Correspondents’ Issue is no exception.

Public health issues have to be dealt with on an international scale and require the involvement of specialists working in every field that can contribute useful scientific insights or applied know-how. Accordingly, our newsletter aims to reach a very broad and diversified readership. Our growing circulation indicates that we are succeeding — this year it is up to 10,000 printed copies per issue, as compared with roughly 9000 copies a year ago.

The SIGHT AND LIFE offices in Basel are run by a small staff and with a relatively limited infrastructure. For that reason the reports and advice supplied by our correspondents are vital. These colleagues — every one of them a respected expert in his field — are our eyes and ears in countries around the globe. With the help of the Newsletter, information can be shared much more quickly and thus deployed more effectively in the fight against vitamin A deficiency.

Finally, on a personal note, I would like to add that, for my colleagues and myself in Basel, working with our correspondents is an enriching experience and one that we would hate to do without.
Vitamin A and its relatives: marvellous molecules in key life processes

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1. Introduction

The discovery of vitamin A, not far short of 100 years ago, ushered in a new era in biochemistry and medicine, the vitamin era. In evolutionary terms, however, vitamin A – in a slightly different form – was early on the scene in one of the most ancient bacteria. It was helping to obtain energy from light when the environment lacked oxygen. Probably quite a few million years earlier coloured precursors of vitamin A, carotenoids, were helping bacteria to trap light for photosynthesis – the very foundation of the extravagant diversity of life that was to come.

Photosynthesis goes on inside chloroplasts, which share with mitochondria an amazing chapter in evolutionary history. It is best not to go into this now but leave it for those unfamiliar with it to enjoy later.

In other ways carotenoids continue to surprise us with the useful things they find to do!

Primitive nervous systems developed for the transduction, or passing on, of various kinds of stimuli from the environment. Probably the most useful and certainly the most studied form is phototransduction in the astonishing variety of eyes. Here also relatives of vitamin A are indispensable to trap photons.

As the end of the 20th century approached, other vitamins were discovered and vitamin A was just one vitamin among many others. It was something the body needed but could not make on its own and therefore had to get from the diet.

Some of the close relatives of vitamin A are now openly classed as hormones involved in cells throughout the body with nuclear receptors and gene transcription. Even here light may be playing a part.

Within the space of a short article like this it will be possible only to scratch the surface of these

![Figure 1. A Vitamin A (retinol); B all-trans-β-carotene; C all-trans-retinoic acid; D all-trans-retinal; E 11-cis-retinal; F 3-dehydroretinal.](attachment:figure1.png)
very complex processes, but if it whets the reader’s appetite for more then the references cited are designed to satisfy with more detailed accounts.

Some definitions of the classes of compounds to which vitamin A and its relatives belong will serve to introduce them. Although vitamin A (Figure 1 A, retinol, all-trans-retinol) is mentioned first it is derived in nature from a handful of carotenoids, most commonly from \( \beta \)-carotene (Figure 1 B, \( \beta \)-carotene, more correctly \( \beta,\beta \)-carotene). Comparison of Figures 1 A and B shows that two molecules of retinal might be obtained from fission of the central chain. This is indeed what the enzyme \( \beta \)-carotene-15,15'-dioxygenase commonly brings about, although eccentric cleavage does occur and may result in some interesting products (1).

Vitamin A is a generic term to designate any compound possessing the biological activity of retinol. Of particular interest here are all-trans-retinoic acid (Figure 1 C), the main form active in DNA transcription, all-trans-retinal (Figure 1 D) and 11-cis-retinal (Figure 1 E) involved in the visual cycle in most animals, and 3-dehydroretinal (retinal 2) (Figure 1 F) in the visual cycle of a few others.

The term retinoids applies to any naturally occurring form of vitamin A and to many synthetic analogues of retinol, with or without biological activity.

More than 600 carotenoids (excluding isomers) are known (2). Some carotenoids have formally lost part of the 40 carbon skeleton. Apocarotenoids have lost carbon atoms from the ends of the molecule and norcarotenoids from within the chain. Those that contain one or more oxygen function are known as xanthophylls and the parent hydrocarbons are carotenes. All are polyisoprenoids (small multiples of isoprene, \( \text{C}_5\text{H}_{10} \)), possess an extensive system of conjugated double bonds, usually contain 40C atoms, commonly show internal symmetry and often have one or two cyclic structures at the end of their conjugated chains (\( \beta \)-carotene see above, Figure 2 A lycopene, Figure 2 B lutein).

The most striking feature of carotenoids is the long system of alternating double and single bonds. It gives them their distinctive molecular shape, chemical reactivity, and light-absorbing properties. Geometrical isomerisation occurs and most carotenoids exist in the more stable trans-form, rather than the cis-form. Carotenoids as a group are very hydrophobic, but cis-isomers may be more readily solubilised, absorbed and transported. The usual indication of carotenoid breakdown is bleaching; also seen in coloured relatives of vitamin A.

For vitamin A activity to occur a carotenoid must include at least

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*Figure 2. A Lycopene; B Lutein; C \( \beta \)-ionone.*
one unsubstituted β-ionone ring and a polyene sidechain. The other end of the molecule may have a cyclic or an acyclic structure. It may be lengthened but not shortened to less than an 11-carbon polyene chain. Chain lengthening decreases activity. Without carotenoids life would not only be impossible but the worlds of trees, flowers, fruits, birds and other life forms would lack their spectacular red, orange, and yellow colours.

As light plays a central role in much of the activity of these molecules, it is important to present certain features of its nature (3). Electromagnetic radiation of all kinds is a wave of electric and magnetic fields propagating at the speed of light (approximately 186,282 miles/sec) through empty space. A photon is a quantum of light, virtually massless, and of what is known as “spin 1” spinning like a twisting screw. As will be seen from Figure 3, only a very small part of the entire electromagnetic spectrum is made up of the visible spectrum composed of the familiar colours.

The electromagnetic spectrum

Figure 3. Electromagnetic radiation spans an enormous range of frequencies or wavelengths. The spectrum is customarily designated by fields, waves and particles in increasing magnitudes of frequencies, with the visible spectrum occupying a very small fraction; (adapted from reference 3).
of the rainbow. It spans approximately wavelength 350–800 nm (a nanometre is 1 billionth of a metre). Some animals see ultraviolet light, but this will not be pursued further.

Colour of an object can result in several different ways. One of the most important of these depends on the selective absorption of light by molecules whose size or vibrational wavelengths, or both, lie within the range of the visible spectrum. Selective absorption of visible light results from retardation in the relative speed or vibrational frequency of the many rapidly vibrating electron pairs found in a compound. Sufficient modification in the frequency of vibration imparts to the whole molecule a special motion, or chemical resonance, that absorbs entering light rays of matching frequency with the evolution of heat. The residual, unabsorbed light is transmitted to the eye.

The colour reflected by a pigment includes all the wavelengths of visible light except the absorbed fraction. The observed colour of a compound depends on the dominant wavelength reflected. Thus a substance absorbing shorter visible light (i.e. violet and blue) will appear yellow or orange and so on.

2. Photosynthesis

This fundamental life process takes place not only in higher plants and trees, but also in various kinds of single cell organisms (4). These include anoxygenic photosynthetic bacteria, cyanobacteria and algaebacteria, as well as algae and plankton. Phytoplankton are responsible for nearly half of all the photosynthesis going on in the world and produce about 50% of the oxygen in the atmosphere. Factors, both man-made and natural, that interfere with their growth are of obvious importance for life on earth.

Fossil evidence suggests that the first known organisms resembled present-day blue-green algae or cyanobacteria and dated from about 3.6 billion years ago. For about the first 1.6 billion years these were possibly the only form of life. Carotenoids were most likely among the pigments assisting in photosynthesis at that time.

Figure 4. Photosynthesis at different wavelengths. (A) The ability of light of different wavelengths to support photosynthesis; (B) The absorption spectra for three photosynthetic pigments: chlorophyll a, chlorophyll b, and β-carotene; (adapted from reference 4, page 748).
We now have a clear understanding of the genetics and molecular biology of the process of biosynthesis of carotenoids common to all members of the plant kingdom with this capability (5).

The prime movers in photosynthesis are the green pigments chlorophylls $a$ and $b$, but their role in trapping light is considerably enhanced by the additional presence of various carotenoids. Just how well chlorophylls and carotenoids work together is shown in the two parts (A) and (B) of Figure 4. There is a striking similarity between the two curves showing (A) the relative rate of photosynthesis and (B) the amount of light absorption at different wavelengths. In these graphs the only carotenoid shown is $\beta$-carotene and on this evidence it appears to contribute more than the chlorophylls. Thus it is not surprising that the concentrations of carotenoids and chlorophylls in any given source are often proportional, and edible dark green leaves are usually among the rich sources also of provitamin A.

The entire process of photosynthesis goes on inside an intracellular structure called the chloroplast (6) (Figure 5), where the carotenoids of plants are always found. The outcome of the process is very simple; light energy, trapped by chlorophyll, carotenoids and some other pigments, is converted into that of carbohydrates.

From carbon dioxide in the atmosphere and water, in the plant carbohydrates for food are provided not only for the plant but also for animal predators, including man. Oxygen is released into the atmosphere as a waste product. The details are very complex and are not part of the present story. A few Nobel prizes have been earned along the way.

In the present context only a brief account of the part played by the carotenoids is appropriate. Inside the chloroplast there are two photosystems (PSI and PSII). The carotenoid and chlorophyll particles are located on these photosystems that are visible as particles in the thylakoid membranes (Figure 5). PSI is light-dependent, but PSII is light-independent, although the process takes place in the light!

In the previous section we saw how light of different wavelengths, and therefore colours, is absorbed. When a molecule of a photosynthetic pigment, like a carotenoid, absorbs light it is said to become “excited”. The energy from photons boosts electrons to a higher energy level. This energy is “trapped” and converted to chemical energy. It is unstable and the first stage of photosynthesis involves movement of excited electrons between molecules within photosystems. Water is split into hydrogen and oxygen, and oxygen is released as a waste product. In the sec-

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**Figure 5. The structure of a chloroplast with simplified membrane structure. Adapted from Introduction to Plant Physiology, 1995, Hopkins-W.G.**
ond light-independent stage carbon dioxide is reduced to carbohydrates, using the chemical energy in the form of adenosine triphosphate (ATP) and hydrogen.

The story of photosynthesis is by no means over. Some of the most exciting research goes on in the largest oceans on the smallest organisms and will affect the future of life on earth (7).

3. Other functions of carotenoids

Less well understood as far as their mechanisms are concerned and receiving less publicity than their role in facilitating photosynthesis, other functions of carotenoids are attracting increasing attention these days.

1) Carotenoids in higher plants are capable of channelling light energy away from chlorophyll in an aerobic atmosphere (8). The xanthophyll cycle (Figure 6) appears to be especially involved in this process. Most plants are able to alter their carotenoid composition in thylakoid membranes (Figure 5) in response to growth under deep shade versus full sunlight. The proportion of carotenoid/chlorophyll molecules is greater in sun than shade and the proportion of xanthophyll cycle carotenoids (violaxanthin, antheraxanthin, and zeaxanthin) versus non-xanthophyll cycle carotenoids increases in sun compared with shade. It is generally agreed that the main site of energy dissipation is the light-harvesting antennae on the thylakoid membranes.

2) In plants and microorganisms carotenoids may protect against the potential formation of singlet oxygen by a triplet energy transfer mechanism (9). More recently it has been shown that a singlet energy transfer mechanism, from excited chlorophyll to zeaxanthin, can also prevent oxidative disruption of the photosynthetic process (5). Besides carotenoids a balanced diet contains other antioxidant substances, such as some essential elements and phytophenols and many other compounds for which no essential nutritional function is known. Many trials, both curative and preventive, in animals and in humans, are underway in relation to many diseases, particularly common chronic conditions such as various forms of cancer, atherosclerosis and the degenerative eye diseases cataract and age-related macular degeneration (10). Of special interest in the present context is the evidence that the two carotenoids zeaxanthin and lutein are preferentially deposited in the retina in man (8). Other carotenoids are absent. Near the centre of the retina there is a yellow spot, the macula lutea, where these pigments are concentrated. In the centre of the macula is the fovea, where all the photoreceptors are cones and visual acuity is at its greatest (Figure 7). Zeaxanthin tends to

\[ \text{Figure 6. The xanthophyll cycle, responsible in higher plants for dissipation of excess energy absorbed from sun-exposed sites and consequently a high demand for photo-protection; (adapted from reference 8).} \]
predominate centrally and lutein peripherally. This corresponds with the distribution of rods and cones respectively. These findings may have significance in relation to the common blinding disease age-related macular degeneration. Much more work is needed before human dosing with carotenoids on a wide scale can be safely recommended. Under some circumstances carotenoids have been shown to be prooxidant (8).

3) The fall of leaves of deciduous trees in autumn is brought about by a carotenoid derivative, abscisic acid, which causes a nipping off of the leaf at the junction with the stem, a process known as abscission. The xanthophyll xanthoxin gives rise to abscisic acid, which is a major plant anti-growth hormone (Figure 8) (11). This is brought about by cell membranes being made more permeable to water, thus preventing the freezing of cell water. The extracellular water in plants freezes first. The resulting osmotic dehydration of cells means that the cell fluids become more concentrated in solutes and their freezing point decreases.

4) The attractive red, orange and yellow colours of many autumn leaves are due to their xanthophyll content. The colours become obvious only after the intense green of chlorophyll has been broken down with a fall in temperature in autumn. An experiment was carried out by observation of the behaviour of the insects, aphids, known to damage deciduous trees in the autumn. It was shown that autumn colouration was stronger in species of trees facing a high diversity of damaging specialist aphids. This was interpreted to mean that the bright colours were a warning to pests that the tree would fight any infestation. The heavier an infestation was likely to be, the more the tree would have to gain by signalling its intentions (12).

