

Biochemistry of Vitamin A and Carotenoids

JAMES A. OLSON

Our understanding of the biochemistry of vision developed much more rapidly than that of cellular differentiation. Since the identification in 1987 of nuclear receptors for retinoic acid that induce gene expression, however, we have been rapidly gaining insight into the nature of the latter process. Our past and present knowledge about vitamin A and carotenoids has been summarized elsewhere.¹⁻³

Nature, insofar as possible, protects us from the ill effects of both inadequate and excessive intakes of the vitamin. The physiological and biochemical processes underlying this protection are described in this chapter.

Chemistry and Nomenclature

Chemical Aspects

Vitamin A and more than 600 carotenoids have been crystallized and fully characterized by a variety of chemical and physical methods. Furthermore, vitamin A and many of its analogs, as well as selected carotenoids, have been synthesized chemically from simple, readily available precursors. Mainly because of the structure of conjugated double bonds that are characteristic of both vitamin A and carotenoids, these substances are sensitive to oxidation.^{2,3}

Vitamin A is now considered chemically as a subgroup of the retinoids, which are defined as a class of compounds consisting of four isoprenoid units joined

in a head-to-tail manner and customarily containing five conjugated double bonds^{4,5} The term “vitamin A” is used as a generic descriptor for retinoids exhibiting qualitatively the biologic activity of retinol The numbering system for all-*trans* retinol is depicted in Figure 8-1 (point A) Other naturally occurring retinoids of biologic interest, shown in this figure, are B through J Retinoids K and L are synthetic compounds with high biological activity³

The nomenclature of carotenoids primarily is based on β -carotene or, more formally, on β,β -carotene^{5,6} The formulas and numbering system for β -carotene and α -carotene are given in Figure 8-1 (points M and N) The term provitamin A carotenoids is used as a generic descriptor for all carotenoids exhibiting qualitatively the biologic activity of vitamin A³⁻⁶

A large number of geometric isomers both of retinol ($n = 16$) and of β -carotene ($n = 272$) can exist All sixteen of the vitamin A isomers and several of the β -carotene isomers have been synthesized Interestingly, the all-*trans* and three of the four mono-*cis* isomers of vitamin A—the 9-*cis*, 11-*cis* and 13-*cis* forms—are known to play specific physiologic roles in nature

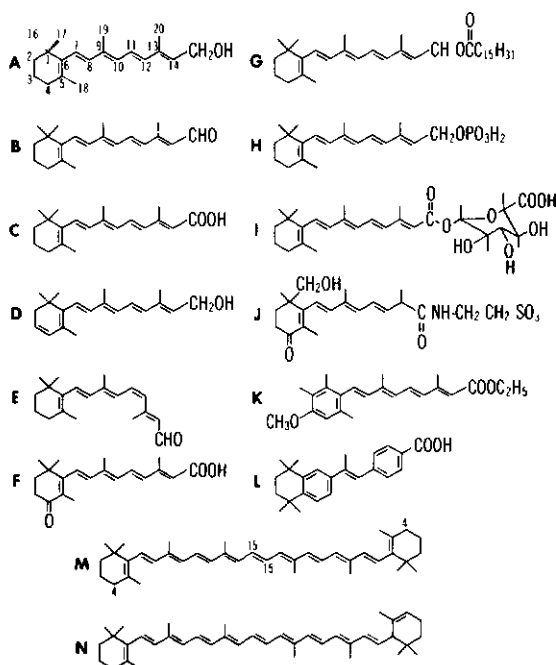


Fig. 8-1. Formulas and numbering systems for retinoids and carotenoids A, all-*trans* retinol, B, all-*trans* retinal, C, all-*trans* retinoic acid, D, 3-dehydroretinol (vitamin A₂), E, 11-*cis* retinal, F, 4-oxoretinoic acid, G, retinyl palmitate, H, retinyl phosphate, I, retinoyl β -glucuronide, J, retinotaurine, K, trimethylmethoxyphenyl analogue of ethyl retinoate, L, tetrahydro, tetramethylnaphthylisopropenylbenzoic acid, M, all-*trans* β -carotene, N, α -carotene (From J A Olson³)

Vitamin A and carotenoids are soluble in most organic solvents, but not in water. In their extraction from plasma and tissues, therefore, the cell structure must be disrupted, the proteins denatured, and the lipid fraction dissolved in some solvent, such as hexane or dichloromethane, which is immiscible with water. In crystalline form or when dissolved in oil containing an antioxidant, vitamin A is stable for long periods, providing it is kept in the dark in a sealed container under a dry nitrogen or argon atmosphere. Carotenoids, although less stable than retinol, are also preserved well under similar conditions.^{2,3}

Vitamin A and the carotenoids are sensitive to oxidation, isomerization, and polymerization when dissolved in dilute solution under light in the presence of oxygen, particularly at elevated temperatures. The destruction of these compounds is particularly rapid when they are adsorbed as a thin surface film in the presence of light and oxygen.³

Vitamin A is stable when stored in frozen liver tissue in the dark at a temperature below -20°C , and in frozen serum stored at -70°C in sealed vials under ideal conditions. Carotenoids present in stored frozen serum or tissues tend to be more sensitive than vitamin A to destruction, but are stable at -70°C in the dark under argon.³

Vitamin A and carotenoids are usually separated by high-pressure liquid chromatography and detected by UV-visible absorption spectrophotometry,^{7,8} although other physical and chemical procedures for detection exist. Vitamin A shows a characteristic ultraviolet (UV) absorption spectrum with an absorption maximum (λ_{max}) of 325 nm and a molecular extinction coefficient of $53,000 \text{ cm}^2\text{M}^{-1}$ ($E_{1\%}^{1\text{cm}}$ of 1850) in hexane.

Carotenoids also show characteristic absorption spectra, β -carotene, for example, has a λ_{max} of 450 nm in hexane with a molecular extinction coefficient of $136,900 \text{ cm}^2\text{M}^{-1}$ ($E_{1\%}^{1\text{cm}}$ of 2550).

Biologic Aspects

Whenever appropriate, vitamin A and individual retinoids and carotenoids are preferentially expressed in molar terms in accord with the Systeme International (SI). Thus, serum concentrations of retinol are given in micromolar terms ($\mu\text{mol/liter}$) rather than in micrograms per deciliter ($\mu\text{g/dl}$), and liver concentrations are given as micromoles per gram, not as micrograms per gram. In this expression, $1 \mu\text{g}$ retinol is equal to $0.003491 \mu\text{mol}$ or, conversely, $1 \mu\text{mol}$ of retinol equals $286.46 \mu\text{g}$ of retinol.³ SI units are less applicable to food sources.

In nutrition, the primary unit of biologic activity for vitamin A is $1 \mu\text{g}$ of all-*trans* retinol, whether present as the free alcohol or as one of several natural or synthetic fatty acyl esters.

To express both preformed vitamin A and provitamin A carotenoids in foods as a single nutritive value, the retinol equivalent (RE) was created. One μg RE is equal to $1 \mu\text{g}$ of all-*trans* retinol, to $6 \mu\text{g}$ of all-*trans* β -carotene, or to $12 \mu\text{g}$

of other provitamin A carotenoids in foods. The bioavailability of carotenoids varies greatly, however, depending on their physical state in foods. Thus, carotenoids in oil are well utilized, but those in uncooked whole vegetables are poorly absorbed.

A unit of historical value, which is still extensively used in food composition tables and in labeling of vitamin A supplements, is the International Unit, or IU. One IU equals 0.300 μg of all-*trans* retinol, or a corresponding amount of retinol in ester linkage. Thus, whether the vitamin A in a given solution is present as free retinol, retinyl acetate, or retinyl palmitate, the number of IUs will be the same.³ One IU of all-*trans* β -carotene was defined at 0.6 μg . The nutritional equivalency of β -carotene relative to vitamin A is three times higher when this nomenclature is used than when RE is employed. Thus, it is useful to distinguish IU as IU_a for vitamin A, and as IU_c for β -carotene. The RE system, which is better based physiologically, is less confusing and, consequently, is preferred.

The all-*trans* isomers of both vitamin A and provitamin A carotenoids are the most nutritionally active forms, *cis*-isomers usually show 50% or lower activities relative to the all-*trans* forms.

Physiologic Processes

Digestion and Absorption

Preformed vitamin A and carotenoids in the diet are largely released from protein during proteolysis in the stomach. Vitamin A and carotenoids tend to aggregate with lipids into globules, which then pass into the small intestine. The upper intestine is the major site of lipid hydrolysis. Dietary fat, protein, and their hydrolytic products stimulate, through cholecystokinin, the secretion of bile, which first emulsifies lipids and then forms micelles. Bile salts also stimulate pancreatic lipase, which hydrolyzes triglycerides and other esters that hydrolyze retinyl esters and cholesteryl esters. Retinyl esters are hydrolyzed primarily by an enzyme located in the brush border of intestinal mucosal cells. Hydrolysis of retinyl esters greatly enhances the bioavailability of vitamin A. The product, retinol, in a bile salt-containing micelle is well absorbed (70% to 90%) by mucosal cells of the small intestine. Vitamin A seems to be absorbed by a carrier-mediated process at low concentrations, but mainly by diffusion from the micellar phase at high doses.^{3,9-11}

Hydrocarbon carotenoids are not as well absorbed as retinol, possibly because of their awkward length and their specific requirement for bile salts. In the presence of fat, 30%–50% of moderate amounts (< 15 mg) of carotenoids are absorbed by humans. As the amount increases, however, the absorption efficiency declines. Highly polar carotenoids are poorly absorbed.

Plasma Transport

Chylomicra

Within intestinal cells, newly formed chylomicra contain retinyl ester, cholesteryl ester, some retinol, phospholipids, much triglyceride, and apolipoproteins A-1, A-4, B, and several others. In the complex conversion of the secreted chylomicra into chylomicron remnants in the plasma, the triglyceride content is markedly reduced by the hydrolytic action of lipoprotein lipase, the predominant apolipoproteins on the chylomicron remnant become B and E, and the relative concentrations of retinyl ester increase per remnant particle^{3,9-11}

Retinol-Binding Protein (RBP)

Human RBP, a single polypeptide chain with 182 amino acids in a known sequence, has a molecular weight of 21,230. The protein contains an eight-stranded anti-parallel β -barrel at its core, within which all-*trans* retinol is bound¹². Thus, little if any bound retinol is exposed at the surface of the protein. The 1:1 molar complex of all-*trans* retinol and RBP is called "holo-RBP". Within the plasma, holo-RBP is found in large part as a 1:1 complex with transthyretin (prealbumin), which specifically binds one thyroxine molecule per tetramer¹³.

