Filipino betel chewers. Chewing of betel nut, also a common practice of rural Malaysians and other Asians, has been known to be an important cause of oral cancers. These investigators studied the effect of these supplements on the frequency of micronuclei in cells scraped from inside the cheeks of the subjects. Micronuclei formation is a measure of chromosome breakage in earlier cell divisions, and it is known to be increased by carcinogenic stimuli. It was found that after 3 months of supplementation, there was a threefold decrease in the mean proportion of cells with micronuclei in 37 of the 40 subjects studied. In 11 unsupplemented betel chewers in a nearby cluster of houses, there was no change in the mean proportion of micronuclei.

Munoz et al.[237] reported that a randomized double-blind intervention trial was carried out in Huixian, Henan Province, People's Republic of China, to determine whether combined treatment with retinol, riboflavine, and zinc and could lower

the prevalence of precancerous lesions of the esophagus. A group of 610 individuals, aged 35 to 64 years, with equal numbers of each sex, was randomly allocated to weekly vitamins plus zinc treatment or to placebo. Examination conducted at the end of the treatment, about 14 months later, showed that the prevalence of esophageal lesions among the vitamins plus zinc treatment group was not different from the placebo group.

Several other long-term follow-up studies have been initiated, results of which are still awaited.[34,36] In a large cooperative study sponsored jointly by NCI and the National Heart, Lung and Blood Institute, approximately 20,000 male physicians over 40 years of age are being followed for at least 5 years to evaluate the potential of β-carotene in lowering cancer incidence. NCI sponsored another trial to assess the protective effects of orally administered β-carotene in combination with canthaxanthin in a population of albino Africans in Tanzania. This study is in line with the suggestion that carotenes are able to prevent the development of skin cancer induced by UV light in this high-risk population. NCI is in fact sponsoring some 22 cancer chemoprevention human trials, and most of these involve the use of β-carotene and/or retinol, and a few using *trans*-retinoic acid, or 13-*cis*-retinoic acid.[205]

X. CONCLUDING REMARKS

Vitamin A deficiency remains a serious problem in many developing countries of the world, affecting mainly young children. An estimate is that at any one time, 20 to 40 million preschool children suffer from at least mild deficiency of the vitamin. Such numbers are mind-boggling and beyond the imagination of people living in countries relatively free of the problem. A large proportion of these affected children are from countries in south and east Asia.

The main source of vitamin A in the diet of the rural communities in developing countries is carotenoids, the precursors of vitamin A, from vegetables and fruits. Pre-formed vitamin A in meat, liver, and eggs is frequently out of reach of the economically deprived. Many of these countries have abundant vegetation, rich sources of carotenoids. There should indeed be no vitamin A deficiency problem. This would be correct, provided young children do consume vegetables; the vegetables and fruits consumed contain those carotenoids possessing vitamin A activity; the carotenes ingested are efficiently absorbed and utilized in the body. These are suppositions that do not hold true for these most vulnerable segments of the population. It is known that vegetable consumption among young children is poor. Whatever small amounts of carotenoids ingested together with a diet poor in fat and protein makes these precursors of vitamin A poorly absorbed. At the same time, it is also not clear which of the fruits and vegetables are richest in β-carotene. These are obviously important areas for studies and intervention.

A new frontier in vitamin A research has been the examination of a possible association between carotenoids and retinoids and the development and prevention of cancer. Research in this area can be likened to a huge jigsaw puzzle, with the pieces slowly being put into their places. Evidence has been accumulating from various sectors to support this association. Publications in this field have been increasing at a very rapid rate. Nevertheless, it appears that it will take some time before a clear picture can be seen. It is still too early for recommendations to be made for routine retinol or carotenoid supplementation to prevent cancer.

Since the discovery of vitamin A as a fat-soluble growth factor in the early part of this century, research into carotenoids and retinoids has attracted the attention of many scientists. Volumes have been written about them, and many more are certain to follow since many questions remain to be answered. Even the biochemical mechanisms of action of the vitamin and precursors remain largely unknown. There is thus a great venue for studies, for scientists looking for excitement in research.

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