4. The chloroplast and eusymbiosis

All known organisms are divided into two groups, prokaryotes and eukaryotes. A prokaryote is a simple organism that does not contain a true nucleus surrounded by a nuclear membrane, and division occurs by simple fission. In an eukaryote a cell has a true nucleus as in all higher organisms. In addition, eukaryotic cells contain a variety of internal organelles, and among these two are special, mitochondria and chloroplasts. These are of about the same size as bacteria and, like bacteria, have a circular genome outside the nucleus as well as the usual nuclear genome with which the former collaborates (Figure 5). The resemblance of mitochondria and chloroplasts to bacteria led to the idea that they might be free-living bacteria that were engulfed by a primitive eukaryote and flourished inside this as endosymbionts. This is now generally accepted. Chloroplasts are able to adapt to environmen-
tal changes such as a low or high level of light. When grown in complete darkness they lack chlorophyll but retain carotenoids. Thus many chloroplasts are light-regulated and carotenoids play an active part under these circumstances (13).

Lynn Margolis, the American biologist who has probably contributed more experimental evidence to support the concept of endsymbiosis than anyone else, has written, “Eukaryotes evolved symbiotically from eating, invading, infecting and cohabiting bacteria” (14).

5. Rhodopsin and the simplest known light-driven proton pump

The archaebacterium *Halobacterium halobium* under normal conditions in the presence of abundant oxygen oxidises fuel molecules to generate ATP, as do most aerobic organisms. However, when oxygen is scarce it synthesises large amounts of a membrane protein called bacteriorhodopsin, which takes part in a light-driven proton pump (15). Light isomerises the all-trans-retinal chromophore to the 13-cis-form, at the same time pumping a proton (H+ atom) from the cytosol to the outside.

The complex atomic details of this proton pump have been worked out in recent years. At the root is the photoisomerisation of retinal that produces a bend in the molecule of the chromophore of only 2 Å (Ångström = a hundred millionth of a centimetre) (Figure 9). Something very similar is seen in the visual cycle (see section 6 below).

6. Photo-transduction

This word refers to the process whereby the energy of photons is converted in photoreceptor cells into a nerve signal for transmission of the message onwards towards the brain for interpretation. There are many other examples of transduction of stimuli, including hearing, taste, smell and so on, but that of sight has been studied much more than any other.

The mammalian eye

The retina is composed of ten layers and the photoreceptors form the outermost of these underneath the retinal pigment epithelium (Figure 10). This means that before the process of phototransduction can begin, light has to pass through all other nine layers. This strange arrangement applies only to the mammalian retina.

There are two types of photoreceptor; rods for vision in poor light and cones for vision in bright light and for distinguishing between light of different colours. Cones and rods have differences in both structure and function (Figure 11). While the chromophore is the same in both, namely 11-cis-reti-
nal, the proteins attached to it are different. Rhodopsin is a very well characterised G-protein (see below) in the rods (Figure 12), and the cones have one of three slightly different versions of a similar protein, iodopsin. These three kinds of cone photoreceptor are either blue, green, or red light absorbing.

The importance of G-proteins has become appreciated only in recent years. Many receptors linked to effector systems by trimeric guanosine triphosphate (GTP)-binding proteins (G-proteins) have been described. The chromophores of mammalian vision, rhodopsin and iodopsin, and bacteriorhodopsin (Figure 9) are only a few among many G-proteins. These proteins regulate the activity of a specific plasma membrane enzyme or ion chan-


Figure 10. Schematic diagram of the ten layers of the retina; (adapted from reference 26, page 691).
nel (in the case of rhodopsin) in response to receptor binding.

Several different types of G-proteins couple to specific plasma-membrane enzyme-effector systems leading to the generation of a soluble second messenger. Cyclic adenosine monophosphate (AMP) was the first discovered. In an analogous system guanylate cyclase produces cyclic guanosine monophosphate (GMP) that specifically activates a cyclic GMP-dependent protein kinase. This is used in the visual system.

The photoreceptors of the human retina are so sensitive that a single one of them can be triggered by a single photon. In a rod this generates a current of approximately 1–3 pico-amperes; in a cone only about 10 femto-amperes, or about 1/100 that of a rod. However, the response time of a cone is about four times faster than that of a rod. Cones are better suited for discerning rapidly changing events; rods for low-light visual acuity.

The process of phototransduction goes on in the outer segment of the rods and cones and the signal generated passes down a fine cilium to the inner segment (Figure 11). Inner segments of both rods and cones contain many mitochondria and ribosomes. They generate ATP rapidly and actively synthesise proteins.

The outer segments of the rods have received much more atten-
tion than those of the cones, probably because they are much more numerous. In the human eye there are about 100 million rods to 3 million cones. The outer segment of each rod contains a stack of about 1000 discs. In the present context the story of the complex process of vision will be taken little further than the phase of phototransduction, in which the relatives of vitamin A are involved.

Before light excitation 11-cis-retinal locks the receptor protein (opsin) in its inactive form. The primary event in visual excitation is the photoisomerisation of the 11-cis-isomer of the Schiff-base of retinal to the all-trans-form (Figure 13). As can be seen in Figure 13 the energy in a photon has been converted into the energy of atomic motion to the extent of 5 Å (Ångström). Much of the isomerisation of retinal takes place within a few picoseconds giving rise to a series of intermediates. One of these, meta-rhodopsin II, called photoexcited or activated rhodopsin, triggers an enzymatic cascade that acti-

Figure 13. The primary event in visual excitation is the isomerisation of the 11-cis-isomer of the Schiff-base of retinal to the all-trans-form. This markedly alters the geometry of retinal. The Schiff-base linkage of retinal moves approximately 5 Å (Ångström) in relation to the ring portion of the chromophore; (adapted from reference 15, page 335).

Figure 14. Phototransduction of a single molecule of rhodopsin leads to a series of enzyme activation and regeneration responses that underly the opening of many channels. Adapted from The Eye: basics sciences in practice, page 194.
vates a G-protein (transducin) cascade leading to cyclic GMP hydrolysis (Figure 14) which in turn closes cation-specific channels to generate a nerve signal. Later, light-induced lowering of the calcium level coordinates recovery and adaptation.

The rhodopsin molecule alters its “colour”, its light-absorbing properties, from magenta, through orange to yellow and ultimately to white or “bleached”. Now it has become opsin and dissociated all-trans-retinal. The opsin undergoes regeneration by binding another molecule of 11-cis-retinal. In addition to restoring light-absorbing capacity this is critical for shutting off the pigment’s catalytic activity and thus allowing full dark adaptation to occur. The details of how 11-cis-retinal is reformed in the eye from all-trans-retinol have recently been investigated (16). Figure 15 gives details of this revised visual cycle and a key feature is the demonstration of a role of light in 11-cis-retinal formation within the retinal pigment epithelium. This pathway is an alternative to dark isomerisation, previously known, which yields 11-cis-retinol.

Some variations on the main theme

a) Colour vision in primates

It seems that in the earliest vertebrates that arose about 520 million years ago there was a single photopigment in cone-like photoreceptors. By the time the earliest four-legged animal emerged the single pigment had diverged into five distinct families, with a potential for one class of pigment in the rods and tetrachromatic colour vision. Most mammals now are dichromatic and some are monochromatic (no colour or day vision and seeing only at night). Primates are unique now in being trichromatic, with red, green and blue cones. Convincing evidence has been put forward that this evolved to make possible the gathering for food of coloured fruits and young leaves (17).

b) A variety of chromophores

Throughout the animal kingdom hundreds of visual pigments have been characterised by their absorption maximum ($\gamma_{\text{max}}$). The $\gamma_{\text{max}}$ range from 432 nm for the green rod of the frog to 625 nm for the red-absorbing cone of the goldfish. The protein component, and not the chromophore, is mainly responsible for these differences.

The main chromophores of the visual pigments are derived from one or other of the two forms of vitamin A: vitamin A1 (all-trans-retinol) or vitamin A2 (all-trans-3-dehydroretinol). The chromophores are respectively retinol and 3,4-didehydroretinol. Throughout the animal kingdom so far only three other retinal congeners
have been identified; all are versions of hydroxyretinal and are especially common in insects. These chromophores are thought to be derived from the cleavage of $\beta$-carotene to retinal followed by hydroxylation in the retina (18).

c) Freshwater fish and some amphibians

It has long been known that the visual pigments of freshwater fish differ from those of marine fish and indeed almost all other animals. Freshwater fish and some amphibians have 3-dehydroretinal as chromophore and porphyropsin for visual pigment with $\gamma$ max near 540 nm. Most other animals have all-trans-retinal and rhodopsin, as in man, with $\gamma$ max near 500 nm.

There is evidence that the retinas of freshwater fish and some amphibians can convert retinal into 3-dehydroretinal. This process may be regulated largely by properties of light in the natural environment and is sometimes under hormonal control. Age and diet also play a part. The difference in $\gamma$ max of the different chromophores appears to be an adaptation to permit fish to make use of the longer wavelengths of light found in fresh water as compared with sea water.

Euryhaline fish, that is to say those that tolerate a wide range of salinity in water, have a mixture of both rhodopsin and porphyropsin. In the frog Rana pipiens, the chromophore in the tadpole is 11-cis-3-dehydroretinal. In the adult this is replaced by 11-cis-retinal.

d) Fruit fly (Drosophila melanogaster) and other flies

It is reported recently that the visual pigment chromophore of this and possibly other flies is 3-hydroxyretinal and that this arises from cleavage of $\beta$-carotene to retinal followed by hydroxylation (18). It is suggested that this all goes on in the retinas.

7. Photoentrainment

This term is applied to the phenomenon whereby the regular daily change in the quantity and quality of light that enters the eyes regulates a wide variety of metabolic processes throughout the body, known as the circadian rhythm or biological clock (19). In mammals this clock is located within a paired nucleus in the brain above the crossing of the optic nerves, the suprachiasmatic nucleus or SCN (Figure 16). Until recently little attention has been paid to the way in which light is captured and processed by the eyes to bring about photoentrainment. It was usually assumed that this was done by the ordinary photoreceptors, the rods and cones.

Studies on mice with genetic defects and patients with eye dis-
ease that caused loss of vision without affecting the circadian system suggested that there might be separate photoreceptors for this function. Recently an expansive photoreceptive “net” in the inner retina of the mouse has been discovered consisting of melanopsin-containing retinal ganglion cells (20). Melanopsin is the only opsin known to exist in the retinal ganglion cell layer. If this, and no other, is the photoreceptive net for the circadian system then photoentrainment is not part of our story because it does not involve a relative of vitamin A. The chromophore is melanin in this case. Confirmation of this inference comes from a recent study in mice mutated in plasma retinol-binding protein, made deficient in vitamin A. The eyes of most mice contained no detectable retinal. Photic signalling to the SCN was fully intact, making it unlikely that this process is mediated by a retinal-dependent receptor.

8. Nuclear retinoid receptors

This function of the relatives of vitamin A is probably the most surprising of all. Twenty years or so ago there was no firm evidence for the mechanisms behind the functions of vitamin A at the cellular level. We had to rely on deducing this from evidence provided by the deficiency state. It was very clear that vitamin A in some form played a vital role in such processes as development, cell proliferation and differentiation. The fundamental mechanism of action of retinoic acid in cell differentiation was clarified with the discovery of the first retinoic acid receptor, RAR (now RAR-α 1), a nuclear transcription factor shown to be activated by all-trans-retinoic acid. At present six retinoid receptors of RAR and RXR gene subfamilies have been identified, belonging to the larger superfamily of steroid/thyroid/retinoid hormone nuclear transcription factors. Detailed information has been provided in numerous reviews (22, 23).

Nearly all cells express at least one member of the RAR and RXR subfamilies. Hundreds of genes have been shown to be involved and the control retinoic acid exerts over tissue and organ development is being made increasingly clear (24).

Among the multitude of emerging functions of retinoic acid a single example in the developing eye of the embryo may be out-
lined to illustrate something of the importance of this field for human health and disease in the future (25). The eye is known to be one of the regions of the developing embryo that is richest in retinoic acid. Several alcohol dehydroge-

nase enzymes are present in the retina and they are responsible for synthesising retinoic acid locally. This they do in such a way that the concentration of retinoic acid forms a gradient of concentra-
tion greatest at the ventral part and least at the dorsal. Gradients also occur over time. In the mature retina aldehyde dehydroge-
nase expression persists but the amount of retinoic acid syn-
thesised varies and is influenced by ambient light levels (Figure 17). This phenomenon is due to changing levels of retinal, which is released in the retinas of ver-
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References

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tification of an enzyme-cleaving β-


Please update your address and help to avoid unnecessary work and expenses.
Knowledge that vitamin A plays an important role in vision can be traced back approximately 3500 years to writings in Egyptian temples that indicate Egyptian physicians prescribed the topical use of ox liver, a source rich in vitamin A, as a means for curing night blindness. Scientific investigations into vitamin A actions in vision were initiated in the late 19th century and reached an apogee with the award of a Nobel Prize in 1967 to George Wald (1). The work of Wald and many other investigators had established by the early 1960s that the 11-cis-retinal form of vitamin A served as the chromophore for the visual pigment rhodopsin. The visual cycle of vitamin A as it was understood at the time of the award of the Nobel Prize to Wald is shown in Figure 1. Figure 1 is reproduced directly from the written text of Wald’s Nobel Prize lecture (1). This early work defined the metabolism and transport processes that take place in the retinal pigment epithelium (RPE) and photoreceptors that are necessary to make 11-cis-retinal available for visual pigment formation. These processes were collectively known as the visual cycle of vitamin A. It appeared in the late 1960s through the mid-1990s that the knowledge regarding the visual cycle of vitamin A was fairly complete. Most of the enzymatic processes involved in the visual cycle were thought to have been identified. It seemed that the sole important question that Wald and his contemporaries failed to answer was how the all-trans form of vitamin A, which is present in the circulation and all tissues outside of the eye, is isomerised to the 11-cis-form that is needed in the eye for vision. By the late 1980s, this question too appeared to have been answered and research interest in the visual cycle waned (2, 3). However, owing to the use of advanced molecular and genetic approaches to study vision, it has become clear that the visual cycle of vitamin A is far more complex than had been previously thought. In the last decade, many novel gene products (proteins) that play essential roles in the visual cycle have been identified. Some of these novel genes and other genes encoding known proteins involved in the visual cycle have been identified as loci for recognised heritable eye diseases.