In well-nourished adults, the total RBP concentration in plasma is 1.9 $\mu\text{mol/liter}$ to 2.4 $\mu\text{mol/liter}$ (40 $\mu\text{g/ml}$ to 50 $\mu\text{g/ml}$), 80% to 90% of which exists as holo-RBP. In children up to the age of puberty, the total RBP concentration is approximately 60% of the adult level^{3,13}. Protein-energy malnutrition, infections, and parasitic infestations all lower steady state concentrations of holo-RBP. Thus, the vitamin A status of an individual often is not predictable on the basis of holo-RBP concentrations alone (Chapter 11).

Serum Retinoids and Carotenoids

Mean values and ranges of retinoids in serum as a function of age and sex are presented in Table 8-1¹⁴. These values, drawn from the first U.S. National Health and Nutrition Examination Survey in 1971-1974 (NHANES I), clearly show that mean plasma retinol concentrations in young children are approximately 60% of adult values until puberty, when they increase. Male and female children show the same serum retinol values, whereas adult males have values 20% higher than adult females.

The concentrations of specific and total carotenoids in the plasma are highly dependent on diet. Of the six major carotenoids in the plasma of American residents, lycopene is the most common, followed by lutein plus zeaxanthin and then by β -carotene. Of the total plasma carotenoids, the percentage of provitamin

Table 8-1 Serum Retinol Concentrations ($\mu\text{mol/liter}$) as a Function of Age and Sex in American Residents, 1971-1974^a

Age	Total	Males	Females
3-5 yr	1.28 (0.73-2.0)	1.29 (0.70-2.1)	1.26 (0.77-2.0)
n	1414	725	689
6-11 yr	1.31 (0.84-1.9)	1.30 (0.84-1.9)	1.32 (0.84-1.9)
n	1857	930	927
12-17 yr	1.58 (1.0-2.3)	1.62 (1.1-2.3)	1.53 (1.0-2.2)
n	2035	1026	1009
18-44 yr	1.94 (1.2-2.9)	2.08 (1.4-3.0)	1.80 (1.1-2.8)
n	7035	2164	4871
45-74 yr	2.20 (1.3-3.3)	2.29 (1.4-3.5)	2.11 (1.3-3.2)
n	6111	2911	3200

^aThe 5th and 95th percentile values are given in parentheses

Derived from S.M. Pritchard⁴

A carotenoids in adults is usually 40%–50%. The plasma carotenoid patterns in adults have been fairly well studied,^{15,16} but as yet few studies have been conducted in children.^{17,18} Serum carotenoid patterns from these studies are summarized in Table 8-2. In malnourished children in Senegal, serum concentrations of both retinol and total carotenoids were approximately half those in well-nourished American children,^{17,18} although provitamin A carotenoid concentrations were similar (Table 8-2).

In addition to retinol and the cited carotenoids, lower steady-state concentrations ($< 0.1 \mu\text{mol/liter}$) of retinyl esters, retinoic acid, retinyl β -glucuronide, retinoyl β -glucuronide, and at least twelve other carotenoids have been identified in fasting plasma.¹⁹⁻²¹ Besides retinoic acid, some of these minor components may also play significant roles in nutrition and function.

Tissue Uptake and Storage

Uptake of Chylomicron Remnants

By interaction with cell surface receptors on liver parenchymal cells for apolipoprotein E, and possibly for apolipoprotein B, chylomicron remnants are internalized by receptor-mediated endocytosis. Retinyl esters are hydrolyzed, combined with cellular retinol-binding protein (CRBP) in the cytosol of the hepatocyte, and then subjected to several possible metabolic routes.^{3,9,11} Lipid-rich chylomicra are cleared more slowly from plasma than are lipid-poor chylomicra.¹¹ Chylomicron remnants are also taken up well, presumably by a similar mechanism, by bone marrow, and to a lesser degree by other peripheral tissues.

Table 8-2 Mean Serum Carotenoid Values ($\mu\text{mol/liter}$) as a Function of Nutritional Status and Age^a

Years	Place	Sex	Number	State	Nutritional	ROL ^b	Total ^c	Carotenoids ^b					Ref
								β -Car	α -Car	β -Cryptox	Lut ^d	Lyc	
2-4	Senegal	MF	271	Poor		0.61 ± 0.22	0.74 ± 0.61	0.16 ± 0.14	0.03 ± 0.06	0.02 ± 0.02	0.46 ± 0.28	0.07 ± 0.11	17
2-14	Michigan	MF	10	Good		1.42 ± 0.45	1.41	0.20 ± 0.12	0.03 ± 0.02	—	0.38 ± 0.20	0.80 ± 0.39	18
18-45+	Boston, MA	M	137	Good		2.2 (1.3-3.3)	1.05	0.34 (0.13-1.1)	0.08 (0.01-0.20)	—	—	0.63 (0.23-1.2)	15
18-45+	Boston, MA	F	193	Good		1.85 (1.1-2.8)	1.39	0.59 (0.11-1.4)	0.14 (0.02-0.40)	—	—	0.66 (0.23-1.2)	15
59 \pm 10	Washington, DC	M	55	Good		2.6 ± 0.64	1.22	0.31 ± 0.20	0.05 ± 0.04	0.15 ± 0.10	0.32 ± 0.15	0.39 ± 0.23	16
59 \pm 7	Washington, DC	F	55	Good		2.3 ± 0.66	1.39	0.44 ± 0.27	0.07 ± 0.04	0.18 ± 0.10	0.35 ± 0.16	0.35 ± 0.04	16

^aUnder the mean values, values in parentheses are 5th and 95th percentile values whereas \pm values are standard deviations

^bAbbreviations ROL: retinol, β Car, β -carotene α -Car, α -carotene, β -Cryptox, β -cryptoxanthin Lut Lutein, Lyc Lycopene

^cTotal carotenoids are estimated as the sum of measured components

^dLutein values include a minor portion (ca. 20%) of zeaxanthin

Uptake from Holo-RBP

Holo-RBP, derived largely from the liver but also from other tissues, is taken up from plasma by all tissues of the body. Two mechanisms have been postulated: (1) interaction of holo-RBP with a specific cell-surface receptor, followed by internalization of the complex²², and (2) dissociation of holo-RBP in the plasma into apo-RBP and retinol, followed by incorporation of retinol into the plasma membrane^{11,23}. Thereafter, CRBP may draw retinol out of the membrane into the cytosol^{11,23}.

Although the retinal pigment epithelial cells apparently possess cell-surface receptors for holo-RBP on their external surfaces,²² their presence on other cells is much less clear^{11,23}. Whatever the mechanism of uptake, however, retinol and its esters are found in significant amounts in adipose tissue, kidney, testis, lung, bone marrow, and the eye, in addition to the liver, and in smaller amounts in other tissues.

Storage

Retinyl esters in chylomicron remnants are hydrolyzed within hepatocytes to retinol, which then can be esterified with long-chain fatty acid esters and stored in specialized lipid globules.

Alternatively, retinol may be transferred to stellate cells, also termed lipocytes, Ito cells, and fat-storing cells, where retinol is also esterified and stored in vitamin A-containing globules. Under normal physiologic conditions, stellate cells contain 80% to 90% of the stored vitamin A, hepatocytes 10% to 20%, and other liver cells only a few percent. The retinyl ester stored in stellate cells and hepatocytes can be readily and completely mobilized and used by the organism.^{3,9,11}

The major ester of stored retinyl esters is the palmitate, with smaller amounts of the stearate, linoleate, oleate, and others. The major synthetic route is by transacylation from the α -position of phospholipids, although direct acylation via coenzyme A derivatives can also occur.¹¹

Although the liver clearly is the major storage site, most other tissues also possess stellate cells and store retinyl esters. Retinol may be transferred from parenchymal cells to stellate cells as intact holo-RBP,²⁴ as free retinol,¹¹ or by yet unidentified carriers or processes.¹¹

Release from Tissues

Within the hepatocyte, a precursor of RBP—preapo-RBP—is first formed. It is then proteolytically cleaved, with the loss of a peptide, to apo-RBP. All-*trans* retinol combines with apo-RBP in a specific 1:1 molecular complex to form holo-

RBP The latter is transported through the Golgi apparatus and then is secreted into the plasma, probably as a complex with transthyretin³¹¹

Retinol might be released into plasma from liver stellate cells by three routes (1) by its transfer back to parenchymal cells, followed by holo-RBP release, (2) by its direct release into the plasma as an endogenously formed RBP complex, or (3) by its transfer to extracellular apo-RBP at the cell membrane. Whether all of these routes are active and, if so, which route predominates under given physiological conditions is uncertain^{311,24}

Although vitamin A is primarily stored in the liver, all tissues contain some vitamin A. Because messenger ribonucleic acid (mRNA) for RBP has been identified in the kidney, lacrimal gland, adipose tissue, and bone marrow as well as in the liver, nonhepatic tissues may well synthesize and secrete RBP. RBP in plasma from hepatic and extrahepatic tissues, however, seems to be identical. Thus, it has not yet been possible to ascertain the relative amount derived from each tissue.

Recycling and Excretion

Retinol released as holo-RBP from the liver is taken up by peripheral tissues. Much of this retinol is later released from peripheral tissues as holo-RBP, as lipoprotein-bound retinyl esters, or as water-soluble retinyl β -glucuronides. All of these forms are then transported back to the liver. This recycling is extensive and efficient, as shown by *in vivo* kinetic analysis^{24,25}. When vitamin A intakes are very low, the efficiency of recycling increases^{24,25}.

The glucuronides of vitamin A, which are secreted in the bile, are also reabsorbed from the intestinal lumen and transported back to the liver^{39,11}. This enterohepatic circulation also helps to maintain the vitamin A status of an individual during periods of low intakes.

Approximately 5% to 20% of ingested vitamin A and a larger percentage of carotenoids, depending on their nature, bioavailability, and amount, are not absorbed from the intestinal tract and consequently are excreted in the feces. A significant portion (10% to 40%) of absorbed vitamin A is oxidized or conjugated in the liver and then is secreted into the bile. Although, as already mentioned, some of these biliary metabolites, such as retinoyl β -glucuronide, are reabsorbed in the intestine and transported back to the liver, most of the biliary metabolites are excreted in the feces.

Vitamin A that is oxidized and chain-shortened in various tissues ultimately is excreted in the urine. Finally, carbon dioxide that is released by the oxidation and cleavage of the side chain of vitamin A is excreted in the expired air. In quantitative terms, an average of 10% of dietary vitamin A is not absorbed, 20% appears in the feces through the bile, 17% is excreted in the urine, 3% is released as CO₂, and 50% is stored, primarily in the liver³¹¹.

Metabolic Processes

Binding Proteins

In addition to plasma RBP, a set of specific binding proteins for retinol, retinal, and retinoic acid has been identified within cells, as well as in the intercellular matrix between the retinal pigment epithelium and the rod outer segments²⁶ Major specific binding proteins of mammals are listed in Table 8-3

Other retinoid-binding proteins that are at least partly characterized have been identified in the epididymis, uterus, fetal liver, and Sertoli cells Some other proteins, such as several fatty acid-binding proteins, serum albumin, and β -lactoglobulin also bind retinoids and, in particular, retinoic acid Several of these proteins are closely related structurally²⁷

Thus, the retinoids clearly are chaperoned by a set of highly specific proteins *in vivo* and can bind as well to other structurally related proteins Specific binding proteins seem to function as transport agents, as sequestering entities, as cofactors in enzymatic transformations, and as cofactors in genetic expression^{3,13} The physiologic role played by nonspecific binding proteins is still unclear

Vitamin A

The major reactions of vitamin A metabolism are esterification, oxidation at C-15, oxidation at C-4, conjugation, isomerization, other miscellaneous oxidative reactions, and chain cleavage Retinol and retinal, as well as other metabolites reversibly converted to them, all possess significant biologic activity Retinoic acid and its glucuronide are active in growth but not in vision or, in most species, in reproduction Except for 14-hydroxy-retinol, more oxidized products—such as 4-hydroxyretinoic acid, 5,6-epoxyretinoic acid, and C-19 metabolites—are largely devoid of biologic activity

Retinoyl β -glucuronide, retinyl β -glucuronide, and retinoic acid, as already mentioned, are normally present in small amounts (3 nmol/liter to 11 nmol/liter, or 1 μ g/liter to 5 μ g/liter) in human plasma Retinoyl β -glucuronide is not hydrolyzed in some cells and only slowly *in vivo* Retinoic acid, besides being physically bound by serum albumin and by cytosolic and nuclear binding proteins, can also be covalently bound to proteins, possibly by means of a coenzyme A intermediate The cellular retinoid-binding proteins play a major role in the oxidation/reduction and transesterification of retinol Intestinal CRBP-II, CRBP, and CRABP have been particularly well studied in this regard^{3,11,28,29}

Stored retinyl esters are hydrolyzed to free retinol by a group of intracellular retinyl ester hydrolases¹¹ Some of these hydrolases depend on the presence of bile salts or other detergents, and some do not Apo-CRBP stimulates hydrolase activity, presumably by serving as an acceptor of released retinol¹¹

Table 8-3 Major Retinoid-Binding Proteins

<i>Name</i>	<i>Abbreviation</i>	<i>Location</i>	<i>Molecular Weight (KDa)</i>	<i>Major Ligands</i>
Retinol-binding protein	RBP	Plasma	21.2	all- <i>trans</i> retinol
Interphotoreceptor retinol-binding protein	IRBP	Extracellular matrix of the eye	135	11- <i>cis</i> & all- <i>trans</i> retinol
Cellular retinol-binding protein, type I	CRBP-I	Cytosol	15.7	all- <i>trans</i> retinol all- <i>trans</i> retinal
Cellular retinol-binding protein, type II	CRBP-II	Cytosol small intestine	15.6	all- <i>trans</i> retinol all- <i>trans</i> retinal
Cellular retinoic acid binding-protein, type I	CRABP-I	Cytosol	15.5	all- <i>trans</i> retinoic acid
Cellular retinoic acid binding-protein, type II	CRABP-II	Cytosol fetal tissues	15.0	all- <i>trans</i> retinoic acid
Cellular retinaldehyde-binding protein	CRALBP	Cytosol eye	36.0	11- <i>cis</i> retinol, 11- <i>cis</i> retinal
Retinoic acid receptor- α	RAR $_{\alpha}$	Nucleus	50	all- <i>trans</i> retinoic acid
Retinoic acid receptor- β	RAR $_{\beta}$	Nucleus	50	all- <i>trans</i> retinoic acid
Retinoic acid receptor- γ	RAR $_{\gamma}$	Nucleus mainly skin	50	all- <i>trans</i> retinoic acid
Retinoid X receptor- α	RXR $_{\alpha}$	Nucleus	51	9- <i>cis</i> retinoic acid
Retinoid X receptor- β	RXR $_{\beta}$	Nucleus	51	9- <i>cis</i> retinoic acid
Retinoid X receptor- γ	RXR $_{\gamma}$	Nucleus	51	9- <i>cis</i> retinoic acid

Carotenoids

Most provitamin A carotenoids can be cleaved by a carotenoid 15,15'-dioxygenase in the cytosol of the intestinal mucosa, of hepatocytes, and of some other tissue cells. β -carotene yields two molecules of retinal, which are in large part reduced and esterified to retinyl ester. The cleavage enzyme requires molecular oxygen and apparently contains a metal, possibly iron, at its catalytic site. β -carotene and some other carotenoids can also be cleaved asymmetrically to yield β -apocarotenals that, in turn, are converted to retinal, or possibly directly to retinoic acid. At the level of retinal, therefore, that is reversibly reduced to retinol by alcohol dehydrogenases in many tissues, the metabolism of carotenoids and that of preformed vitamin A usually coincide. β -carotene can also be oxidized to biologically inactive products by lipoxygenase and other oxidative enzymes. Much less is known about the metabolism and excretion of carotenoids other

than carotene. Each carotenoid, however, seems to show a specific pattern of absorption, metabolism, and transport.^{3,30}

The overall metabolism of carotenoids has recently been reviewed.^{31,32}

Functions of Vitamin A and Carotenoids

Vitamin A

Vitamin A is important for vision, cellular differentiation, morphogenesis, and transmembrane transport (in bacteria). Many other complex physiologic processes in animals, such as growth, reproduction, and the immune response (Chapter 9), seem to be affected by these central functions.

Vision

The role of vitamin A in vision is well defined (Chapter 4).³³ In the outer segment of rod cells in the retina, 11-*cis* retinal forms a protonated Schiff base with a specific lysine residue of the membrane-bound protein, opsin, to yield rhodopsin, with an absorption maximum of 498 nm. Similar complexes exist in human cone cells to give three specific iodopsins that absorb maximally at 420 nm (blue cones), 534 nm (green cones), and 563 nm (red cones).

When a photon of light strikes the dark-adapted retina, the 11-*cis* bond of retinal in rhodopsin is isomerized to the all-*trans* form. This isomerization destabilizes rhodopsin, which passes through a series of different conformational states. The light-activated transformation of rhodopsin ultimately results in a reduction in the sodium ion current into the rod outer segment, which induces hyperpolarization of the membrane. In the probable sequence of steps in this amplification cascade, light converts rhodopsin to the active intermediate, metarhodopsin II. The latter induces the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on a disk protein termed "transducin." Transducin contains three subunits, and the complex of GTP with transducin activates phosphodiesterase, which in turn hydrolyzes cyclic guanosine monophosphate (cGMP) to GMP. As the concentration of cGMP falls, the sodium channel closes, leading to membrane hyperpolarization.³³

The cascade is turned off by the time-dependent decay of metarhodopsin II to opsin and all-*trans* retinal, by the conversion of metarhodopsin II to an inactive phosphorylated form, and by the hydrolysis of bound GTP by the inherent GTPase activity of transducin.

A number of proteins are involved in this complex cascade besides those already cited: rod cell spectrin, the 63-KDa protein and the Na⁺-Ca⁺⁺ 220 KDa exchange protein, the Na⁺ channel, peripherin and the rim protein of the

disc membrane, recovering, phosphodiesterase, arrestin, and many others³³ Specific roles in the cascade have been defined for many, but not for all, of the twenty or more characterized protein components involved in the visual cycle³³

All-*trans* retinal may be isomerized back to the 11-*cis* form in the rod outer segment by light in the presence of certain phospholipids In the dark, however, 11-*cis*-retinol is formed by the action of all-*trans* 11-*cis* retinyl ester isomerase, a membrane-bound enzyme in retinal pigment epithelial cells³⁴ This enzyme might better be called an isomerohydrolase, in that the energy released in the hydrolysis of the retinyl ester bond is directly coupled to the formation of the energy-rich 11-*cis* retinol The 11-*cis* forms of retinol and retinal are then transported on IRBP to the rod outer segment, whereas all-*trans* retinol is shuttled back on IRBP^{33,35}

Both all-*trans* and 11-*cis* retinyl esters are also stored in the retinyl pigment epithelial cells, often in lipid-rich globules Retinyl esters are formed in the eye by a trans-acylation reaction from phospholipids, whereas retinyl ester hydrolases, both in the eye and elsewhere, act preferentially on the *cis*-isomers³⁵

Cellular Differentiation

In vitamin A deficiency, mucus-secreting cells are replaced by keratin-producing cells in many tissues of the body Conversely, the addition of vitamin A to vitamin A-deficient keratinizing cells in tissue culture induces a shift to mucus-producing cells Retinoids also rapidly induce F-9 teratocarcinoma cells, as well as many other cell lines, to differentiate In this process, a number of new proteins appear in the newly differentiated cells Thus, vitamin A and its analogues, both in vivo and in vitro, markedly influence the way in which cells differentiate^{33,36}

The mechanism by which retinoids induce cellular differentiation is becoming clear (Fig 8-2) Within tissue cells, all-*trans* retinol, in association with CRBP, can be oxidized to all-*trans* retinoic acid and presumably can also be isomerized to 9-*cis* retinol, which in turn can be oxidized to 9-*cis* retinoic acid Another likely route for the synthesis of 9-*cis* retinal is by oxidative cleavage of 9-*cis* β -carotene All-*trans* or 9-*cis* retinoic acid is transported on CRABP or on other retinoid-binding proteins to the nucleus, where it is tightly bound to one or more of the three (α , β , γ) retinoic acid receptors (RAR) or to one or more of the three (α , β , γ) retinoid X receptors (RXR) respectively^{37,38} In the activation of retinoic acid-responsive genes, a heterodimer of RAR and RXR, or in some cases a homodimer of RXR, binds to the response element of the gene to initiate transcription RXR also serves as a coregulator for the expression of genes responsive to triiodothyronine, to calcitriol, and perhaps to other hormones, but not to estrogen^{33,37,38} In this latter role, RXR forms a heterodimer with the appropriate nuclear receptor for other hormones, thereby enhancing its affinity for the response element of the gene The quantitative balance among RAR, RXR, and other nuclear proteins may also contribute to gene regulation in vivo

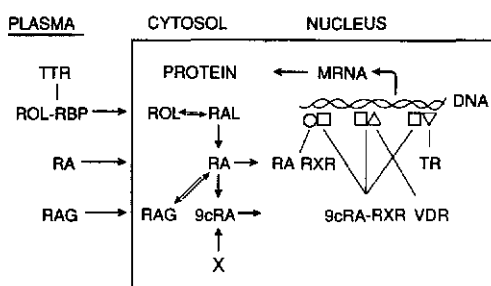


Fig. 8-2. Roles of retinoids in the differentiation of target cells. All retinoids are considered to be all-*trans* isomers unless otherwise specified. Abbreviations are: ROL, retinol; RAL, retinal; RA, retinoic acid; 9cRA, 9-*cis* retinoic acid; RAG, retinoyl β -glucuronide; RBP, plasma retinol-binding protein; TTR, transthyretin; RAR, retinoic acid receptor; RXR, retinoic acid receptor that specifically binds 9cRA; VDR, vitamin D receptor; TR, thyroxine receptor; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; X, unknown precursors (possibly carotenoids) of 9cRA; \square , $\square\Delta$, and $\square\nabla$, heterodimers of various nuclear receptors.