Vitamin A and the visual cycle: An increasingly complex story

Christine M. Donmoyer, Katherine Lai and William S. Blaner, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032 U.S.A.

Figure 1. Visual cycle as depicted by Wald (1968). DPN and TPN refer to cofactors NAD+ and NADH.
The isomerisation of all-trans-vitamin A to 11-cis-vitamin A

The one great-unresolved question regarding the visual cycle of vitamin A that remained unanswered from the era of Wald was how the eye generates 11-cis-retinal (1). Starting in 1987, a series of reports were published that indicated that 11-cis-retinal is formed from all-trans-retinol ester through the enzymatic coupling of ester hydrolysis with isomerisation of the resultant all-trans-retinol to its 11-cis-isomer (2, 3). The retinal pigment epithelium (RPE) enzyme that catalyses this reaction was referred to as the isomerohydrolase (4). Although the enzyme had not been purified or cloned, it was generally accepted that the isomerohydrolase was responsible for catalyzing 11-cis-retinol formation within the eye (4). However, this view began to be questioned in 1998 with the publication of the characterisation of RPE65 knockout mice; these mice were generated through the targeted disruption of the gene for RPE65 (5). The deletion of the gene for RPE65 rendered these mutant mice blind and unable to convert all-trans forms of vitamin A to 11-cis-isomers. RPE65 had been initially described several years earlier as a protein that is expressed solely in the RPE in the eye but no physiological function could be assigned to the protein. The phenotype of the RPE65 knockout mice suggested that RPE65 could be the isomerohydrolase; however when the purified protein was studied, still no definitive evidence could be obtained that RPE65 acted as an isomerohydrolase. Thus, the absence of RPE65 results in a failure to form 11-cis-retinal from all-trans-vitamin A forms yet RPE65 does not act catalytically as an isomerohydrolase.

More recently, another RPE-specific protein, retinal G-protein-coupled receptor (RGR) was identified and demonstrated to be importantly involved in the light-dependent formation of 11-cis-retinal from all-trans-retinal (6, 7). Exposure of all-trans-retinal bound to RGR to light generates 11-cis-retinal (6, 7). Interestingly, RGR-knockout mice regenerate visual pigment more slowly, when exposed to a continuous low level of light, than do wild type mice, but in darkness the rates of rod visual-pigment regeneration are the same for RGR-knockout and

![Figure 2. Current perspective of the visual cycle of vitamin A from Pepperberg and Crouch (2001). RGR, retinal G-protein-coupled receptor; LRAT, lecithin:retinol acyltransferase.](image-url)
It is now thought that the light-dependent isomerisation of all-trans to 11-cis-retinal catalysed by RGR most likely operates in concert with a dark pathway of 11-cis-retinol from all-trans-retinol that probably involves the action RPE65 (8). Thus, based on these recent data, it is now believed that two distinct isomerisation pathways operate as part of the visual cycle of vitamin A. Our current understanding of the visual cycle of vitamin A is summarised in Figure 2.

New understanding of other enzymes involved in transforming vitamin A in the visual cycle

Three enzymes proposed to have essential actions in the visual cycle of vitamin A have been studied to varying degrees in the last decade. Two of these, 11-cis-retinol dehydrogenase (also called RDH5 and cis-retinol dehydrogenase) and lecithin:retinol acyltransferase (LRAT) are present in the RPE and the other, all-trans-retinol dehydrogenase, is present in the photoreceptors (see Figure 2).

11-cis-Retinol dehydrogenase catalyses the conversion of 11-cis-retinol to 11-cis-retinal, which is needed for visual pigment formation (4). Since 11-cis-vitamin A forms are present in only the eye and although it was originally reported that this enzyme is eyespecific (9, 10), it is now recognized that the enzyme is also present in kidney, liver, testis as well as other tissues. It has been proposed that outside of the eye this enzyme plays a role in generating 9-cis-retinoic acid, which is needed for regulating transcription, and in the metabolism of steroids (11). Interestingly, mutant mice completely lacking 11-cis-retinol dehydrogenase display delayed dark adaptation but have otherwise normal vision (12). This observation has been taken to suggest that other enzymes are present in the RPE able to catalyse 11-cis-retinal formation. Indeed, this also appears to be the case for humans, since mutations in the human 11-cis-retinol dehydrogenase gene do not result in complete blindness (see below for more details) (13).

LRAT catalyses the formation of retinyl esters through trans-esterification of all-trans-retinol with a fatty acid group taken from phosphatidyl choline present in the membranes of the RPE cell (3, 4, 14). This enzyme, too, is found in tissues outside of the eye and is thought to play an essential role in the esterification of

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**Table I. Mutations in vitamin A-related genes responsible for human retinal diseases**

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>Disease</th>
<th>Clinical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal pigment epithelial protein 65 (RPE65) (27)</td>
<td>Leber’s congenital amaurosis, early-onset retinal dystrophy</td>
<td>Severely reduced rod function, blindness</td>
</tr>
<tr>
<td>ATP-binding cassette transporter in retina (ABCR) (21)</td>
<td>Stargardt’s disease, cone-rod dystrophy, retinitis pigmentosa, age-related macular degeneration</td>
<td>Central vision loss due to lipofuscin accumulation</td>
</tr>
<tr>
<td>11-cis-Retinol dehydrogenase (RDH5) (13)</td>
<td>Recessive fundus albipunctatus, late-onset recessive cone dystrophy</td>
<td>Stationary night blindness and delayed dark adaptation</td>
</tr>
<tr>
<td>Cellular retinaldehyde-binding protein (CRalBP) (28)</td>
<td>Recessive retinitis pigmentosa, recessive retinitis punctata albscens</td>
<td>Peripheral vision loss</td>
</tr>
<tr>
<td>Lecithin:retinol acyltransferase (LRAT) (18)</td>
<td>Severe early-onset recessive retinitis pigmentosa</td>
<td>Severe retinal dystrophy</td>
</tr>
<tr>
<td>Retinal G-protein-coupled receptor (RGR) (17)</td>
<td>Recessive retinitis pigmentosa</td>
<td>Peripheral vision loss</td>
</tr>
<tr>
<td>Retinol-binding protein (RBP) (24,25)</td>
<td>Recessive RPE degeneration</td>
<td>Night blindness with RPE atrophy and reduced acuity</td>
</tr>
</tbody>
</table>
dietary retinol in the small intestine and in the storage of retinyl ester in the liver (14). In the eye, LRAT action is needed to generate all-trans-retinyl ester pools that can be used by the isomerohydrolase for generation of 11-cis-retinol (14).

The photoreceptor enzyme, all-trans-retinol dehydrogenase, that catalyses the formation of all-trans-retinol from all-trans-retinal formed upon bleaching of the visual pigment rhodopsin has been recently cloned and characterised (15). This enzyme specifically requires all-trans-vitamin A forms as substrates and will not catalyse the inverconversion of 11-cis-vitamin A forms. It is presently unclear whether this enzyme has an important physiological role outside of the eye and the visual cycle.

**Genetic eye diseases and proteins involved in vitamin A metabolism in the eye**

Many of the genes encoding proteins that are active in maintaining the visual cycle are also associated with genetic eye diseases (8, 13, 16-21). A partial listing of these genes is given in Table I. As can be seen from Table I, the genes for RPE65, RGR, 11-cis-retinol dehydrogenase and LRAT have all been identified as sites for mutations that give rise to impaired vision or blindness. Mutations in RPE65 are associated with certain forms of Leber’s congenital amaurosis and account for approximately 15% of the disease in North America (8, 16). In a dog model of Leber’s congenital amaurosis arising due to RPE65 mutation, gene therapy with a recombinant adeno-associated virus carrying wild-type RPE65 has been shown to restore vision (22). RGR gene mutations have been found in some patients with retinitis pigmentosa (17). Mutations in the gene for 11-cis-retinol dehydrogenase have been associated with fundus albinipunctatus, a form of stationary night blindness characterised by a delay in the regeneration of cone and rod photopigments (13). LRAT gene mutations are associated with early-onset severe retinal dystrophy (18). It is interesting to note that, unlike RPE65 or RGR, LRAT is expressed widely throughout the body (including in the liver, intestine and numerous other tissues), yet mutations associated with LRAT seem to result solely in ocular disease.

Several proteins that are present in the retina and important for maintaining the visual cycle have also recently been cloned and characterised. One of these proteins, ABCR, is an ATP-binding cassette transporter present in the rims of photoreceptor outer segment discs (19-21). This membrane transport protein is proposed to play a role in pumping all-trans-retinal from the disc interior after light exposure and in eliminating the vitamin A derivatives and lipofuscin precursors A2PE-H2 and A2PE from photoreceptor outer segments (19-21). Mutations in the ABCR gene have been shown to be responsible for Stargardt’s disease, a blinding disorder that is characterized by delayed dark-adaptation and accelerated deposition of lipofuscin in the RPE (19-21).

Other recent studies indicate that mutations that influence the delivery of vitamin A to the eye can also give rise to ocular disease. Plasma retinol-binding protein (RBP) is the sole circulating transport protein for vitamin A and it is thought that the preponderance of the vitamin A delivered to the eye and other tissues is delivered via RBP (23). Two sisters have been identified in Germany who reportedly lack any circulating RBP (24, 25). These young women display compound RBP mutations that give rise to night blindness and impaired dark adaptation (24, 25). RBP knockout mice similarly display an impaired vision phenotype that can be reversed if the mice are provided significant amounts of vitamin A in their diets (26). Thus, as was the case for LRAT mutations mentioned above, mutations in RBP, a protein that is responsible for delivering vitamin A to all tissues in the body, seem to be manifested solely through impairments in vision.

**Summary**

Overall, in the past decade there has been tremendous growth in our understanding of the genes and proteins that are responsible for maintaining the visual cycle of vitamin A. It is clear that the visual cycle is far more complex and involves many more protein species than was ever suspected by Wald and his contemporaries. In addition, it is clear that many of the proteins involved in the visual cycle can be sites for mutations that give rise to heritable eye diseases. After many years of relatively little research interest in the visual cycle, this research area is again one of great activity. The research cycle
seems to have rotated back to a period when significant new insights into the actions of vitamin A in the visual cycle are again being made. Indeed, the present time is one where a whole new layer of complexity has been added to our basic understanding of the visual cycle of vitamin A.

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Effects of processing and storage on food carotenoids

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Introduction

Many carotenogenic foods are seasonal and processing at peak harvest is necessary to minimise losses, make the products available all year round and permit transportation to places other than the site of production. Processing and storage of foods should, however, be optimised to prevent or reduce harmful effects and accentuate the benefits.

Alteration or loss of carotenoids during processing and storage of foods occur through physical removal (e.g. peeling), geometrical isomerisation and enzymatic or non-enzymatic oxidation. Necessary measures should be taken to ensure maximum retention of carotenoids. Although industrial processing is more often focalised, losses on home preparation can also be, at times even more, considerable. On the other hand, processing may enhance bioavailability.

Retention of carotenoids during processing and storage

Considerable retention or loss of carotenoids during processing and storage of food has been reported in numerous papers. However, data are somewhat conflicting and often difficult to interpret because of the following reasons: (a) processing and storage conditions are not, or are only partially, described; (b) different foods are processed differently, making comparisons of processing methods difficult; (c) different conditions (e.g. time and temperature) are used for the same method of processing; (d) the procedure followed for calculating losses is not specified or calculation is faulty (1). Additionally, an inherent problem that should not be overlooked is the possibility of isomerisation and oxidation of carotenoids taking place during analysis and/or during storage of samples prior to analysis. These reactions may be erroneously attributed to the processing or storage of foods. But despite some experimental inadequacies and discrepancies in results, some conclusions can be drawn (1):

• Carotenoid biosynthesis may continue, raising the carotenoid content in fruits, fruit vegetables and root crops even after harvest, provided these plant materials are kept intact and are not treated in any way that would inactivate the enzymes responsible for carotenogenesis. In leaves and other vegetables, post-harvest degradation of carotenoids appears to prevail, especially at high storage temperature and under conditions that favour wilting.

• Carotenoids are naturally protected in plant tissues; cutting, shredding, chopping and pulping of fruits and vegetables increase exposure to oxygen and bring together carotenoids and enzymes that catalyse carotenoid oxidation.

• The stability of carotenoids differs in different foods, even when the same processing and storage conditions are used. Thus, optimum conditions for carotenoid retention during preparation/processing differ from one food to another. Carotenoids per se have different susceptibilities to degradation.

• The major cause of carotenoid destruction during processing and storage of foods is enzymatic or non-enzymatic oxidation. Isomerisation of trans-carotenoids to the cis-isomers, particularly during heat treatment, alters their biological activity and discolours foods, but not to the same extent as oxidation. Enzymatic degradation of carotenoids may be a more serious problem than thermal decomposition in many foods.

• Reported increases in carotenoid content during cooking or thermal processing are not likely to be true increases but are artefacts of the analytical/calculation procedure, due to
loss of carotenoids in fresh samples because of enzymatic activity during sample preparation for analysis, greater extractability of carotenoids from processed samples, and unaccounted loss of water and leaching of soluble solids during processing.