The RAR and RXR receptors, like other nuclear hormone receptors, possess six protein domains with specific functions. At the N-terminal end, domains A and B serve as physiologic activators of the receptor, domain C, which is highly conserved, contains zinc-sulfhydryl interactions ("zinc fingers") that bind to DNA, domain D is a hinge region that provides the necessary conformation of the receptor, domain E binds the ligand, and domain F, at the C-terminal end, enhances dimerization. All nuclear retinoid receptors contain approximately 460 amino acids and have molecular weights of approximately 50 kDa.^{3,37,38}

Retinoic acid directly activates the genes for CRBP-I, CRBP-II, RAR β , Hox-1.6, laminin B1, and transglutaminase in diverse types of cells. The sequence by which other genes are subsequently activated is not clear. Various isoforms of transforming growth factor β (TGF β) can be induced or suppressed by retinoic acid, depending on conditions. Although attention has centered on the activation of gene expression, retinoids can also suppress transcription.^{3,37,38}

Some retinoids may stimulate differentiation by a different pathway, for example, retinoyl β -glucuronide does not bind to CRBP, CRABP, or nuclear RAR, but nonetheless is highly active biologically.^{39,40} Similarly, B lymphocytes differentiate in response to 14-hydroxy-retro-retinol, but not to all-*trans* retinoic acid.⁴¹ Retinoids also show some physiologic effects in enucleated cells. Thus, probably by several mechanisms, retinoids seem to play a central role in the development and maintenance of many tissues.^{3,37,38}

Morphogenesis

Both a deficiency and an excess of vitamin A and of most other retinoids adversely affect embryogenesis.^{42,43} In a more physiologic context, pattern formation in the

skin might well be affected by gradients of vitamin A.⁴⁴ The hypothesis that all-*trans* retinoic acid might well be one of a presumed host of morphogens that control embryologic development originated with the demonstration that an implant containing all-*trans* retinoic acid, when placed in the anterior part of the developing chick limb bud, mimics the activity of the naturally occurring zone of polarizing activity (ZPA).^{42,45}

There are two major hypotheses to explain the dramatic effects of retinoic acid and of many other retinoids on the embryo: (1) that a morphogenic gradient of retinoic acid exists in the developing limb, which in turn provides signals to cells at a given position in the embryo, thereby inducing them to differentiate in a specific way or to migrate in a given direction, and (2) that retinoic acid induces the differentiation of a specific group of cells—the ZPA, which in turn provides as yet unidentified signals to nearby cells, thereby causing them to act in specific ways. Although evidence supports both hypotheses, the second suggestion seems to be the most viable.⁴⁶

Both cytosolic retinoid-binding proteins and nuclear retinoic acid receptors appear in different groups of cells at different times in development. Retinoic acid also induces the formation of many Hox genes, which have been closely linked to developmental processes.^{36,42} Further, retinoic acid induces programmed cell death, or apoptosis, in the developing embryo. Apoptosis is a normal process in development, but can also cause terata if not regulated.^{36,42,43} Thus, clarification of the mechanisms of action of retinoids in development, whatever they might be, will enrich our understanding of these complex but important processes.

Other Functions

The Immune Response Vitamin A has long been termed the “anti-infective” vitamin based on the increased number of infections noted in vitamin A-deficient animals and humans.¹ In vitamin A deficiency, both specific and nonspecific protective mechanisms are impaired: the humoral response to bacterial, parasitic, and viral infections, cell-mediated immunity, mucosal immunity, natural killer cell activity, and phagocytosis. In addition to vitamin A, both nutritionally active (β -carotene) and nutritionally inactive (canthaxanthin) carotenoids enhance the immune response in animals by as yet undefined mechanisms. The roles of vitamin A in these various processes have been recently reviewed.^{47,48} This important topic is considered in detail in Chapter 9.

Intercellular Communication Both retinoids and, at much higher concentrations, carotenoids enhance communication between cells by inducing the formation of connexin 43, a gap-junctional protein.⁴⁹ These interesting observations may be relevant both to cellular patterns in tissues and to the suppression of neoplastic growth.

Transmembrane Transport Bacteriorhodopsin, a light-sensitive, retinal-containing protein similar to rhodopsin, is found in the purple patches on membranes of *Halobacterium halobium*.⁵⁰ In response to light, this protein also undergoes a series of conformational changes ultimately linked to the transfer of a proton from the cytosol to the external medium.⁵⁰ During this cycle, however, the 13-*cis* and all-*trans* isomers of retinal are involved, rather than the 11-*cis* and all-*trans* forms as in the vertebrate visual cycle. Several related light-sensitive retinal-binding proteins with different functions have been identified in this and other like organisms.

Whether retinoids play somewhat similar roles in animal cells is not clear.

Complex Tissue Involvements

Vitamin A is essential, either directly or indirectly, for the proper functioning of most organs of the body. For example, reproductive processes in both males and females and bone development and maintenance are particularly dependent on adequate vitamin A status. Whether these complex physiologic processes have unique needs for vitamin A or rely primarily on the action of vitamin A in cellular differentiation is not clear. Because vitamin A influences the synthesis and secretion of a variety of cytokines and growth factors, some of its effects may well be induced via the action of such factors on cells.⁵¹

Carotenoids

Carotenoids are necessary for many biological processes, show interesting, usually beneficial effects against abnormal conditions and diseases, and have been associated as possible protective factors against several chronic diseases. Their manifold activities can be classified as functions, actions, or associations.^{52,53}

Functions

Carotenoids function in many ways in nature—as accessory pigments in photosynthesis, as protectants against light-induced photo-oxidation of chlorophyll and of other readily peroxidized molecules in plants and bacteria, and as protective coloration in birds, insects, and other species. The only well-established function of carotenoids elucidated in humans to date is the formation of vitamin A. Only 50 of the approximately 600 characterized carotenoids in nature, however, serve as provitamin A molecules. The carotenoid-dependent coloration of many fruits, vegetables, and flowers, which both please the senses and stimulate appetite, might also be considered as a human function, but largely on aesthetic rather than on technical grounds.

Actions

Chemical Carotenoids, because of their physical properties, show both chemical and biological actions that are not shown by vitamin A. Thus, carotenoids are very effective in quenching singlet oxygen and in serving as antioxidants.⁵⁴ Carotenoids can quench singlet oxygen without being modified chemically, can consume free radicals and hence serve as antioxidants, or can be autoxidized without a reduction in free-radical concentrations. All three of these reactions occur biologically. The products of both antioxidant and autoxidant reactions are a set of epoxides, aldehydes and ketones, often produced by carbon-carbon bond cleavage.⁵⁴⁻⁵⁶ Carotenoids are much more effective than vitamin A in these reactions because they possess a much longer conjugated double bond system, e.g., eleven in β -carotene versus five in retinol.

β -Carotene, and presumably other carotenoids, can also interact with other antioxidants, such as α -tocopherol and ascorbic acid. Therefore, β -carotene may serve as part of an antioxidant network within cells.⁵⁷

Biological Carotenoids show a variety of protective effects in experimental systems, namely a reduction in free-radicals, in photoinduced neoplasm, in mutagenesis, in sister chromatid exchange, and in cell transformation.⁵⁸⁻⁶⁰ In humans, treatment with β -carotene reduced leukoplakia, the number of micronuclei in the buccal mucosa of betel-nut chewers, and photo-induced skin disorders in inherited light-sensitivity diseases.^{61,62} β -carotene supplements can also enhance various facets of the immune response in the elderly and can block the immunosuppressive actions of ultraviolet light and of HIV infection in human subjects.⁶¹ Thus, carotenoids seem to show moderately beneficial effects in humans with various types of diseases and stresses.

Associations In epidemiologic studies, carotenoid ingestion has been associated with protective effects against several types of cancer, and particularly against lung cancer, as well as against senile cataract.^{61,63} In an intervention trial, the incidence of cardiovascular incidents in a small, high-risk group of physicians treated with β -carotene was reported to be lower than in a similar placebo group.⁶¹

One must bear in mind, however, that these results are preliminary and often depend on the ingestion of pharmacologic rather than physiologic amounts, in quantities that could not be readily approached from dietary sources. Potential benefits observed among individuals consuming larger dietary intakes may be related to other differences in their diet, such as being lower in fat than diets predominantly containing animal foods, or other physical activity, and smoking and drinking behavior. These potential beneficial effects of carotenoids seem unrelated to their provitamin A activities.

Recommended Intakes

In defining dietary requirements for vitamin A, four options are available (A) an average intake that satisfies the needs of 50% of subjects, (B) an intake that satisfies the needs of nearly all subjects, (C) an intake that provides suitable reserves for 50% of subjects, and (D) an intake that provides suitable reserves for nearly all subjects

A reserve of 0.07 $\mu\text{mol/g}$ wet weight of liver, or approximately 160 μmoles for a 70-kg person, serves as the basis for establishing the intake needed under option D. Experimentally, the intake of vitamin A needed to satisfy option C is approximately twice that needed for option A. Because the coefficient of variation for plasma levels of vitamin A in humans and for dietary requirements in animals is approximately 20%, two standard deviations are usually used to correct option A to option B and option C to option D. Thus, the relative intakes needed to satisfy options A, B, C, and D are in the ratio 1:1.4:2.2:8, respectively.⁶⁴ The recommended nutrient intakes adopted by the FAO/WHO,⁶⁵ proposed for the European Community⁶⁶ and approved by the National Academy of Sciences in the United States,⁶⁷ are given in Table 8-4.

The Food and Agriculture Organization and the World Health Organization have selected options B and D in a two-tier system, termed "basal and safe levels" of vitamin A requirements.⁶⁵ Similarly, the Nordic Committee on Food defined a two-tier system for micronutrients, called "lower limits" and "recommended intakes."⁶⁸ Other countries, including the United States, have selected single values for a given age and sex, based mainly on option D.

Table 8-4 Recommended and Proposed Dietary Intakes of Vitamin A in μg Equivalents

Category	Age (years)	FAO/WHO ⁶⁵		European Community ⁶⁶			USA ⁶⁷ RDA ^a
		Basal	Safe	Lowest Threshold	Average Requirement	Population Reference	
Infants	0-0.5	180	350			—	375
	0.5-1.0	180	350			350	375
Children	1.0-10	200-250	400			400-500	400-700
Males	10-14/15	300-350	500-600			600	1000
	14/15-70+	300-400	600	300	500	700	1000
Females	10-14/15	270-330	500			600	800
	14/15-70+	270	500	250	400	600	800
Pregnancy		+100	+100			+100	+0
Lactation	0-0.5	+180	+350			+350	+500
	> 0.5	+180	+350			+350	+400

^aRecommended dietary allowance

The proposed values for the European Community are a three-tier system based on statistical considerations. The population reference intake (PRI) is similar in nature to the recommended nutrient intakes (RNI, RDA) established in many countries, i.e., option D. The average requirement (AR) in all likelihood is option C, and the lowest threshold intake (LTI), which is defined as an intake below which nearly all individuals will be unable to maintain metabolic integrity according to the criterion chosen, is closest to option A. In any case, values for AR and PRI for European adults, if adjusted for higher reference body weights than those used by FAO/WHO, are both very similar to those adopted by the FAO/WHO⁶⁵ and nearly identical to the calculated average requirements and RNI values for vitamin A suggested for use in the United States⁶⁹. Both of the latter recommendations, by the way, are based on an adequate total body reserve^{65,69}.

For infants and children, the selected "safe" (PRI, RNI, RDA) intake is essentially the same in all recommendations: 350 μg –375 μg retinol equivalents. This recommendation is based, however, on the mean daily volume and the concentration of vitamin A present in the breast milk of well-nourished mothers (0.5 μg retinol equivalents/ml \times 700–750 ml milk/day) rather than on a scientific assessment of requirement. In this regard, breast-fed Indian infants grow normally on approximately 100 μg retinol equivalents, largely as vitamin A, although they probably possess little or no reserves⁶⁵. Human milk also contains carotenoids (0.2 $\mu\text{g}/\text{ml}$ –0.3 $\mu\text{g}/\text{ml}$), of which 20%–30% are vitamin A precursors¹. Because the transfer of vitamin A to the fetus is regulated by the placenta, the vitamin A reserves in newborn infants, even from well-nourished mothers, are low. Thus, breast milk plays a crucial role in providing sufficient vitamin A for growth, vision, and cell differentiation.

The bioavailability of carotenoids can vary greatly, as already mentioned, being highest when given in an oily or detergent solution and lowest when provided in uncooked vegetables⁶³. The difficulty in correctly assessing the vitamin A value of ingested carotenoids has been thoughtfully discussed⁷⁰. In part because of the difficulties involved in accurately assessing both the dietary intake of carotenoids and their utilization, a variety of other assessment indicators has been developed^{71,72}. The assessment of vitamin A status is specifically considered elsewhere in this volume.

Biochemical Consequences of Inadequate Intakes

Vitamin A deficiency ultimately affects most tissues of the body⁷³. In humans, the eye is affected in two major ways: (1) a reduction in the rhodopsin concentration in the retina, which is noted clinically by abnormal dark adaptation (night-blindness), and (2) keratinization of the epithelial layers of the conjunctiva and

cornea, which in severe cases can lead to rupture of the cornea and loss of sight. The pathological consequences of these changes, and the specific signs associated with them, are discussed in Chapter 4.

Physiologic Dynamics

Vitamin A deficiency does not affect to any major extent the absorption of the vitamin from the intestinal tract. The cleavage of provitamin A to vitamin A in the gut, however, may be depressed by accompanying protein-calorie malnutrition. In the presence of some dietary fat, vitamin A and carotenoids are transported more or less normally in the form of chylomicra in the plasma. On the other hand, the efficiency of storage of vitamin A in the liver is markedly depressed when initial liver stores are very low. This reduced storage may be due in part to a lack of uptake of vitamin A by hepatocytes as well as to its reduced transfer from hepatocytes to liver stellate cells. Humans that suffer from vitamin A deficiency may well show similar defects in the storage of vitamin A. For example, a few vitamin A-depleted, malnourished Indonesian children who received 200,000 IU of vitamin A as a single dose developed signs of vitamin A deficiency within a two- to three-month period.⁷³ Thus, by apparently activating the liver storage mechanism by an initial low oral dose of vitamin A, a subsequent large oral dose was better utilized by preschool Indonesian children than was a single large dose alone.⁷⁴

During the initial stages of depletion of vitamin A reserves, the vitamin A content of the liver falls, vitamin A concentrations in the plasma and retina remain at normal levels, and in some tissues like the kidney, the concentrations actually increase. The excretion of metabolites of vitamin A is reduced, and recycling mechanisms become more efficient. Moreover, the turnover rate of plasma retinol becomes slower. That vitamin A deficiency is very difficult to induce in adult human volunteers with initially adequate total body reserves attests to the effectiveness of these conservation mechanisms.

As depletion becomes more severe, however, homeostatic mechanisms no longer can cope with the situation. Plasma retinol values fall, although plasma concentrations of RBP, increasingly in the apo form, are less affected. The vitamin A concentration in the saliva also decreases. Apo-RBP, however, builds up in parenchymal cells of the liver.

Retina

The rods and cones of the retina tenaciously maintain their vitamin A levels until the body is fairly well depleted. Nonetheless, an early sign of vitamin A deficiency in both humans and experimental animals is nightblindness, i.e., impaired dark adaptation. The sensitivity of dark adaptation is directly related to the amount of rhodopsin present in the eye. Consequently, as the vitamin A in

the eye falls, the total amount of rhodopsin present also decreases, and then dark adaptation becomes abnormal. This defect in rhodopsin formation is further exacerbated by protein-calorie malnutrition and by zinc deficiency. In protein-calorie malnutrition, the pigment epithelial cells show abnormalities, and in zinc deficiency, the concentration of opsin seems to be depressed. Thus, multiple nutritional deficiencies, which are commonly found in human populations suffering from vitamin A deficiency in animals, enhance these abnormalities in dark adaptation. In prolonged, severe vitamin A deficiency, the outer segments of the rod irreversibly deteriorate and blindness results.⁷³

Cornea and Conjunctiva

Since the cornea and conjunctiva are avascular, the manner in which vitamin A is transferred to corneal cells has aroused considerable interest. Possible routes of transfer are (1) the migration of holo-RBP from blood capillaries in the limbus region, (2) the direct uptake of vitamin A from the tear fluid, and (3) the transfer of vitamin A from the aqueous humor.

Holo-RBP has been identified in the cornea at about 2% of its concentration in the plasma, and a gradient in holo-RBP concentration from the limbus to the center of the cornea exists. Retinol is present in human tears at a concentration of 0.1 $\mu\text{mol/liter}$, approximately 5% of that in plasma. The concentration of retinol in the aqueous humor, however, is barely detectable. Thus, diffusion from the limbus and the tear fluid seem to be the major sources of vitamin A for the cornea.

In vitamin A deficiency, plasma values of vitamin A are very low, and vitamin A disappears from the tear fluid. As a result, the amount of vitamin A delivered to the cornea markedly falls, which gives rise to the histological changes described elsewhere (Chapter 4).

Skin

Vitamin A is essential for the normal differentiation and maintenance of the skin. Holo-RBP diffuses into the dermis and then into the epidermis from capillaries in the skin.⁷⁵ The concentration of RBP in the intercellular fluid of the skin is one-third to one-half that in the serum, whereas the concentration of retinol is somewhat lower. Vitamin A and carotenoids are found in higher concentration in the subcutis than in the plasma, although significant amounts are present in the epidermis and dermis as well. Of particular interest is the presence in the epidermis of dehydroretinol, an analog not found in the serum. Upon entering cells of the skin, retinol presumably is bound by CRBP, and after its oxidation to retinoic acid, by CRABP. CRABP has also been identified in the cytosol of the sebaceous follicle.^{73,75}

Vitamin A has a marked effect on the terminal differentiation of human keratinocytes. Under normal conditions, human keratinocytes synthesize keratins with molecular weights of 40,000 and 52,000, as well as many others.⁷⁶ When vitamin A is absent, these “small” keratins are replaced by larger keratins (molecular weight $\geq 67,000$) characteristic of the stratum corneum. Retinoids stimulate basal cell proliferation but inhibit the transcription of several epidermal keratins, probably by interacting with RAR α or RAR γ in the skin.^{76,77} TGF α and TGF β also influence skin development, but in different ways. Hair follicles, which are particularly sensitive to vitamin A, become obstructed and enlarged in the deficient condition and are replaced by mucus-secreting glands in vitamin A excess. In all likelihood, vitamin A deficiency sets in motion a large number of changes in skin structure and metabolism that ultimately lead to the observed pathologic signs.^{3,75-77}

Because vitamin A is absorbed quite well through the skin, local application yields improvement of specific skin lesions. Of course, general improvement in the vitamin A status of an individual also ameliorates skin abnormalities.

Other Epithelial Tissues

The trachea, salivary gland, and vaginal epithelium, as well as many other epithelial tissues, are adversely affected by vitamin A deficiency. In general, mucus-secreting cells tend to be replaced by squamous and keratinized epithelium. This dramatic shift from mucus-secreting to keratinized cells in the trachea and in the vagina has been used as a quantitative biological assay for the effectiveness of retinoids in animals. Changes that occur in the tracheal epithelium as a result of vitamin A deficiency have been carefully documented by electron microscopy.⁷⁸

Toxicity

One of the most commonly used strategies for counteracting vitamin A deficiency in less-industrialized countries has been the oral administration of periodic large doses—usually 0.21 mmoles (60 mg, or 200,000 IU_a), of retinyl palmitate in oil. Transient side effects have been noted in some children at this or at even lower doses (Chapter 15). Thus, safety is an important issue in dealing with intervention strategies, particularly in regard to very young children and to pregnant and lactating women, even perceived toxicity may reduce public acceptance of the program.

Carotenoids, even when taken in extremely large doses for long periods, are generally nontoxic. The only known exception is canthaxanthin, which can induce retinopathy when it is ingested in large amounts for long periods.⁷⁹

Acute

When a single dose of more than 0.7 mmol of vitamin A (> 200 mg, or > 660,000 IU_a) is ingested by adults or when a dose larger than 0.35 mmol (> 100 mg or > 330,000 IU_a) is ingested by children, the results may be nausea, vomiting, headache, increased cerebrospinal pressure, vertigo, blurred (double) vision, muscular incoordination, and (in infants) bulging of the fontanelle. Some infants can be adversely affected by single doses of only 0.1 mmol. These signs are generally transient and subside within one to two days (Chapter 15)^{80,81} When the dose is extremely large, it is soon followed by drowsiness, malaise, inappetence, reduced physical activity, skin exfoliation, itching (particularly around the eyes), and recurrent vomiting.