- In home preparation, losses of carotenoids generally increase in the following order: microwaving < steaming < boiling < sautéing. Deep-frying, prolonged cooking, combination of several preparation and cooking methods, baking and pickling all result in substantial losses of carotenoids.
- Whatever the processing method chosen, retention of carotenoids decreases with longer processing time, higher processing temperature and cutting or puréeing of the food. Reducing processing time and temperature, and the time lag between peeling, cutting or puréeing and processing improves retention significantly. High-temperature, short-time processing is a good alternative.
- The heat treatment in blanching may provoke some losses of carotenoids, but the inactivation of oxidative enzymes will prevent further and greater losses during holding before thermal processing, slow processing and storage.
- Freezing (especially quick-freezing) and frozen storage generally preserve the carotenoids, but slow thawing can be detrimental, particularly when the product has not been properly blanched.
- Peeling and juicing result in substantial losses of carotenoids, often surpassing those of heat treatment.

- Traditional sun-drying, although the cheapest and most accessible means of food preservation in poor regions, causes considerable carotenoid destruction. Drying in a solar dryer, even of simple and inexpensive design, can appreciably reduce losses. Protecting the food from direct sunlight also has a positive effect.
- Natural or added antioxidant and sulfiting may reduce carotenoid degradation.
- Exclusion of oxygen (e.g. through vacuum or hot filling, oxygen-impermeable packaging, inert atmosphere), protection from light and low temperature diminish carotenoid decomposition during storage.

**Geometrical isomerisation**

Being highly unsaturated, carotenoids are prone to isomerisation and oxidation (Figure 1). Isomerisation of trans-carotenoids, the usual configuration in nature, to the cis-isomers is promoted by contact with acids, heat treatment and exposure to light. This results in some loss of colour and alteration of biological activity. The principal cis-isomers of β-carotene are shown in Figure 2.

The release of organic acids during slicing or juicing of fruits is sufficient to provoke trans-cis isomerisation. However, occur-

---

**Figure 1. Possible scheme for the degradation of carotenoid.**
rence of this isomerisation has been better demonstrated in thermal processing.

A 10–39% increase in the percentage of total cis-isomers of provitamin A carotenoids was observed on heat treatment (canning) of several fruits and vegetables (2). Canning of sweet potato caused the largest increase, followed by processing of carrot, tomato juice, collard, tomato, spinach, peach and orange juice. The principal cis-isomers in processed red, yellow and orange fruits and vegetables were 13-cis-(and 13'-cis-), although 9-cis- and 15-cis-isomers were also detected. In processed green vegetables (in which β-carotene was the only provitamin A carotenoid detected), 9-cis-β-carotene predominated, followed by 13-cis-β-carotene, an unidentified cis-isomer and 15-cis-β-carotene.

Table 1. Cis-trans-β-carotene isomer concentrations (µg/g dry weight) in raw and processed sweet potatoes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>13-Cis</th>
<th>All-Trans</th>
<th>9-Cis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw product</td>
<td>22</td>
<td>418</td>
<td>-</td>
</tr>
<tr>
<td>Strips (2-min blanch 100°C)</td>
<td>39</td>
<td>460</td>
<td>-</td>
</tr>
<tr>
<td>Strips (10-min blanch 100°C)</td>
<td>70</td>
<td>388</td>
<td>-</td>
</tr>
<tr>
<td>Puree (lye peeled, Fitzmill comminutor with 0.06&quot; screen)</td>
<td>25</td>
<td>461</td>
<td>-</td>
</tr>
<tr>
<td>Steam injection (81°C to gelatinise starch, hold 30 min)</td>
<td>34</td>
<td>461</td>
<td>-</td>
</tr>
<tr>
<td>Steam injection (100°C to inactivate amylases)</td>
<td>37</td>
<td>419</td>
<td>-</td>
</tr>
<tr>
<td>Canned (still retort, 90 min at 116°C)</td>
<td>57</td>
<td>323</td>
<td>11</td>
</tr>
<tr>
<td>Dehydrated (drum dried at 160°C at 25 rpm with contact time of 1.8–2 sec)</td>
<td>101</td>
<td>249</td>
<td>trace</td>
</tr>
<tr>
<td>Microwaved (full power for 7 min until internal temp. of 99°C)</td>
<td>56</td>
<td>284</td>
<td>trace</td>
</tr>
<tr>
<td>Baked (conventional oven at 191°C for 80 min until internal temp. of 99°C)</td>
<td>69</td>
<td>232</td>
<td>trace</td>
</tr>
</tbody>
</table>

Reference: Chandler and Schwartz (5)

![Figure 2. Common geometrical isomers of β-carotene.](image-url)
The isomerisation pattern reported by Lessin et al. was also observed in earlier studies, the $13\text{-cis}$ being the major $cis$-isomer in processed fruits and vegetables, except in processed green vegetables in which the $9\text{-cis}$ prevailed (3-5).

In sweet potatoes, heat induced formation of $13\text{-cis}$-$\beta$-carotene in the different thermal treatments investigated, the quantity formed being related to the severity and length of processing (Table 1) (5). $cis$-isomers also increased during heating of carrot juice (6), $13\text{-cis}$-$\beta$-carotene being formed in largest amount, followed by $13\text{-cis}$-lutein and $15\text{-cis}$-$\alpha$-carotene.

To minimise hydrolytic rancidity in the oil, red palm fruits are sterilised immediately after harvest to inactivate lipases. This treatment (128°C, 66 min) provokes substantial isomerisation of $\alpha$- and $\beta$-carotene, as shown in Table 2 for oils from *Elais guineensis* and *E. oleifera* fruits (7).

Although the $cis$-isomer levels of $\beta$-carotene in cooked (boiled and/or stir-fried) carrot, Indian eggplant and squash were higher than those of the corresponding raw vegetables, those of cooked broccoli, green beans, okra and spinach were lower (8). Since both $cis$- and $trans$-carotenoids undergo oxidation, the latter finding could be a reflection of the turn-over of $cis$-$\beta$-carotene.

Lycopene was found to be relatively resistant to heat-induced geometrical isomerisation during typical food processing of tomatoes and related products (9). In various thermally processed products (juice, paste, soup, sauce), the lycopene $cis$-isomers amounted to 3.56–5.98%, compared to 4.16% in fresh tomato and 5.37% in peeled tomato, and the $cis$-isomer content did not reflect the severity of the heat treatment. On the other hand, appreciable levels of $\beta$-carotene $cis$-isomers did form, the $cis$-isomer percentage ranging from 55.57 to 85.85% in the processed products, compared to 21.77% in the fresh tomato and 23.83% in the peeled tomato.

### Enzymatic oxidation

The highly reactive, electron-rich carotenoid molecule suffers oxidation under food processing and storage conditions, the magnitude of which depends on the carotenoids present, available oxygen, exposure to light, temperature, presence of enzymes, metals, prooxidants and antioxidants.

Enzyme-catalysed oxidation can occur in the steps prior to heat treatment, during peeling, slicing, pulping or juicing. Thus, it is recommended that foods be thermally processed immediately after these operations. Enzymatic oxidation can also take place in minimally processed and in unblanched frozen foods.

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>From fresh fruits</th>
<th>From sterilised fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. guineensis</em> Dura Dumpy</td>
<td><em>E. guineensis</em> Psífera</td>
</tr>
<tr>
<td>13-$cis$-$\alpha$-carotene</td>
<td>4.8/87</td>
<td>0.5/5.5</td>
</tr>
<tr>
<td>All-$trans$-$\alpha$-carotene</td>
<td>296/228</td>
<td>18/14</td>
</tr>
<tr>
<td>13-$cis$-$\beta$-carotene</td>
<td>12/200</td>
<td>8.2/63</td>
</tr>
<tr>
<td>All-$trans$-$\beta$-carotene</td>
<td>576/255</td>
<td>202/88</td>
</tr>
<tr>
<td>9-$cis$-$\beta$-carotene</td>
<td>12/179</td>
<td>1.2/55</td>
</tr>
</tbody>
</table>

Reference: Trujillo-Quijano et. al. (7)
Marketing minimally processed fruits and vegetables is an increasing trend, stimulated by consumers’ demand for high-quality, nutritive, fresh-like and convenient-to-use products. Since drastic processing conditions are not employed, minimally processed products are expected to retain fresh or fresh-like properties and have good nutritive quality. However, tissue disruption by cutting or shredding allows substrate/enzyme interactions and makes these products more susceptible to physiological/biochemical changes than intact raw commodities. Greater exposure of plant components to oxygen also enhances oxidative degradation.

In mini-peeled carrots packed in LDPE films and stored at 2ºC, \( \alpha \)-carotene and \( \beta \)-carotene levels declined 18 and 14%, respectively, within three days after minimal processing with no further losses during 14 days of storage (10). In minimally processed Jalapeño pepper rings packed in polyethylene bags and stored at 4.4ºC, \( \alpha \)-carotene and \( \beta \)-carotene decreased 31 and 24%, respectively, after three days in sealed perforated bags (air atmosphere) (11). With modified atmosphere (5% \( O_2 \), 4% \( CO_2 \)), however, losses of these carotenoids amounted to only 4 and 10%, respectively.

\( \beta \)-carotene, lutein, violaxanthin and neoxanthin concentrations, monitored during five days of storage at 7–9ºC were reduced 14–42%, 19–32%, 12–20% and 8–31%, respectively, in minimally processed endive, kale (Table 3), spinach and a mixture of green onion and parsley packed in polyethylene bags (12). As would be expected of an enzymatic reaction, losses of carotenoids occurred mainly in the first two days of storage. Minimal processing consisted of washing, trimming, cutting, washing in chlorinated water, draining (centrifugation) and packaging. Spinach (small-leaf type) consisted of whole leaves, endive and kale were shredded, parsley and onion were finely chopped.

In frozen (-18ºC) *Eugenia uniflora* pulp, \( \beta \)-cryptoxanthin, \( \gamma \)-carotene and lycopene were considerably reduced during the first two months of storage at -15ºC, stabilising thereafter (13). The extent of loss was much greater than that usually seen in thermally processed products. Moreover, carotenoid decomposition in the latter products is usually insignificant during the first several months, increasing rapidly when it ensues.

<table>
<thead>
<tr>
<th>Time after processing (days)</th>
<th>( \beta )-Carotene</th>
<th>Lutein</th>
<th>Violaxanthin</th>
<th>Neoxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29a</td>
<td>45a</td>
<td>20a</td>
<td>13a</td>
</tr>
<tr>
<td>1</td>
<td>25b</td>
<td>37b</td>
<td>17b</td>
<td>9b</td>
</tr>
<tr>
<td>2</td>
<td>23b</td>
<td>35b,c</td>
<td>16b</td>
<td>9b</td>
</tr>
<tr>
<td>3</td>
<td>24b</td>
<td>33c</td>
<td>16b</td>
<td>9b</td>
</tr>
<tr>
<td>5</td>
<td>25b</td>
<td>33c</td>
<td>16b</td>
<td>9b</td>
</tr>
</tbody>
</table>

* Shredded kale packed in polyethylene bags, stored at 6–9ºC. Values in the same column with different letters are significantly different (\( \rho \leq 0.05 \)); Reference: Hess and Rodriguez-Amaya (12).
activation of lipoxygenase. Reduction of lutein, however, was roughly the same for blanched and unblanched beans. In contrast, the β-carotene and lutein levels in the frozen pepper fluctuated around more or less constant values over the 12 months.

Papaya slices without previous treatment were vacuum-packed in plastic bags and frozen in air-blast freezer operating at -40°C. The bags were left in the freezer until the center of the slices reached -24°C and then stored at -18°C for 12 months (15). The carotenoid content decreased significantly, the reduction being markedly higher in the female papaya slices than the hermaphrodite papaya slices. The difference was attributed to greater enzymatic activity in the female papaya slices.

Non-enzymatic oxidation

In contrast to the wealth of information on lipid oxidation, present-day knowledge of carotenoid oxidation is still fragmentary. It is often accompanied by isomerisation, both cis- and trans-isomers being subject to oxidation (Figure 1). It is generally accepted that the initial stages of oxidation involve epoxidation and cleavage to apocarotenals (Figure 1). Subsequent fragmentations result in compounds of low molecular masses, similar to those produced in fatty acid oxidation. Now devoid of colour and biological activity, these compounds contribute to the desirable flavour of wine and tea but can be responsible for the off-flavour of dehydrated carrot. Full structural elucidation of the intermediate and final products of the oxidative process, as well as delineation of the mechanisms for their formation, are urgently needed.

Epoxidation and transformation of the 5,6-epoxide groups to the 5,8-furanoid groups of β-carotene is illustrated in Figure 3. Introduction of an epoxide group at one of the β-rings reduces the vitamin A activity by about half, while the epoxidation of both rings eliminates this activity.

Transformation of the xanthophyll violaxanthin is often observed in food processing (Figure 4). In mango (cultivar Tommy Atkins) slices, the carotenoid composition was practically maintained during processing (16). The only significant change was the increase in luteoxanthin, compatible with the conversion of 5,6-
to 5,8-epoxide. More evident changes occurred on processing mango (cultivar Golden) purée. Auroxanthin, not found in the fresh fruit, appeared while violaxanthin and luteoxanthin decreased, again reflecting the transformation of 5,6- to 5,8-epoxide. In commercially processed mango juice (three brands), the notoriously unstable violaxanthin, the principal carotenoid of the major mango cultivars in Brazil, was not detected while auroxanthin was found in appreciable amount (17).

In bottled papaya (cultivar Solo) purée, no significant loss of β-carotene, ζ-carotene, γ-carotene and lycopene occurred during processing (18). There was a small statistically significant decrease in β-cryptoxanthin and cryptoflavin, an epoxy derivative of β-cryptoxanthin, appeared after processing. During 14 months of storage, β-carotene and lycopene showed an insignificant downward trend. β-cryptoxanthin did not change significantly during the first 10 months, but showed a small significant decrease after 14 months. The epoxy carotenoids auroxanthin and flavoxanthin were formed during storage.

The apocarotenals that can be derived from β-carotene are shown in Figure 5. Formation of apocarotenals can occur sequentially or at random. The isolation of β-apo-8'-carotenal, β-apo-10'-carotenal and β-apo-12'-carotenal, along with β-cyclocitral and acetaldehyde, in a model system suggests sequential cleavage (19). Apocarotenals are rarely detected in foods, indicating a fast turn-over.

**Factors influencing carotenoid degradation**

In model systems, carotenoid decomposition has been shown to depend on carotenoid structure, nature of the system, available oxygen, exposure to light, water content or activity, temperature, atmosphere, presence of antioxidants, prooxidants, free radical initiators and inhibitors, and sulphites (20). The situation is more complex in foods, considering the complicated interplay of the factors mentioned above, along with the varied nature and composition of foods, processing treatment, packaging and storage conditions, activity of lipoxygenase and other enzymes, and coupled oxidation with lipids.

![Figure 4. Transformation of violaxanthin during processing and storage of foods.](image_url)
Ample evidence that carotenoids in foods vary in their susceptibility to degradation can be found in the literature, although discrepancies in the trend followed by specific carotenoids can be noted. The effects of light and temperature have also been well demonstrated.

Lutein decreased slightly but β-carotene was stable during freezing of winter squash (21). Under frozen storage, lutein was stable while β-carotene decreased 32% after three months. No loss of carotenoids was observed on freeze-drying. During storage of freeze-dried squash at 30°C, loss of β-carotene reached 15, 20 and 53% after one, two and three months, respectively. However, after 3 months of storage at 3°C, reduction of β-carotene was only 10%.

In acidified, pasteurised carrot juice stored for three months, reduction of lutein, α-carotene and β-carotene concentrations increased with increasing storage temperature and was also greater under illumination than under dark storage (22). The formation of 13-cis-isomers appeared to be favoured under lighted storage and the 9-cis-isomers in the dark.

In vegetable juice containing mainly tomato and carrot juice, after four days of exposure to 230 ft-c of light at 4°C, only 25% of the initial α- and β-carotene remained, while 75% of lycopene was still present (23). Carotene loss was extensive after eight days. The control samples (held in darkness) showed no or negligible destruction of carotenoids. β-carotene was also found to be more sensitive than lycopene during heat-based processing of tomato (hot-break extract and tomato paste) (24).

The relative stability of lycopene observed above is unexpected, considering that lycopene degrades rapidly in oil and low-moisture model systems (25, 26). In fact, because of this instability, loss of lycopene during analysis is considered a major analytical problem (27-30).

The lycopene content in tomato pulp was found to decrease during heating under different processing conditions (31). The apparent rate constant for lycopene degradation increased with increase in the concentrations of lycopene, acids, sugars and total solids. After four months of storage, lycopene

![β-apo-8'-carotenal](image1)

![β-apo-10'-carotenal](image2)

![β-apo-12'-carotenal](image3)

Figure 5. β-Apocarotenals derived from β-carotene.
loss was greater in freeze-dried fibre-rich tomato pulp than in oven-dried samples. Lycopene degradation increased with exposure to air, light and high storage temperature.

After six weeks of light exposure at room temperature or storage at 6°C in the dark, 60–70% of all-trans-lycopene of total lycopene was retained in two commercially produced spray-dried tomato powders (32). Thus, light and increase of storage temperature from 6°C to room temperature were not important factors in the stability of lycopene in these powders. At 45°C, however, only 40% retention was observed after 6 weeks.

**Influence of processing on bioavailability**

It is recommended that strategies to increase the dietary intake of carotenoid-containing foods include measures to enhance bioavailability (33). For a long time the major concern about processing in relation to carotenoids had been preventing losses. In recent years, attention has shifted to the effect of processing on the bioavailability of carotenoids.

Cis-isomers have long been attributed lower vitamin A activity than the trans-provitamin A carotenoids. In recent years, trans-β-carotene was found to be preferentially absorbed over 9-cis-β-carotene in humans (34-36) and ferrets (37). On the other hand, cis-lycopene was observed to be more bioavailable than trans-lycopene in ferrets (38). Aside from geometrical isomerisation, processing has another effect on bioavailability. Carotenoids in nature are protected by the cellular structure, the destruction of which renders the carotenoids vulnerable to degradation as discussed above. On the other hand, this natural protection limits the bioavailability. Processing denatures proteins and breaks down the cell walls, making the release of carotenoids from the food matrix easier.

Indeed, enzymatic disruption of the matrix enhanced the bioavailability of β-carotene from whole-leaf and minced spinach, the serum total β-carotene response over a 3-week period in human subjects (n=10 for control group; n=12 per spinach group) was significantly different between the whole leaf and the enzymatically liquefied spinach groups, and between the minced and the liquefied spinach groups (39). The lutein response, however, did not differ among the spinach groups. In another study involving eight healthy females, daily consumption of processed carrots and spinach over a 4-week period resulted in an increase in plasma β-carotene concentration, averaging three times that associated with the ingestion of the same amount of β-carotene in the raw vegetables (40).

Determining the carotenoid in the chylomicrons of five subjects given a single dose of fresh tomato or tomato paste, ingested together with corn oil, consumption of tomato paste resulted in 2.5-fold higher total and all-trans-lycopene peak concentrations and 3.5-fold higher total area under the curve than consumption of fresh tomato (41). In an earlier study, ingestion of heat-processed tomato juice (cooked in an oil medium) resulted in a 2- or 3-fold increase in lycopene serum concentrations one day after ingestion (42). An equivalent consumption of unprocessed tomato juice caused no rise in plasma concentrations. Carotenoid response in triglyceride-rich lipoproteins after single consumption (n=11) and plasma carotenoid concentrations after four days of daily consumption (n=33) showed that matrix disruption of canned peeled, whole tomatoes by mechanical homogenisation and/or heat treatment enhanced carotenoid bioavailability (43).

Current knowledge therefore suggests that processing conditions should be optimised to prevent appreciable losses of carotenoids while enhancing their bioavailability.

**Summary**

The major reactions undergone by the highly unsaturated carotenoids during processing and storage of foods are geometrical isomerisation and oxidation. Isomerisation of trans-carotenoids to cis-carotenoids, promoted by contact with acids, heat treatment and exposure to light, diminishes the colour and alters the biological activity. The major cause of carotenoid loss, however, is enzymatic and non-enzymatic oxidation, which depends on the availability of oxygen and the carotenoid structure. It is stimulated by light, heat, metals, enzymes and peroxides and is inhibited by antioxidants. Data on percentage losses of carotenoids during food processing and storage are somewhat
conflicting, but carotenoid degradation is known to increase with the destruction of the food cellular structure, increase of surface area or porosity, length and severity of the processing conditions, storage time and temperature, exposure to light, permeability to $O_2$ of the packaging. Contrary to lipid oxidation, for which the mechanism is well established, the oxidation of carotenoids is not well understood. It involves initially epoxidation and cleavage to apocarotenoids. Subsequent fragmentations result in a series of compounds of low molecular masses. Completely losing their colour and biological activities, the carotenoids give rise to volatile compounds which contribute to the aroma/flavour, desirable in tea and wine and undesirable in dehydrated carrot. Processing also influence the bioavailability of carotenoids, through geometrical isomerisation and disruption of the cellular structure.

**Acknowledgment**

The author acknowledges with gratitude the financial support given by MCT-FINEP-CNpq through the project PRONEX No 4196091500.

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Using immunisation contacts to deliver vitamin A

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Status

Children’s Summit Goal: To achieve the sustainable elimination of vitamin A deficiency by 2010.

• Globally, 136 countries have a vitamin A deficiency (VAD) problem of public health significance. 140–250 million children under 5 years are at risk.
  • Issue of child survival, not just blindness; delivery of vitamin A supplements shown to reduce all cause mortality by 23%, and reduce measles and diarrhea morbidity.
• In 2000, 61 countries included vitamin A with polio or measles national immunisation days (NIDs).
• 49 countries distributed vitamin A with routine immunisation contacts.
• 34 of these countries used both strategies.
Key issues

- While much success has been achieved linking vitamin A delivery with NIDs the use of routine immunisation contacts has not yet been fully realised.
- For sustainability, integration of vitamin A with routine immunisation services (that is, post-partum maternal doses of vitamin A given with first immunisation contact, and vitamin A given to children with measles immunisation at 9 months) is needed.

Challenges

1) Advocacy to increase implementation: Need to seize opportunities to promote vitamin A as part of the global effort to strengthen routine immunisation systems.

2) Monitoring and reporting: Of the 49 countries distributing vitamin A with routine immunisation contacts, only 21 countries provided vitamin A coverage on the WHO/UNICEF Joint Reporting Form for the year 2000, and only 10 had coverage above 80%. This points to difficulties in implementation and monitoring which partners (particularly WHO and UNICEF) need to address through strong technical support.

3) Collaboration with national nutrition programs: Successful implementation depends on good collaboration between national immunisation and nutrition programs. High-level Ministry of Health support is needed to ensure the active engagement of all departments and the development of a national plan for the elimination of vitamin A deficiency.

Looking to the future:

- Increasing immunisation-linked opportunities to deliver vitamin A: In April 2002, WHO-coordinated research trials commenced in Tanzania and Ghana to evaluate the expanded delivery of vitamin A with DTP 1, 2 and 3 contacts. (results expected in December 2003).
Enriching breast milk with vitamin A

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Vitamin A and child survival

Three meta-analyses of large field trials published almost a decade ago provided the rationale for public health interventions that increase vitamin A intake as strategies to reduce child mortality. Beaton et al. (1993) presented the best known of these and determined that improving vitamin A status reduced mortality in children aged 6–59 months by an average of 23%.

Two other important findings from this work are less well known. First, the mortality reduction resulted from improved vitamin A intake, not just from high-dose capsules – in fact the intervention with the greatest mortality reduction (54%) was low-dose weekly supplementation. Another field trial was based upon MSG fortification. Second, the mortality reduction was independent of age, that is the 23% reduction was constant across all ages from 6–59 months. Because mortality rates are higher in young children than in older ones, the constant 23% reduction means that more lives are saved in younger children. In the eight field trials analysed by Beaton et al. 70% of the lives saved were in children 6–24 months old (see Table I). The implication of this finding is that vitamin A interventions will have greater impact in terms of number of lives saved by focusing on younger children.

Vitamin A in breast milk

Since vitamin A is so important to child survival, it is surprising that children, even in rich countries, are born with virtually no stores of the vitamin. Thus breast milk, as the infant’s first and ideally only food, is absolutely critical to vitamin A nutrition. Having higher vitamin A concentration than almost all complementary foods, breast milk remains the primary source of the vitamin through the first twelve months, and in many countries remains a major source throughout the second year of life. An infant’s intake of vitamin A from breast milk is determined by the concentration of vitamin A in the milk and by the volume consumed.

The concentration of vitamin A in colostrum is particularly high but this decreases rapidly over the first two or three weeks of lacta-
It is not widely recognised that the vitamin A concentration of mature human milk is dependent upon the mother’s vitamin A status and her current intake of the vitamin. The concentration of vitamin A in mature human milk in developing countries is only about half that in developed countries (Newman, 1994). In areas where vitamin A intake is low and deficiency is common, breast milk will not necessarily provide sufficient vitamin A to meet the needs of the infant, even when the breast-feeding practices of the mother are ideal. Reviewing the importance of maternal vitamin A status for young children, Underwood (1994) concluded that subclinical vitamin A deficiency problems were becoming manifest from six months of age forward, even among breast-fed infants, because lactating women had inadequate vitamin A status. The breast milk may have provided enough vitamin A for immediate functional requirements of the infant including growth, but not enough to build any stores. Building a store of the vitamin by six months is a key protection for the infant against infections such as measles and diarrhoea that deplete vitamin A at a time when intakes of the vitamin may decrease.

The volume of breast milk consumed by the infant is determined by the breast-feeding practices of the mother. Exclusive, frequent breast-feeding for six months will result in the greatest consumption of milk and should be encouraged. In addition to increasing intake of vitamin A, increasing breast milk consumption enhances protection against illness by providing immune factors and a hygienic source of nutrition. Reducing the incidence of infections improves vitamin A status because infections, in addition to causing losses of vitamin A, decrease food intake, and also interfere with vitamin A absorption (Linkages: Facts for Feeding, Breastfeeding and Vitamin A, 20001).