Young monkeys, when given lethal doses by intramuscular injection, fall into a deep coma, often have convulsions and respiratory irregularities, and finally die of either respiratory failure or convulsions.⁸² The median lethal dose (LD₅₀ value) of vitamin A injected intramuscularly in a water-miscible form in the young monkey is 0.6 mmol (168 mg) retinol/kg body weight. Extrapolated to a 3-kg child and a 70-kg adult, the total LD₅₀ dose would be 1.8 mmol (500 mg) and 41 mmol (11.8 g) respectively. A newborn child, who mistakenly was given 0.09 mmol (25 mg) daily, or 28 μmol/kg, for eleven days died of apparent vitamin A toxicity.⁸³ The total dose received was 0.31 mmol/kg, or half of the LD₅₀ value for young monkeys. Such enormous amounts of vitamin A are present only in high-potency preparations of vitamin A or in large amounts (~500 g) of livers particularly rich in vitamin A (> 0.035 mmol/g or > 10 mg/g).

Chronic

Chronic toxicity is induced by the recurrent intake of vitamin A in amounts at least ten times the RDA, that is, 13 μmol (3.75 mg retinol equivalents or 12,500 IU_a) for an infant or 35 μmol (10 mg retinol equivalents or 33,300 IU_a) for an adult. A health-food enthusiast who ingested 26 μmol (25,000 IU_a) of vitamin A as a supplement daily plus a similar amount in food over a prolonged period showed severe signs of toxicity.⁸¹

Approximately 50 signs of chronic toxicity have been reported, of which the most frequent are alopecia, ataxia, bone and muscle pain, cheilitis, conjunctivitis, headache, hepatotoxicity, hyperlipemia, hyperostosis, membrane dryness, pruritus, pseudotumor cerebri, various skin disorders, and visual impairment.^{80,81} When the supplemental intake of vitamin A is eliminated, these signs usually disappear over a period of weeks to months, but not always.

In chronic hypervitaminosis A, holo-RBP in the plasma is not much elevated, whereas retinyl esters are usually increased markedly.³⁹ Factors that enhance toxicity include alcohol ingestion, low protein intake, viral hepatitis, other dis-

eases of the liver and kidney, and possibly tetracycline use. Elderly individuals may be more sensitive because of a slower rate of storage in the liver and a reduced plasma clearance of administered vitamin A. Tocopherol, taurine, and zinc are protective in tissue culture cells, but they may or may not be effective *in vivo*.⁸¹

Some individuals seem to suffer from vitamin A intolerance, that is, the appearance of signs of toxicity upon routinely ingesting moderate amounts of vitamin A. This relatively rare condition, which seems to be genetic, mainly affects males.⁸⁴

Both all-*trans* and 13-*cis* retinoic acid, as well as many synthetic retinoids, induce similar toxic states. Indeed, acidic forms of retinoids are more toxic than the alcoholic forms and are much more toxic than some conjugated derivatives.³⁹ Mechanisms of toxicity are ill defined.

Teratogenic

Vitamin A and other retinoids are teratogenic, both in experimental animals and in women.^{81,86,87} In experimental animals, all-*trans* retinoic acid is four times more teratogenic than all-*trans* vitamin A or its ester.⁸⁵ A single extremely large dose of either retinyl ester or retinoic acid, or exposure for as short as a week on high daily doses of retinoic acid (0.1 mmol to 0.3 mmol, or 30 mg to 90 mg), during early pregnancy can induce spontaneous abortions or major fetal malformations. Long-term daily intakes of greater than 26 μmol of retinyl ester (25,000 IU_a or 7,500 RE) have also been associated with birth defects in human fetuses, but causality has not been established.⁸⁷ Common defects are craniofacial abnormalities, including microcephaly, microtia, and harelip, congenital heart disease, kidney defects, thymic abnormalities, and central nervous system disorders. The extent of induction of RAR β 2 by retinoids correlates well with their teratogenic actions.⁸⁸ Permanent learning disabilities have been noted in otherwise normal rat pups whose dams received nonteratogenic doses of vitamin A, as well as in children exposed to large doses of 13-*cis* retinoic acid (Accutane) early in fetal life.⁸⁹ Synergism between vitamin A and other teratogens, such as alcohol and drugs, at nonteratogenic doses of each is probable. Thus, women who are pregnant, or who might become so, should carefully control their intake of vitamin A, in regard both to rich food sources, such as liver, and to vitamin A supplements.

Healthy women who routinely ingest diets containing adequate fruits and green leafy vegetables do not require supplements of vitamin A during pregnancy.⁸¹ In cases where supplementation is advisable, the total daily intake should not exceed 10 μmol (approximately 3 mg or 10,000 IU) of vitamin A.^{39,81,90} It should be noted that vitamin A deficiency, just as its excess, adversely affects the reproductive process.

Conclusions

Vitamin A and carotenoids are essential for basic physiologic processes in many living organisms. As a consequence, nature has devised ingenious ways to protect us both from an inadequacy and from an excess of vitamin A. The biochemistry of vitamin A and carotenoids in mammals deals largely with nature's clever devices: the efficient absorption of preformed vitamin A, its transport, in large part, on protein chaperones within the body, special mechanisms for its storage, efficient recycling, effective homeostasis, and a regulated catabolic and excretory system. Because carotenoids, until recently, have served as major dietary sources of vitamin A, their bioconversion to vitamin A has evolved as a slow and regulated process, thereby protecting us in large part from the toxic effects of dietary excess.

But vitamin A inadequacy, ironically, remains a major nutritional problem in the world, despite nature's care in trying to prevent it. The reasons underlying this important problem, and some of the strategies designed to address it, are the major focus of this book.

Acknowledgments

This chapter is based in part on generous support from the National Institutes of Health (DK-39733, CA-46406, HD-27994), the U.S. Department of Agriculture (CDFIN/ISU 91-34115-5903), the Thrasher Research Fund (2808-2) and the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50011, U.S.A., Journal Paper J-15533, Project No. 3035.

References

- 1 Moore T. Vitamin A. Amsterdam: Elsevier, 1957: 645.
- 2 Isler O, ed. Carotenoids. Basle: Birkhauser Verlag, 1971: 932.
- 3 Olson JA. Vitamin A, retinoids and carotenoids. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea & Febiger, 1993: 287-307.
- 4 IUPAC-IUB Joint Commission on Biochemical Nomenclature. Eur J Biochem 1982, 129: 1-5.
- 5 Nomenclature Policy. J Nutr 1990, 120: 12-19.
- 6 IUPAC-IUB Commission on Biochemical Nomenclature. Biochemistry 1971, 10: 4827-4837.
- 7 Furr HC, Barua AB, Olson JA. Retinoids and carotenoids. In: Nelis HJ, Lambert WE, De Leenheer AP, eds. Modern chromatographic analysis of the vitamins. 2nd ed. New York: Marcel Dekker, 1992: 1-71.

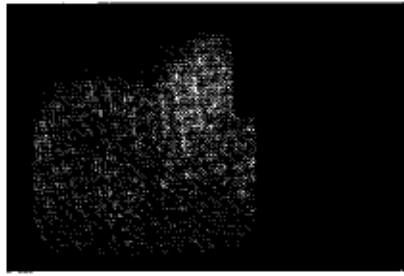
- 8 Furr HC, Barua AB, Olson JA Analytical methods In Sporn MB, Roberts AB, Goodman DS, eds The retinoids biology, chemistry and medicine 2nd ed New York Raven Press 1994 178–209
- 9 Olson JA Vitamin A In Machlin LJ, ed Handbook of vitamins 2nd ed New York Marcel Dekker 1990 1–57
- 10 Ross AC Overview of retinoid metabolism J Nutr 1993,123 346–350
- 11 Blaner WS, Olson JA Retinol and retinoic acid metabolism In Sporn MB, Roberts AB, Goodman DS, eds The retinoids biology, chemistry and medicine 2nd ed New York Raven Press 1994 229–255
- 12 Cowan SW, Newcomer ME, Jones TA Crystallographic refinement of human serum retinol binding protein at 2A resolution Proteins Struct Funct Genet 1990,8 44–61
- 13 Soprano DR, Blaner WS Plasma retinol-binding protein In Sporn MB, Roberts AB, Goodman DS, eds The retinoids biology, chemistry and medicine 2nd ed New York Raven Press 1994,257–281
- 14 Pilch SM, ed Assessment of the vitamin A nutritional status of the U S population based on data collected in the health and nutrition examination surveys Bethesda, MD Life Sci Res Office, Fed Am Soc Exp Biol 1985 49
- 15 Kaplan LA, Stein EA, Willett WC, Stampfer MJ, Stryker WS Reference ranges of retinol, tocopherols, lycopene and alpha- and β -carotene in plasma by simultaneous high performance liquid chromatographic analysis Clin Physiol Biochem 1987, 5 297–304
- 16 Stacewicz-Sapuntzakis M Bowen PE, Kikendall JW, Burgess M Simultaneous determination of serum retinol and various carotenoids their distribution in middle-aged men and women J Micronutr Anal 1987,3 27–45
- 17 Rankins J, Green NR, Tremperer W, Stacewicz-Sapuntzakis M, Bowen P, Ndiaye M Undernutrition and vitamin A deficiency in the Department of Linguere, Louga Region of Senegal Am J Clin Nutr 1993,58 91–97
- 18 Homnick DN, Cox JH, DeLoof MJ, Ringer TV Carotenoid levels in normal children and in children with cystic fibrosis J Pediatr 1993,122 703–707
- 19 Khachik F, Beecher GR, Gol MB, Lusby WR, Smith JC Jr Separation and identification of carotenoids and their oxidation products in the extracts of human plasma Anal Chem 1992,64 2111–2122
- 20 Barua AB, Kostic D, Olson JA Simplified procedures for the extraction and simultaneous HPLC analysis of retinol, tocopherols and carotenoids in human serum J Chromatog 1993,617 257–264
- 21 Barua AB, Batres RO, Olson JA Characterization of retinyl β -glucuronide in human blood Am J Clin Nutr 1989,50 370–374
- 22 Bavik CO, Ericksson U, Allen RA, Peterson PA Identification and partial characterization of a retinal pigment epithelial membrane receptor for plasma retinol-binding protein J Biol Chem 1991,266 14978–14985
- 23 Creek KE, St Hilaire P, Hodam JR A comparison of the uptake, metabolism and biologic effects of retinol delivered to human keratinocytes either free or bound to retinol-binding protein J Nutr 1993,123 356–361
- 24 Blomhoff R, Green MH, Norum KR Vitamin A physiological and biochemical processing Annu Rev Nutr 1992,12 37–57
- 25 Green MH, Green JB, Lewis KC Variation in retinol utilization rate with the vitamin A status of the rat J Nutr 1987,117 694–703
- 26 Ong DE, Newcomer ME, Chytil F Cellular retinoid-binding proteins In Sporn MB,

- Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994,283–317
- 27 Banaszak L, Winter N, Xu Z, Bernlohr DA, Cowan S, Jones TA Lipid binding proteins a family of fatty acid and retinoid transport proteins *Adv Protein Chem* 1993,45 89–149
 - 28 Ong DE Retinoid metabolism during intestinal absorption *J Nutr* 1993,123 351–355
 - 29 Napoli JL Biosynthesis and metabolism of retinoic acid roles of CRBP and CRABP in retinoic acid homeostasis *J Nutr* 1993,123 362–366
 - 30 Zeng S, Furr HC, Olson JA Metabolism of carotenoid analogs in humans *Am J Clin Nutr* 1992,56 433–439
 - 31 Erdman JW, Bierer TL, Gugger ET Absorption and transport of carotenoids *Ann NY Acad Sci* 1993,691 76–85
 - 32 Olson JA Absorption, transport and metabolism of carotenoids in humans *Pure Appl Chem* 1994,66 1011–1016
 - 33 Hargrave PA, McDowell JH Rhodopsin and phototransduction *Int Rev Cytology* 1992,1373 49–97
 - 34 Rando RR Isomerization reactions of retinoids in the visual system *Pure Appl Chem* 1994,66 989–994
 - 35 Saari JC Retinoids in photosensitive systems In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994 351–385
 - 36 Gudas LJ, Sporn MB, Roberts AB Cellular biology and biochemistry of retinoids In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994 443–520
 - 37 Petkovich M Regulation of gene expression by vitamin A. the role of nuclear retinoic acid receptors *Annu Rev Nutr* 1992,12 443–471
 - 38 Mangelsdorf DJ, Umesono K, Evans RM Nuclear receptors for retinoids In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994 319–324
 - 39 Mehta RG, Barua AB, Moon RC, Olson JA Interactions between retinoid beta-glucuronide and cellular retinol- and retinoic acid-binding proteins *Intern J Vitam Nutr Res* 1992,62 143–147
 - 40 Sami BP, Barua AB, Hill DL, Shih T-W, Olson JA Retinoyl β -glucuronide lack of binding to receptor proteins of retinoic acid as related to biological activity *Biochem Pharmacol* 1992,43 919–922
 - 41 Buck J, Dergumi F, Levi E, Nakanishi K, Hammerling U Intracellular signalling by 14-hydroxy-4,14-retro-retinol *Science* 1991,254 1654–1655
 - 42 Hofman C, Eichele G Retinoids in development In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994,387–441
 - 43 Armstrong RB, Ashenfelter KO, Eckoff C, Levin AA, Shapiro S General and reproductive toxicology of retinoids In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994 545–572
 - 44 Olson JA The biological role of vitamin A in maintaining epithelial tissues *Israel J Med Sci* 1972,8 1170–1178
 - 45 Summerbell D, Maden M Retinoic acid a developmental signalling molecule *Trends Neurosci* 1990,13 142–147

- 46 Slack JMW Embryonic induction *Mech Develop* 1993,41 91–107
- 47 Ross AC, Hammerling U Retinoids and the immune system In Sporn MB, Roberts AB, Goodman DS, eds *The retinoids biology, chemistry and medicine* 2nd ed New York Raven Press 1994 521–543
- 48 Friedman A, Sklan D Vitamin A and immunity In Klurfeld DM, ed *Nutrition and immunology* New York Plenum Press 1993 197–216
- 49 Hossain MZ, Zhang L-X, Bertram JS Mechanistic studies of cancer chemoprevention by retinoids and carotenoids In Livrea MA, Packer L, eds *Retinoids progress in research and clinical applications* New York Marcel Dekker 1993 361–381
- 50 Mathies RA, Liu SW, Ames JB, Pollard WT From femtoseconds to biology mechanism of bacteriorhodopsin's light-driven proton pump *Annu Rev Biophys Biophys Chem* 1991,20 491–518
- 51 Bollag W, Peck R Modulation of cell proliferation and differentiation by retinoids, cytokines and their combination experimental and clinical aspects In Livrea MA, Packer L, eds *Retinoids progress in research and clinical applications* New York Marcel Dekker 1993 311–328
- 52 Olson JA Biological actions of carotenoids *J Nutr* 1989,119 94–95
- 53 Krinsky NI The biological properties of carotenoids *Pure Appl Chem* 1994,66 1003–1010
- 54 Liebler DC Antioxidant reactions of carotenoids *Ann NY Acad Sci* 1993,691 20–31
- 55 Handelman GJ, van Kuijk FJGM, Chatterjee A, Krinsky NI Characterization of products formed during the autoxidation of β -carotene *Free Rad Biol Med* 1991,10 427–437
- 56 Mordt RC, Walton JC, Burton GW, Hughes L, Ingold KU Lindsay DA, Moffatt DJ Oxidative degradation of β -carotene and β -apo-8' carotenal *Tetrahedron* 1993, 49 911–928
- 57 Packer L Antioxidant action of carotenoids in vitro and in vivo and protection against oxidation of human low-density lipoproteins *Ann NY Acad Sci* 1993,691 48–60
- 58 Krinsky NI Actions of carotenoids in biological systems *Annu Rev Nutr* 1993,13 561–587
- 59 Bendich A Olson JA Biological actions of carotenoids *FASEB J* 1989,3 1927–1932
- 60 Olson JA Molecular actions of carotenoids *Ann NY Acad Sci* 1993,691 156–166
- 61 Bendich A Biological functions of dietary carotenoids *Ann NY Acad Sci* 1993, 691 61–67
- 62 Mathews-Roth MM Carotenoids in erythropoietic protoporphyria and other photosensitivity diseases *Ann NY Acad Sci* 1993,691 127–138
- 63 Singh VM Role of β -carotene in disease prevention with special reference to cancer In Ong ASH, Packer L, eds *Lipid-soluble antioxidants biochemistry and clinical applications* Basel Birkhauser Verlag 1992 208–226
- 64 Olson JA Marabou Conference Needs and sources of carotenoids and vitamin A *Nutr Rev* 1994,52 S67-S73
- 65 Food and Agriculture Organization Requirements of vitamin A, iron, folate and vitamin B₁₂ Report of a Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Series No 23 Rome, Italy FAO Press 1988 107
- 66 Anonymous Proposed nutrient and energy intakes for the European Community, a report of the Scientific Committee for Food of the European Community *Nutr Rev* 1993,51 209–212
- 67 Food and Nutrition Board Recommended dietary allowances, 10th ed National Research Council Washington DC National Academy Press 1989 284

- 68 Nordic Committee on Food Nordic nutrition recommendations, 2nd ed Stockholm Nordic Council of Ministers 1989 16
- 69 Olson JA Recommended dietary intakes (RDI) of vitamin A in humans *Am J Clin Nutr* 1987,45 693–703
- 70 Solomons NW, Bulux J Plant sources of provitamin A and human nutriture *Nutr Rev* 1993,51 199–204
- 71 Olson JA Measurement of vitamin A status *Netherlands J Nutr* 1992,53 163–167
- 72 Underwood BA, Olson JA, eds A brief guide to current methods of assessing vitamin A status Washington DC Intern Vitam A Consult Gp, ILSI-Nutr Fndn 1993 37
- 73 Olson JA Physiological and metabolic basis of major signs of vitamin A deficiency In Bauernfeind JC, ed Vitamin A deficiency and its control Orlando, FL Academic Press 1986 19–67
- 74 Humphrey JH, West KP Jr, Muhilal, See LC, Natadisastra C, Sommer A A priming dose of oral vitamin A given to preschool children may extend protection conferred by a subsequent large dose of vitamin A *J Nutr* 1993,123 1363–1369
- 75 Vahlquist A, Torma H Vitamin A metabolism and retinoic acid receptors in relation to epidermal disorders and retinoid therapy In Livrea MA, Packer L, eds Retinoids progress in research and clinical applications New York Marcel Dekker, 1993 361–381
- 76 Fuchs E Keratin genes, epidermal differentiation and animal models for the study of human diseases *Biochem Soc Trans* 1991,19 1112–1115
- 77 Stellmach V, Leask A, Fuchs E Retinoid-mediated transcriptional regulation of keratin genes in human epidermal and squamous cell carcinoma cells *Proc Natl Acad Sci USA* 1991 88 4582–4586
- 78 McDowell EM, Keenan KP, Huang M Effects of vitamin A-deprivation on hamster tracheal epithelium a quantitative morphological study *Virchow's Arch B* 1984, 45 197–219
- 79 Weber U, Goerz G, Baseler H, Michaelis L Canthaxanthin retinopathy followup of over 6 years *Klin Monatsbl Augenheilkd* 1992,201 174–177
- 80 Bauernfeind JC The safe use of vitamin A Washington DC Intern Vitam A Consult Gp, Nutr Fndn 1980 44
- 81 Hathcock JN, Hattan DG, Jenkins MY, McDonald JT, Sundaresan PR, Wilkenin VL Evaluation of vitamin A toxicity *Am J Clin Nutr* 1990,52 183–202
- 82 Macapinlac MP, Olson JA A lethal hyper-vitaminosis syndrome in young monkeys (*Macacus fascicularis*) following a single intramuscular dose of a water-soluble preparation containing vitamins A, D₂ and K *Int J Vitam Nutr Res* 1981,51 381–341
- 83 Bush ME, Dahms BB Fatal hypervitaminosis A in a neonate *Arch Pathol Lab Med* 1984,108 838–842
- 84 Olson JA Upper limits of vitamin A in infant formulas, with some comments on vitamin K *J Nutr* 1989,119 1820–1824
- 85 Adams J Structure-activity and dose-response relationships in the neural and behavioral teratogenesis of retinoids *Neurotoxicol Teratol* 1993,15 193–202
- 86 Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW, Lott IT, Richard JM, Sun SC Retinoic acid embryopathy *New Engl J Med* 1985,313 837–841
- 87 Rosa FW Retinoid embryopathy in humans In Koren G, ed Retinoids in clinical practice New York Marcel Dekker 1993 77–109
- 88 Soprano DR, Harnish DC, Soprano KJ, Kochhar DM, Jiang H Correlations of RAR

- isoforms and cellular retinoid-binding proteins in RNA levels with retinoid-induced teratogenesis *J Nutr* 1993,123 367–371
- 89 Adams J Neural and behavioral pathology following prenatal exposure to retinoids
In Koren G, ed *Retinoids in clinical practice* New York Marcel Dekker
1993 111–128
- 90 Underwood BA The safe use of vitamin A by women during the reproductive years
Washington DC Int Vitam A Consult Gp, ILSI Nutr Fndn 1986 4



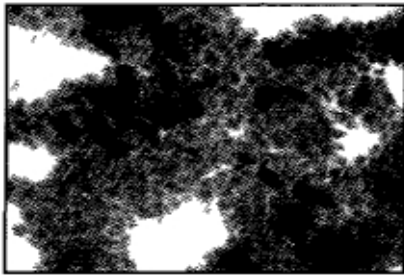
1



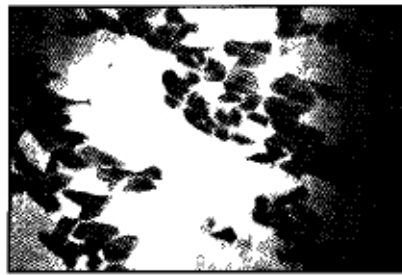
2

Plate 1. Xerophthalmic retinopathy in a 24-year-old otherwise well-nourished Indonesian woman who presented with nightblindness, constricted visual fields, and severe conjunctival and corneal xerosis