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Mortality rate/1000</th>
<th>Lives saved/1000 covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>6–11</td>
<td>27.8</td>
<td>6.2</td>
</tr>
<tr>
<td>12–23</td>
<td>25.0</td>
<td>5.8</td>
</tr>
<tr>
<td>24–35</td>
<td>12.0</td>
<td>2.8</td>
</tr>
<tr>
<td>36–47</td>
<td>4.8</td>
<td>1.1</td>
</tr>
<tr>
<td>48–59</td>
<td>4.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table I. Impact of age and mortality rate on vitamin A effect expressed as lives saved per 1000 children covered (data from Beaton et al., 1993)

Figure 1: Prevalence of adequate breast milk vitamin A, by period of lactation, Guatemala Sugar Fortification, (adapted from Arroyave et al., 1979).
Effective strategies for enriching breast milk with vitamin A are available

Three options for enriching breast milk are available: postpartum dosing, fortification, and increasing diet diversity. At this time, the evidence of effectiveness for the first two of these is stronger than that for the third.

a) Postpartum dosing

Postpartum dosing increases breast milk vitamin A primarily by improving the vitamin A status of the mother. It is a critical component of a comprehensive vitamin A strategy that often does not receive the focussed effort needed to achieve adequate coverage. Combined with appropriate breast-feeding practices it will ensure that breast milk provides adequate vitamin A to both meet the immediate needs of infants and establish the stores needed to support survival in the crucial second six months of life (MOST FAQ 4). Placebo-controlled studies of the impact on breast milk retinol by single postpartum doses of 200,000 IU to 300,000 IU have been conducted in Bangladesh, India, Indonesia, and Thailand. Larger and longer lasting improvements in milk vitamin A content and vitamin A status of infants were observed when higher doses were used. The lack of impact on vitamin A status of infants reported from some studies using 200,000 IU has been attributed to the dose being inadequate. Soon to be released IVACG guidelines will recommend doubling the current postpartum dose to 400,000 IU (two doses of 200,000 IU at least one day apart) delivered as soon after delivery as possible, but not more than six weeks after delivery (see SIGHT AND LIFE Newsletter 1/2001, pages 20–23).

b) Fortification

Consumption of foods fortified with vitamin A increases breast milk retinol both by increasing every day intake of the vitamin and by improving status. The efficacy of increasing breast milk vitamin A through fortification of sugar was demonstrated in a controlled study in El Salvador by Arroyave et al. (1974). The effectiveness of this approach was demonstrated subsequently through an INCAP study evaluating the impact of the national sugar fortification program in rural Guatemala (Arroyave et al., 1979). Figure 1 shows the dramatic impact fortification had on breast milk vitamin A. In several studies authors have observed vitamin A concentrations in milk decreasing with duration of breast-feeding and this has been interpreted as the vitamin being “drained” from maternal stores. By presenting the vitamin A levels in milk by period of lactation, Arroyave et al. identified that the greatest impact was in mothers who had been lactating for 5–6 months, the group that had the

1 Available at the Linkages web site at www.linkagesproject.org
2 See Frequently Asked Questions on the MOST Project website at www.mostproject.org

Prolonged breastfeeding.
lowest vitamin A levels at baseline. The INCAP evaluation also documented increased concentrations of serum retinol among preschool children resulting from this national fortification program (Arroyave et al., 1981). In a study in Indonesia, MSG was fortified with vitamin A and marketed through ordinary channels in five “program” villages. Five nearby villages served as controls. After 12 months of intervention, serum and breast milk levels of vitamin A had increased dramatically in the program villages but did not change in the control villages (Muhilal et al., 1988).

c) Increasing diet diversity

Interventions that increase diet diversity are appealing because they will improve overall food security and the intake of many, not single, micronutrients. However, the evidence of their effectiveness, or even efficacy, is not strong. A recent compilation of experiences by Helen Keller International/Asia-Pacific (2001) highlighted the broader benefits of promoting homestead food production including income generation and nutritional benefits for women. Homestead food production in Bangladesh was shown to increase intakes of vitamin A in both women and children, but “harder” biochemical evidence of effectiveness is not yet available. Ruel and Levin (2000) reviewed the literature on the impact of food-based strategies on vitamin A deficiency but reported being unable to find data with sufficient scientific rigor to allow firm conclusions to be drawn about effectiveness. The lack of strong evidence that these strategies might reduce vitamin A deficiency does not necessarily mean that the interventions have no impact, but rather that these impacts are extremely difficult to measure. Recent small studies in Tanzania (Lietz et al., 2000) and Honduras (Canfield et al., 2001) have demonstrated the potential benefits for vitamin A status of supplementing pregnant and lactating women with red palm oil. Diet-based strategies hold enormous potential for broadly-based benefits and warrant further investments for their development and rigorous evaluation.

Conclusion

Enriching breast milk with vitamin A and promoting exclusive breast-feeding for the first six months are critically important components of any integrated intervention to reduce mortality resulting from vitamin A deficiency during the period when mortality rates are very high.

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Vitamin A, growth faltering in infancy and gut integrity

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Summary

It is well established that vitamin A is needed for epithelial differentiation, the production of goblet cells and the maintenance of an effective epithelial barrier against pathogenic attack. Studies from The Gambia now suggest that failure to maintain the integrity of the epithelia in the gut is closely linked to the widely observed problem of growth faltering. The events leading to this discovery are outlined in this short review.

Growth faltering is a common problem in socially deprived areas of the world and may be caused by a combination of factors. Infection has been implicated and diarrhoea is often one of the first signs. If diarrhoea is accompanied by mucosal damage then catch-up growth following an episode cannot occur until the injury is repaired.

We studied the gut integrity of 120 Gambian infants using the dual sugar permeability test. Infants were recruited at 2 months of age and followed for the next 13 months. Gut integrity was measured monthly and we found the integrity of the mucosa progressively deteriorated following the introduction of weaning food. Seasonally, the data showed that integrity was least impaired from April to June, coinciding with the time of maximum vitamin A (VA) intake – the mango season. The results suggest that VA status may influence gut integrity during the vulnerable weaning period.

Growth faltering

Growth faltering is a common problem in many developing countries and in socially deprived areas throughout the world. Data from The Gambia illustrates facets of the problem which are common to many countries. Infants are born with a mean expected weight-for-age of approximately 93%, which improves during the first 3 months of life. However, between 3 to 6 months a precipitous fall in mean expected weight- and length-for-age occurs, which stabilises at about 75% of expected values when the infants are 12 to 15 months of age (Figure 1) (1). The precipitous fall in weight and length gain, known as growth faltering, is associated with the introduction of complimentary weaning foods and closely linked to worsening gut integrity. The process appears to be complex, and causes suggested have included disease, feeding patterns, specific nutrient deficiencies, growth factors and/or psycho-social conditions.

Figure 1: Growth performance of Gambian infants expressed as percent of expected weight-for-age according to NCHS standards.
Infection and growth faltering

Infections and infestations have been implicated as some of the main causes of growth faltering. In overcrowded, unhygienic or deprived environments, even trivial acute infections following in quick succession may not allow children sufficient time for catch-up growth between episodes of disease. In developing countries, conditions such as malaria, diarrhoea, respiratory infections, Helicobacter pylori and rotavirus are commonly associated with growth faltering.

Diarrhoea, in particular, is often one of the first signs of the onset of growth faltering and usually occurs shortly after weaning foods are introduced. Episodes of diarrhoea cause short-term growth faltering in both weight and height and the ability of children to catch-up with their expected growth trajectory after such episodes can vary depending on the cause of the diarrhoea.

It is important that diarrhoea be regarded as a symptom not a disease. The pathophysiology of diarrhoea differs according to the “cause”, hence the impact of the illness on weight and height growth and the extent of mucosal damage will also vary. Episodes associated with a systemic inflammatory reaction can result in severe growth faltering during the acute phase of the illness but in the absence of mucosal injury, catch-up growth could be rapid. If the diarrhoea is accompanied by mucosal damage then full catch-up might not be expected until the injury is repaired.

Measuring mucosal damage using the dual sugar permeability test

Little is known about the time taken for restoration of normal mucosal structure and function following injury because of difficulties of measurement. However, non-invasive techniques based on the permeation of poorly absorbed, non-metabolised sugars are available methods of assessing the integrity of the small intestine. Most of my own experience in this field has been using two sugars, lactulose and mannitol. The sugars permeate the mucosa by unmediated diffusion and the rate is determined by molecular size (cross-sectional diameter not weight) of the probes, lactulose is 9.5 Å in size and permeates more slowly than mannitol (6.7 Å). The differential excretion, following an oral dose of two sugars of differing molecular size, can be used as a measure of mucosal integrity.

Under normal circumstances, as a child grows, the surface area of the gut increases, the amount of mannitol absorbed and excreted into urine increases and the ratio of lactulose:mannitol (L/M) decreases. In industrialised countries, the L/M ratio of a normal infant will fall from 0.12 to 0.02 over the first 12 months of life. In the presence of disease, however, bacteria attach to the mucosal surface and alter cell permeability. The villous structure of the gut becomes flattened and the surface area decreases. In these circumstances, the amount of mannitol absorbed and excreted decreases and the L/M ratio increases.

Intestinal disease, growth and the dual sugar permeability test in Gambian infants

To explore the relation between intestinal disease and growth performance, the dual sugar permeability test was used in a prospective longitudinal study in The Gambia (2). Infants (n=120) were recruited as they reached 2 months and followed through the next 13 months to 15 months of age. Weight and length were measured monthly and morbidity was recorded weekly. Gut integrity was measured using the dual sugar permeability test on a monthly basis. Briefly, the test involved giving a solution of 400 mg of lactulose (disaccharide) and 100 mg of mannitol (monosaccharide) in 2 ml of water. Children received 2 ml per kg
body weight up to a maximum dose at 10 kg weight. Urine was collected over the following 5 hours using a urine bag. Urinary lactulose and mannitol were measured using enzymatic techniques and the L/M ratio was calculated as an expression of mucosal integrity.

Results showed that for the first 3 months of life, growth was normal as expected (Figure 1). Beyond this, both weight and length deteriorated and by 14 months of age, mean length and weight Z-scores (±SD) were −2.13 (±0.88) and −2.03 (±0.99), respectively (i.e. approximately 75% of expected values for reference children of the same age).

Intestinal permeability ratios were normal and very close to those of infants of similar age in the UK (L/M=0.12) up to 3 months, but beyond this age the L/M ratio rose progressively, peaking in the 9–12 month age group (L/M=0.5). Intestinal permeability to lactulose did not change with increasing age suggesting that the main alteration in the L/M ratio was due to a change in mannitol. There was a marked decline in mannitol absorption from 3 months of age onwards and the reduction in mannitol uptake indicated a decrease in mucosal absorptive area as a result of partial villous atrophy. The results imply that a gradual deterioration in small bowel mucosa took place throughout the first year of life and this deterioration strongly coincided with poor growth. Statistically, the relationship can be explained by age-corrected regression equations between change in weight (kg/month) or length growth (cm/month) and the log of the permeability ratio (approximately 1000 data points), which suggested that ~40% of growth faltering in both weight and length can be explained by abnormal L/M ratios. The effect of diarrhoea on gut integrity was short-term and not significant. Abnormal permeability ratios were measured in 700 out of 922 tests (76%) but infants only had diarrhoea for 14% of the time. When regression equations between change in weight growth (kg/month) and percentage of time with diarrhoea were calculated, no significant relationship was found. Our data therefore showed no long-term relationship between diarrhoeal disease and growth but that a high prevalence of intestinal damage was a major determinant of poor growth.

Vitamin A and gut integrity

VA is known to play a role in maintaining integrity of epithelial tissues such as the gut mucosa and in assisting in the body’s response to inflammatory stress. In The Gambia, there is a marked seasonal fluctuation in the availability of VA, as most VA is derived from β-carotene during the mango season. Therefore, in view of the seasonality of VA intake, the longitudinal data on gut integrity and growth were re-analysed (3).

Figure 2 shows a combined histogram in which changes in growth rate (mean monthly...
change in weight z-score) and gut integrity (L/M ratio) were plotted against month of the year. Intestinal permeability was found to be least impaired from April to June, the time of maximum VA intake. The poorest L/M ratios were found during the rainy season (July to September). The data therefore suggested that gut integrity and growth can be explained in terms of food availability (April to June) and disease prevalence during the rainy season (July to October) (4).

During November the main harvest occurs and food is plentiful until the end of May. The dry season is from November to May and is the healthiest time for Gambian people, although respiratory disease and rotavirus do occur during this time period. Rain falls from June to October and coincides with the period of hardest work in the fields and of the highest prevalence of diarrhoea and malaria. Figure 2 shows the greatest weight loss and poorest gut integrity occurring between June and September. Food is also in short supply from June to September and miscellaneous leaves may be used to make sauces to supplement the main staple. The leaves probably supply some VA but by far the largest supply of VA is obtained during the mango season (April to June), consequently the VA supply of carotenoid-rich food is very seasonal. In this context it is of interest to note that Quadro et al. working in Brazil infants found a strong inverse correlation between serum vitamin A and urine L/M ratios ($r=-0.46, p=0.12$) and a highly positive correlation between serum retinol and urinary mannitol ($r=0.66, p<0.01$) (7).

The first results confirm our observations of better gut integrity in Gambian infants during the mango season, and that poor VA status is associated with reduced mucosal surface area.

Additional data illustrating the close link between infection and gut integrity was obtained from measuring an acute phase protein, $\alpha$-1-antichymotrypsin (ACT). Figure 3 shows that the prevalence of sub-clinical infection was at its lowest from April to June corresponding to the time of best gut integrity, most rapid growth and also with the plentiful supply of mangoes.

Role of vitamin A in maintaining gut integrity

Although VA deficiency is not considered to be a clinical problem in The Gambia, several studies have shown large seasonal fluctuations in plasma retinol concentrations and in the dietary intake of VA (5, 6). Fluctuating plasma values in Gambian infants indicate that VA status may not always be adequate to prevent the deterioration in gut permeability observed during weaning. However, we do not know
what causes the initial loss of gut integrity. Before the introduction of weaning foods, Gambian infants have normal gut integrity and growth is near to normal, but both deteriorate subsequently. Very few cases of abnormal gut integrity are found in Western infants, but bacterial contamination of weaning foods is much lower than in The Gambia and nutritional status in Western infants is probably better, hence both factors are probably important in determining gut sensitivity to damage. The introduction of locally-produced weaning foods to an immature infant may therefore initiate tissue damage, perhaps due to dietary antigens in combination with bacterial contamination leading to inflammation, increased bacterial translocation and an increased risk of systemic infection.

Gambian infants are fed a form of cereal gruel made from millet or rice as a first weaning food. Food antigens like lectins, occurring in common dietary staples such as cereal grains and legumes, can have potent anti-nutritional properties, influencing the structure and function of both enterocytes and lymphocytes. Lectins are glycoproteins which can bind to the surface glycans on gut brush border epithelial cells causing damage to the base of the villi, disarrangement of the cytoskeleton, increasing endocytosis and shortening of the microvilli resulting in abnormal permeability. Such damage to the mucosa may facilitate the passage of food antigens and pathogenic bacteria. Once damaged a new bout of infection or further exposure to dietary antigens would prevent repair and precipitate further damage.

Food availability during the dry season probably stimulates growth but the increase in dietary VA may be particularly important in reducing levels of infection as reflected by the ACT concentrations (Figure 3). The lowest monthly mean ACT was in April, the height of the mango season, and the ACT values for April, May and June were not significantly different. The L/M ratios mirrored the ACT concentrations. The mechanism by which VA influences infection is not clear. A direct effect of VA on immune cells is possible but gut tissue is unique in being able to use nutrient from both the serosal and luminal side of the mucosa. It is possible that dietary VA may act directly on the gut enterocytes to restore integrity thereby preventing further bacterial translocation and increasing resistance to further infection.

Conclusion

The gut integrity of infants in developing countries is often abnormal and this is closely linked to failure to grow. We found biochemical measurements of gut integrity were best at the time of year when dietary VA was most abundant. However, the mechanism for this interaction is not known. VA may have a direct effect on gut cells or increased intake may increase circulating concentrations of retinol, which may have systemic effects. Vitamin A is also known to be important for the proper functioning of the immune system. In infants the immune system is immature, but undoubtedly developing rapidly during the first 12 months of life. Interaction between immune tissue, vitamin A and gut integrity has yet to be fully described.

References

What makes a successful supplement distributor?

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Many countries now have successful ongoing community-based vitamin A supplementation programs. The increased emphasis on these programs has been fueled by the demonstration that such programs can improve child survival in environments where vitamin A deficiency is prevalent. However, the real challenge now lies in how to sustain high supplement coverage over time in countries where fortification or promotion of vitamin A-rich foods that can impact on status are unlikely to be appropriate strategies for many years to come. Community-based supplement distributors can play an important role in motivating participation in supplementation programs, and in creating the demand for such programs within the community. What attributes and circumstances make some distributors more successful than others in producing high coverage for supplementation programs?

One study addressed this question using data about male village-based distributors and their coverage rates within the bi-annual government supplementation program in Aceh province, Indonesia, in 1983 (1). This study found that the most successful distributors were farmers with minimal education, and that smaller villages further from the main roads and without any stores had higher coverage that larger, more urbanized villages. The study did not identify any characteristics of the program participants that resulted in higher coverage.

Maternal supplementation with iron folate, vitamin A or other micronutrients poses a different set of coverage challenges since daily, or at least weekly supplementation is most appropriate from both a safety and efficacy perspective during pregnancy. Identifying the characteristics of a successful distributor is likely even more important for sustaining coverage with a more frequent dosing regimen than it is for the twice yearly campaign style approach that supports a child survival impact (2). We tried to answer the question of what makes a successful antenatal distributor in the context of a community trial to examine the impact of weekly maternal vitamin A or β-carotene supplementation on maternal and infant health and survival in Sarlahi district, Nepal (3).
The Nepal Nutrition Intervention Project Sarlahi (NNIPS) is a series of randomized community trials examining the role of different micronutrient supplements on health and survival of women and children in a rural subsistence environment in South Asia. The first trial examined the impact of thrice yearly large-dose vitamin A supplementation on child survival (2). The second of these trials involved weekly supplementation of 45,000 women of childbearing age and its impact on maternal and infant survival (4), (5). The third and fourth trials examine the impact of daily supplements of other micronutrients for women and children, respectively.

To undertake weekly supplementation in the second study, we identified and trained a cadre of over 400 local village-based women distributors who would be responsible for supplementing a group of an average of 110 (range from 32 to 292) women in their neighborhood. The distributor visited each woman on her list weekly and gave the supplement directly to her. She then recorded whether the supplement was taken, whether the woman refused to take it, or whether no supplement was given because she was unable to find the woman at home during that week. Distributors spent about 4 hours per week delivering supplements, and were paid a relatively small amount of monetary compensation, the equivalent of USD 15-20 per month. The employment was part-time and the timing flexible, so as to allow the distributors to fulfill their household obligations, and to visit participants when they were most likely to be at home. In addition, the distributors met every Friday to review their work, discuss problems and their solutions, hand in completed forms, receive new forms, report pregnancies and deliveries, and replenish supplement stores. Supplementation continued for three years, from 1994 through 1997.

All women who applied for the job of supplement distributor were interviewed before any employment decision was made. Demographic and socio-economic characteristics were obtained from each applicant. Characteristics of the neighborhood for which the distributor was responsible were identified by project supervisors prior to the start of the trial. The coverage level for each distributor was defined as the percentage of doses actually

<table>
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<tr>
<th>Table I. Predictors of low and high weekly supplement coverage, Sarlahi, Nepal, 1994-1997</th>
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<td>Low coverage</td>
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<td>&lt; 50%</td>
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<td>Weekly market present</td>
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<tr>
<td>Literacy</td>
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<td>Low socio-economic status</td>
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<td>Age &lt; 20 years</td>
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<td>Mean hrs/week housework</td>
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<td>Mean no. women supplemented</td>
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One of the over 400 local village-based women responsible for supplementation and collection of data in Sarlahi, Nepal.
consumed out of the total number of possible doses for all women on the distributor’s list over the duration of the trial. The coverage ranged from 16% to 86%, with the average being 61% of all possible doses actually consumed. High coverage was defined as 70% or more, and low coverage as less than 50% of all possible doses consumed.

As shown in the Table I, distributors with high coverage were less literate than those with low coverage (98% versus 94%). Women with high coverage reported spending one hour less per week distributing supplements, but they also had an average of 13 fewer women to supplement each week, compared to those with low coverage. Distributors with low coverage reported doing 5 more hours per week of housework than those with higher coverage. Villages with a weekly market had lower coverage than those without such a market. A measure of household socio-economic status that included ownership of land, cattle, ox-carts and type of housing was not found to be associated with either low or high coverage, after taking literacy, age, workload and presence of a weekly market into account.

This study found strikingly similar characteristics of predicted coverage among village-based male distributors of twice yearly vitamin A supplements in Indonesia as among female distributors of weekly supplements in Nepal. Those with lower levels of education had better performance than those with more education, perhaps because they could interact more comfortably with the program participants, whose level of education was closer to their own. Similarly, coverage was lower in communities that had a weekly market or had village stores, perhaps reflecting villages where there were more distractions for participants and distributors alike. Work conditions were somewhat associated with low coverage in that those with more competing work demands at home had lower coverage rates. Those with low coverage also had more women to supplement, and consequently reported spending more time distributing supplements. A lower workload at home and on the job did not appear to predict high coverage, but a higher workload did seem to have a negative impact on coverage.

There is increasing interest by UNICEF and others in daily antenatal micronutrient supplementation. Creative ways of designing, implementing and sustaining such programs at the community level will be needed if this type of supplementation can be shown to have a positive impact on maternal and infant health. Studies like this can be helpful in identifying the types of community workers who are most likely to be successful program implementers.

References


Vitamin A program in India – why the controversy?

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A Vitamin A supplementation program has been in operation in India since 1970. Under this programme, sponsored by the Ministry of Health and Family Welfare (GOI), children aged between 9 months to 3 years are given six monthly doses of vitamin A, and the administration of the first two doses is linked with routine immunisation. Although the supplementation programme was initially started as a short-term measure to prevent blindness in children, it has been going on for the last three decades and its continuation has become a subject of national debate. The recent reports of child deaths after the administration of vitamin A during a mass campaign in Assam triggered a fresh controversy over the vitamin A program (1–3). The controversy is not confined to the campaign approach for vitamin A distribution, but the very existence of vitamin A deficiency (VAD) as a public health problem in India and the need for supplementation are questioned (4). Such debates often confuse the policy makers and cause a set-back to the ongoing programme, which is already suffering from tardy implementation. An attempt is made here to review the available data and answer some of the questions raised by the critics.

Is VAD a public health problem in India?

Clinical deficiency: Severe deficiency of vitamin A is known to produce corneal xerophthalmia/keratomalacia and blindness in children. Such cases are rarely seen in a community survey and require a large sample size for accurate estimates of prevalence. Hospital records show a significant decline in keratomalacia cases in the last two decades (5) and clinicians vouch for its rarity (6). However, clinical signs of mild xerophthalmia like Bitot’s spots and night blindness are still seen among children in deprived communities. The first repeat survey of the National Nutrition Monitoring Bureau (NNMB) conducted during 1988–1990, in the same villages as those surveyed earlier during 1975–1979, showed that the prevalence of Bitot’s spots has declined from 1.8% to 0.7% (7). But the second repeat survey conducted in 1996–1997 showed no further improvement (8) and the prevalence is still above 0.5% (Figure 1), the WHO cut-off level for a public health problem. The national averages do not give a full picture because the prevalence rates vary widely, not only between the states but also within a state. Nevertheless, they provide useful information on time trends.

Figure 1. Prevalence of Bitot’s spots in pre-school children.
India Nutrition Profile (1999) is often quoted to show low prevalence of clinical deficiency in the population, but the prevalence rates of Bitot’s spots published in this report cannot be used because they are based on pooled data of all age groups (9). In a few states like Haryana, Assam and Orissa, for which the data on preschool children are given separately, the prevalence is relatively higher. A survey in five north-eastern states (Assam, Bihar, Orissa, West Bengal and Tripura) showed the prevalence of Bitot’s spots to be 0.7–2.2% and that of night blindness 1.2–4.0%, indicating a public health problem in all the five states (10). The survey also showed high prevalence of night blindness among pregnant women (3.2–16%). The district-wise data collected in the state of Uttar Pradesh showed Bitot’s spots in 5.6% of children (11). There was a wide variation in the prevalence between the districts and even within a district from cluster to cluster, ranging from 0.2% to 13.7%. A recent survey of the Indian Council of Medical Research (ICMR) (1998) covering 16 districts, mostly in northern and eastern regions, showed that the prevalence of Bitot’s spots ranged from 0–4.7% and that of night blindness from 0.4–4.8% (12). Low prevalence of Bitot’s spots observed in a number of districts surveyed is used to argue that VAD is no longer a public health problem, but the prevalence of night blindness, though a subjective sign, cannot be ignored. If both indicators are used, VAD is a significant problem in 7 districts and if the prevalence of corneal scars (>0.05%) is also considered, 11 out of 16 districts have a significant problem. It must be recognised that all the available clinical and biochemical indicators are subject to limitations. And therefore WHO has recommended that at least 2 indicators should be used for assessing the vitamin A status of a population (13).

Sub-clinical deficiency: It is well recognised that xerophthalmia represents an advanced state of deficiency. In communities where clinical signs of VAD are seen, sub-clinical deficiency can be expected to be more common. Large-scale data on serum retinol levels are not available to assess the extent of biochemical deficiency. But the community studies carried out in Andhra Pradesh (14), Tamilnadu (15) and Uttar Pradesh (11) indicate that 30–50% of children have retinol levels below 20 µg/dl, the WHO cut-off indicating a public health problem. These observations are corroborated by the dietary data. Green leafy vegetables, milk and milk products are the major sources of vitamin A in Indian diets. Surveys carried out in different parts of the country show low consumption of these foods (16). The average intake of vitamin A is around 300 µg in women and 120 µg in children, and more than 80% have intakes less than 50% of the recommended dietary allowance (RDA).

Thus the available data show that though the severe forms of blinding malnutrition have declined in the last two decades, milder grades of VAD still exist in many parts of India. National surveys provide only state level information and the limited data available from district surveys show a wide variation between the districts. The magnitude of the public health problem varies depending upon the areas surveyed and the indicators used.

Is mild VAD a public health concern?

Apart from causing ocular signs VAD is known to produce systemic changes, of which the most significant effects are alterations in epithelial integrity and immune status. Evidence for an association between VAD and infection was documented by Scrimshaw et al. some 30 years ago (17). Since then, supporting data from animal experiments and observational studies in humans have been published (18). Positive association between mild xerophthalmia and the risk of respiratory infection was reported in Indian children (19), while Indonesian children showed an association with both diarrhoea and respiratory infection (20). Children with clinical signs of VAD were found to be at greater risk of death than those without (21).

A subsequent intervention trial in Indonesia showed a 34% reduction in mortality among children receiving six monthly doses of 200,000 IU vitamin A (22). This effect was seen even in children without clinical signs, highlighting the importance of sub-clinical deficiency. Controlled trials in other countries also resulted in a significant reduction in mortality—19% in Ghana (23) and 30% in Nepal (24). The reduction was attributed to a fall in deaths related to diarrhoea and measles. However, studies in India (25) and Sudan (26) using the same dose showed no effect. Trials of weekly supplements in India (15) and food fortification in Indonesia (27) showed higher reduction
in mortality, indicating that the beneficial effect was due to improvement in vitamin A status by whatever means. A meta-analysis of data from eight intervention trials in pre-school children showed an average of 23% reduction in total mortality (28). However, this conclusion has been challenged because the results are not consistent (29). Subsequent studies in infants less than 6 months old have also shown variable results. Administration of a single dose of 50,000 IU of vitamin A to neonates in Indonesia resulted in a significant reduction in mortality risk (30), while a similar trial in Nepal showed no effect (31). In a WHO multicentre trial in Peru, Ghana and India, vitamin A supplements (25,000 IU) given along with DPT immunisation at 6, 10 and 14 weeks did not affect morbidity or mortality (32).