Plate 2. Two months after vitamin A therapy the small yellowish-white retinal lesions seen in the patient in Plate 1 have largely disappeared



3



4

Plate 3 Impression cytology specimen (CIC-A) of normal conjunctiva revealing abundant PAS-positive mucus-secreting goblet cells amid a sheet of small, regular epithelial cells (PAS and Harris' hematoxylin, $\times 400$)

Plate 4 CIC-A specimen of abnormal conjunctiva from a vitamin A-deficient child. Epithelial cells are large and irregular (though not yet keratinized) and lack goblet cells (PAS and Harris' hematoxylin, $\times 400$)



5



6

Plate 5. Localized area of dry, granular conjunctival xerosis temporally in a 5-year-old Indonesian boy

Plate 6 Tiny, localized areas of foam or bubbles of conjunctival xerosis in a 4-year-old Indonesian boy



7



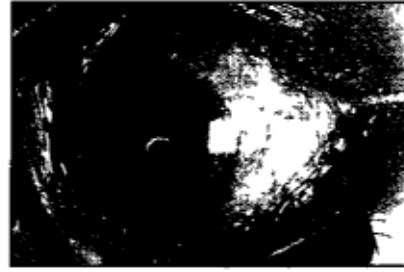
8

Plate 7 Classical foamy Bitot's spot in a 29-year-old Indonesian man that largely resolved within 2 months of vitamin A therapy

Plate 8. Classical Bitot's spot in a 20-year-old Indonesian man. The superior aspect is foamy in character, the inferior aspect is denser and cheesy in appearance. There was no history of nightblindness and the Bitot's spot failed to respond to vitamin A therapy



9



10

Plate 9 Foamy Bitot's spot in a 3-year-old girl from South India. Note the heavily pigmented appearance of the conjunctiva.

Plate 10. Thick, stiffened temporal conjunctiva in a 4-year-old Indonesian girl with advanced conjunctival xerosis. Haziness of the inferior cornea represents mild corneal xerosis.



11



12

Plate 11 Thickened skinlike temporal conjunctiva in a 1-year-old Indonesian boy with early corneal xerosis. The eye had been stained with Rose Bengal.

Plate 12. Extensive punctate keratopathy in a 10-month-old Indonesian boy. The cornea appeared crystal clear to handlight examination, but fluorescein-staining punctate epithelial lesions were apparent on examination with the slit-lamp biomicroscope. The cornea of the other eye was entirely necrotic (Plate 13).



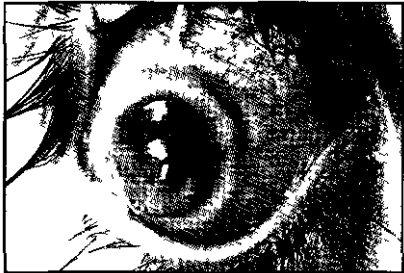
13



14

Plate 13. Fellow eye of subject described in Plate 12. The cornea was entirely necrotic and bulging forward. Dark, pigmented iris is visible in areas where most of the stroma had already sloughed.

Plate 14. Inferior corneal xerosis in a severely malnourished 3-year-old Indonesian boy. The keratinized surface is sharply demarcated. The cornea of the other eye was entirely necrotic (Plate 15).



15



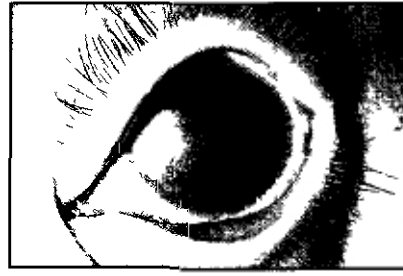
16

Plate 15. Fellow eye of case described in Plate 14. Except for the peripheral 1 mm, the cornea is entirely necrotic, most having already sloughed, resulting in a large central descemetocele. The conjunctiva is moderately inflamed and without evident xerosis.

Plate 16. Heavily keratinized corneal surface in the eye of a 2-year-old Indonesian girl two days after initiation of therapy. A portion of the keratinized surface has already peeled off inferiorly, forming a scroll below. The clear area is now devoid of its keratinized layer. At the initial examination, the identical area of the other eye contained a typical ulcer (Plate 17).



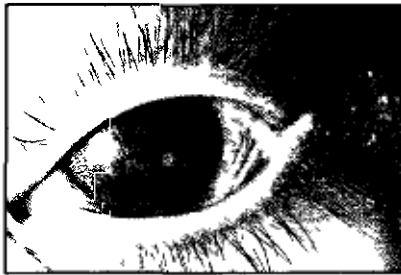
17



18

Plate 17. Fellow eye of case described in Plate 16. A typical, sharp-margined oval ulcer is present inferiorly. The conjunctiva is not inflamed and contains extensive xerosis which is most apparent in the inferior quadrant adjacent to the cornea.

Plate 18. Extensive conjunctival xerosis and heavily keratinized corneal surface in a 3-year-old Indonesian boy.



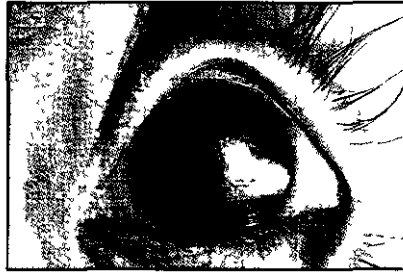
19



20

Plate 19. Same eye shown in Plate 18, two days following initiation of therapy. The heavily keratinized corneal plaque inferiorly has sloughed, leaving a small, round, sharply demarcated superficial ulcer in its place. Crevices of the surrounding keratinized surface converge on the ulcer, most apparently above the inferior limbus. Keratinized plaques are still present in the interpalpebral zone.

Plate 20. External appearance of one eye of the case described in Plate 1. Marked xerosis of both the conjunctiva and cornea is present.



21



22

Plate 21. Heavily keratinized corneal plaque in a 2-year-old Indonesian girl with poor lid closure and severe malnutrition. Conjunctival and corneal xerosis is extensive.

Plate 22. Right eye of a 3-year-old Indonesian girl. A classical punched-out, shallow round ulcer was present anteriorly, with pigment (presumably adherent iris surface left behind following dilatation) posteriorly, and normal-appearing stroma in between. Two pinpoint ulcers are present superiorly. The left eye is shown in Plate 23.



23



24

Plate 23. Left eye of case described in Plate 22. A large, circumscribed area of cornea is missing centrally, with iris bulging through the defect. The surrounding cornea was hazy but viable. The conjunctiva was xerotic and not inflamed.

Plate 24. The left eye of a 4-year-old Indonesian boy. Corneal xerosis is apparent inferiorly, and a classically sharp, round, punched-out three-fourths depth ulcer is evident superonasally. The ulcer reached Descemet's membrane.



25



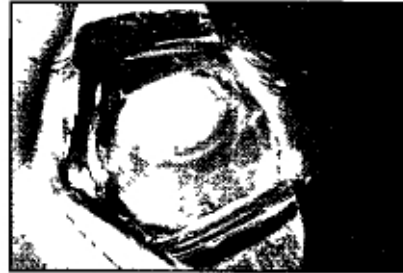
26

Plate 25. A characteristically fluorescein-staining oval, inferior corneal ulcer in a 2-year-old Indonesian girl. A small hypopyon is present and the conjunctiva is inflamed.

Plate 26. A 19-month-old Indonesian boy. The right eye contained extensive conjunctival xerosis and punctate keratopathy. The left eye, shown here one day following initiation of vitamin A therapy, contained three areas of surface abnormality. One area was irregular, composed of mounds (clear epithelial or superficial stromal vesicles) alternating with dell-like depressions. Temporal to this area was a depression. In its center was a sharp, deeper, cylindrical ulcer, the only part that stained with fluorescein. A second, larger depression with a centrally staining ulcer was present more temporally. The bases of the two ulcers, originally clear, were by now moderately opaque. A small hypopyon was present and the conjunctiva was inflamed.



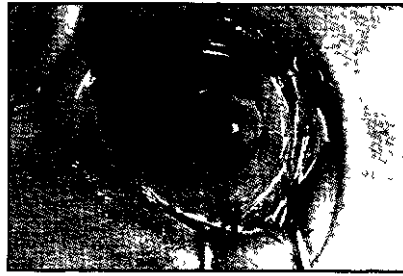
27



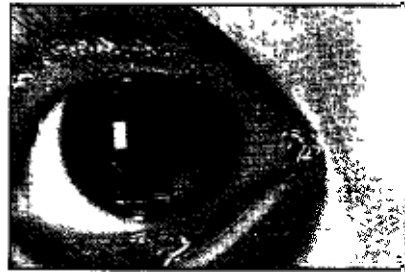
28

Plate 27. Same eye shown in Plate 26, three days after presentation, stained with fluorescein and observed through a blue filter. The superonasal area is less irregular and the adjacent ulcer has largely healed. But now the most temporal ulcer has enlarged, the entire area of depression being deeper and staining edge-to-edge. The hypopyon has increased.

Plate 28. Opaque, necrotic cornea in a 2-year-old Indonesian boy. The conjunctiva is both xerotic and inflamed. The fellow eye contained an area of inferior focal, corneal necrosis.



29



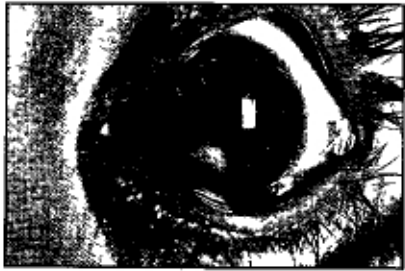
30

Plate 29. Sharply focal necrotic lesion in the right eye of a 2-year-old Indonesian boy. The stroma has largely sloughed, a bulging descemetocele filling the defect. The rest of the cornea is hazy and xerotic. The conjunctiva is inflamed. The other eye had moderately severe corneal xerosis.

Plate 30 Same eye shown in Plate 29 two months later. The defect has healed as a remarkably small, peripheral adherent leukoma, leaving a clear pupillary zone.



31



32

Plate 31. Left eye of an otherwise well-nourished 2-year-old Indonesian boy. A large, nonstaining, focally necrotic, bulging, opaque corneal lesion is present inferiorly. The conjunctiva is both inflamed and xerotic. Abnormalities in the other eye were limited to dense punctate keratopathy.

Plate 32. Same eye shown in Plate 31, one month later. The previous lesion has healed as a surprisingly small adherent leukoma, preserving a clear central pupillary zone.