There are a number of potential explanations for the variability in results across trials. These include age of the children and the dosage schedule; smaller and frequent doses seem to be more protective than large periodic doses. High prevalence of infections resulting in vitamin losses and depletion of stores can shorten the protective period of supplements. Vitamin A is likely to have a greater effect in areas where VAD is highly prevalent. Other factors like concomitant nutritional deficiencies and access to healthcare can also modify the mortality effect. Thus the impact of vitamin A may vary depending upon environmental conditions. An average 23% reduction in mortality may not be applicable to all ecological settings, but the positive impact of vitamin A in some situations cannot be denied.

After reviewing the studies on vitamin A and mortality, a National Consultation on the Benefits and Safety of Vitamin A Administration, held in New Delhi in September 2000, concluded that the data are “not robust” enough to recommend vitamin A supplementation for the purpose of mortality reduction in children (33). In India, infant deaths comprise up to 80% of under-five mortality in some states and therefore it is argued that an intervention with possible effects only beyond infancy will not be of much value for reducing child mortality (34).

It is true that vitamin A is not a panacea for all the illnesses that affect children in developing countries. However, the need for improving vitamin A status cannot be denied. The fact that a majority of the population subsists on inadequate diets, with...
vitamin A intakes less than half the recommended level and a significant proportion of children having clinical and sub-clinical deficiency is a matter of public health concern. The aim of the National Nutrition Policy is not only to prevent blindness in children, but also to eliminate VAD as a public health problem. There is thus a need to accelerate the intervention efforts to achieve the goal.

What are the appropriate strategies for VAD control?

Multiple approaches including vitamin A supplementation, food fortification, dietary diversification and public health measures have been suggested for prevention and control of VAD. Although pilot projects have demonstrated their efficacy and feasibility, large-scale implementation of these programs have met with limited success. This has led to considerable debate as to which of the interventions is most cost-effective and sustainable. The choice is not simple. Each one has its strengths and limitations. For maximum impact and efficacy, each strategy should be considered in the context of a country’s needs/priorities and its capacity to implement and sustain an intervention.

Vitamin A supplementation is the quickest way of improving the vitamin A status of a population and is the choice of strategy in areas where the problem is widely prevalent. Improving the diet, even if it is difficult to achieve in the short term, is of paramount importance, as it contributes to improving the overall nutritional status. Food fortification with vitamin A has proved to be an effective strategy for reducing VAD in some countries. A right mix of interventions tailored to the local circumstances is more likely to succeed in achieving the objective.

In India, the National Vitamin A Prophylaxis Program was started with the primary aim of reducing blindness in children, which was a significant problem at that time. Under this program sponsored by the Ministry of Health and Family Welfare, children aged between 1 and 5 years were given oral doses of 200,000 IU vitamin A every 6 months. Evaluation studies in the late 1970s revealed poor implementation of the program and inadequate coverage in most of the states (35). The program was reviewed several times since then, and efforts were made to correct existing deficiencies. Currently, vitamin A is given only to children aged less than 3 years who are at greater risk, and administration of the first two doses is linked with routine immunisation to improve coverage. 100,000 IU of vitamin A are given along with measles vaccine at 9 months of age and 200,000 IU with DPT booster at 15 months (36).

In recent years, there has been considerable debate on the continuation of the vitamin A supplementation program. Since keratomalacia/blindness is no longer a significant problem, it is argued that there is no need for supplementation and that milder forms of deficiency can be tackled through alternate strategies aiming at dietary improvement (29). It is true that dietary intervention is the most logical approach. Right from the beginning, supplementation was conceived as an interim measure to be discontinued once the dietary improvement was achieved. Unfortunately, the dietary situation has not changed in the last three decades (Figure 2). Vitamin A intake of children is less than half the RDA even today, with a significant proportion of them having clinical evidence of deficiency. Under these circumstances, it is not wise or ethical to withdraw the benefits of supplementation.

There is also a controversy about the universal approach currently adopted, because VAD is not uniformly spread throughout the country (29, 34). The cost of a vitamin A supplement is estimated to be Rs. 3.20 (0.06 USD) per child per year, which is a negligible proportion of the total health expenditure (37). A selective approach, covering only the districts where VAD is a public health problem, would be a more cost-effective strategy. It requires district mapping for VAD signs all over the country. This is possible if the states take responsibility for conducting surveys and monitoring the program. When such data are not available, priority should be given to backward areas, identified by the ecological indicators.

The mode of delivery of the vitamin has also been a subject of intense discussion. Under the national programme, children are given vitamin A along with routine immunisation (measles, polio and DPT). While the international agencies have been vigorously promoting supplementation linked with routine as well as campaign-based immunisation, it is regarded not as a short-term
measure but as a low-cost sustainable strategy to combat VAD in developing countries (38). Efforts are also made to expand the program to cover pregnant and lactating women, and infants below 6 months, though studies have failed to demonstrate clear benefits in these groups (39). These efforts have met great resistance in the Indian context. In recent years, Pulse Polio Immunisation (PPI) has been implemented as a national campaign, offering an opportunity for delivering vitamin A. But the Indian Academy of Paediatrics disapproved linking vitamin A with PPI, primarily due to lack of sufficient evidence for the benefit of supplements in infancy, chances of destabilisation of routine services and the fact that PPI is a temporary program (40). Of the two states which included vitamin A in the PPI campaigns during 1990–2000, improved coverage was achieved in Orissa but not in Uttar Pradesh due to poor logistic support (11). Considering the inconsistent results and the fact that PPI itself is coming to an end, the National Consultation on Vitamin A also recommended that vitamin A should not be linked with PPI. Instead, the ongoing program of supplementation linked with routine immunisation should be strengthened to achieve high coverage (>90%) for at least the first two doses (33). There is also a need to strengthen the education component of the program to improve the diet as a long-term goal.

Dietary improvement is, undoubtedly, the most logical and sustainable strategy to prevent VAD. Its contribution to improvement in overall nutrition justifies continued efforts in this direction. There is a general consensus on this at both the national and international levels. However, past efforts concentrated on supplementation and not much attention was paid to planning and implementation of food-based programmes. It is often stated that green leafy vegetables (GLV) and fruits are available in plenty during the season and well within the economic reach of even the poor (29). Availability alone, however, does not ensure programmatic success. This requires a change in dietary habits and increased access to vitamin A-rich foods. In recent years, efforts have been made to achieve these objectives through educational and horticultural interventions. However, these are mostly small-scale projects and are yet to be incorporated into national programmes. Even these projects have failed to demonstrate a significant impact on vitamin A status because they focussed on GLV as the main source of vitamin A (41). Bioavailability of β-carotene is lower from GLV than from other vegetables and fruits. Young children cannot consume leafy vegetables in sufficient quantities to meet the vitamin A requirement. Based on feeding trials with selected vegetables, a factor of 26 (instead of 6) has been suggested for conversion of β-carotene to vitamin A (42). However, this is debated because bioavailability of carotene varies widely and depends not only on the food source but also on the way it is prepared, as well as on the level of other dietary components like fibre and fat. A detailed discussion of this issue is beyond the scope of this paper. But it must be admitted that promotion of GLV alone is unlikely to eliminate VAD. Dietary diversification programmes must include a variety of vegetables and fruits as well as animal foods like milk and eggs. We should not just settle for something “cheap”, but make all efforts to improve the quality of diet.

What went wrong with the vitamin A campaign in Assam?

The reported deaths of over a dozen children and a large number of children falling sick after vitamin A administration during a mass campaign in the north-eastern state of Assam has caused considerable anxiety and concern among health professionals (1-3). The campaign was stopped immediately and an inquiry was set up by the Government. Some doctors blamed the stock of vitamin A supplied. However, testing of vitamin A samples from batches used in the campaign showed nothing wrong with the vitamin supplied (43). They conformed to the standards of quality specifications, so the reported adverse reactions were not attributable to the quality of the product supplied.

Some nutritionists have questioned the campaign approach adopted by the state, when the national guidelines recommend vitamin A administration along with routine immunisation (44). Unfortunately, the national immunisation coverage is also low (49%). According to NFHS-2, the national coverage for at least one dose of vitamin A (linked with immunisation) is only 30% and in Assam, it is even lower at 15.4% (45). Recently, some of the states
in India including Assam have initiated a vitamin A campaign, with UNICEF support, to improve the coverage. This is the third round of vitamin A distribution in Assam, and the first two rounds in this state as well as other states of Andhra Pradesh, Karnataka and Orissa were uneventful. During this round, UNICEF has replaced the traditional 2 ml spoons with 5 ml cups for administering vitamin A. It is possible that this switch in the method and inadequate training of health workers might have led to overdosing in some cases. Though the cup had 2 ml markings, health workers cannot be expected to measure the dose accurately, especially in a mass campaign where hundreds of children are covered in a day.

Administration of large doses of vitamin A is known to produce side effects like headache, vomiting and bulging fontanel in 1–2% of children, but these symptoms are mild and disappear within 48 hours (46). According to the newspaper reports, up to 15,000 children, out of the 3 million who received vitamin A during the campaign, became ill (47). This is much less than what is expected (up to 60,000) with vitamin A dosing.

The Assam episode started with the death of a two-year-old child from the Tea garden community, after consuming vitamin A. This triggered panic amongst the parents, and thousands of people rushed with their children to the nearest health centre, some of them complaining of fever, vomiting and diarrhoea. Normally, these symptoms do not attract mass attention. But the media has sensationalised the event in Assam leading to a wave of mass concern. All deaths and illnesses that occurred in children during the following week were attributed to vitamin A. There is no evidence that vitamin A will cause death even if a child had received twice the amount (400,000 IU). This is the dose recommended by the WHO for the treatment of xerophthalmia (48). Vitamin A programmes have been in operation for the past several years not only in India, but also in 60 other countries. So far, not a single case of death attributable to vitamin A dosing was reported. Lethal dose of vitamin A is not known, but a review of the case reports of children getting 300,000–900,000 IU do not suggest severe toxic effects that could be fatal (49). It is not surprising that the investigation conducted by the State Department of Health and UNICEF revealed that in most of the cases death was due to causes unrelated to vitamin A (50). These were cardiac failure, severe anaemia, high fever, foreign body aspiration etc. Considering the current mortality rate of 28/1000 in children aged 1–4 years (45), 15 deaths reported in the week following vitamin A administration are far less than the expected number.

Inadequate training of health workers, lack of supervision and negligence of children who developed symptoms might have contributed to the confusion. There are important lessons to be learnt from this episode. A community may lose faith in government-sponsored public health programmes if adequate precautions are not observed. Major precautions for vitamin A are avoiding massive doses in young infants and ensuring that the dose limit is not exceeded and that the administration is carried out by trained health workers under strict supervision. Adequate steps should be taken to educate the community about the benefits of vitamin A supplementation and the possibility of transient side effects. Extra precautions are needed for treating sick children. WHO has recommended that sick children who are at greater risk, particularly those with measles and severe protein-energy malnutrition should be given an additional dose of vitamin A (48). However, this approach can create problems if adequate precautions are not taken. If a child who is seriously ill dies after receiving the dose, vitamin A may be blamed as the cause of death (as it happened in Assam). Such cases should be referred to the nearest health centre for full treatment. Efficient management is crucial for success of any public health program.

Conclusion

VAD still exists as a public health problem in many parts of India and there is a need for continued efforts to improve vitamin A status of the population. It is unfortunate that the Assam episode led to so much controversy, putting an end to the vitamin A campaign even in other states. However, this should be viewed not as a set-back, but as an opportunity to strengthen the ongoing programme of supplementation linked with routine immunisation and accord higher priority to dietary approaches as a long-term sustainable solution.
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A combined approach to vitamin A deficiency in Thailand

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**Historical background: 1960–1990**

A survey conducted in 1960 by the United States Interdepartmental Committee on Nutrition for National Defense (ICNND) indicated that Thailand had a vitamin A deficiency problem (1). This was further confirmed by various community surveys and hospital records and led to the recognition of vitamin A deficiency as one of the seven national nutrition problems in Thailand’s Fourth National Economic and Social Development Plan (NESDP) during 1977–1981. Since the problem of vitamin A deficiency occurred primarily in pocket areas and its prevalence trailed behind those of protein-energy malnutrition, iron-deficiency anemia and iodine deficiency disorders, the national nutrition programs were not aimed specifically at vitamin A deficiency. Rather, vitamin A considerations were integrated into Thailand’s health and nutrition policies, plans and programs along with other nutritional problems. Under the Fifth NESDP (1982–1986), Thailand launched an intensive programme nationwide to reduce poverty, improve primary healthcare services, and increase household food security among rural poor families (2). Nutrition programs became a part of the Poverty Alleviation Plan, which linked together the activities of four major ministries: Health, Agriculture, Education and Interior. The strategies included programs such as growth monitoring, nutrition education, horticulture, supplementary feeding, school lunch etc. These efforts led to a marked decline in clinical vitamin A deficiency such that in 1985 the World Health Organization (WHO) classified Thailand as a country where vitamin A deficiency was not a public health problem, although sporadic cases might occur (3).

By the end of the fifth NESDP, case reports of xerophthalmia became rare and the country began to enter a transition from severe to moderate degrees of deficiency (4). This was confirmed by a 1990 prevalence survey of vitamin A deficiency in northern and northeastern Thailand which found no case of xerophthalmia, but approximately one-fifth of preschool children in these regions manifested low liver stores (relative dose response test) and abnormal conjunctival epithelium.

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*Figure 1. Ivy gourd plant (Coccinia indica).*
Since vitamin A deficiency problems in the north and northeast regions of Thailand are marginal, an intervention to promote consumption of locally available vitamin A-rich foods seems most appropriate. Despite the fact that vitamin A-rich foods such as yellow and orange fruits and vegetables, dark green leafy vegetables, liver, eggs and whole milk are readily available in most areas, they are underutilised due to such factors as consumption behaviour, seasonal variation and food appeal. Recent experience in northeast Thailand on the social marketing of the ivy gourd plant (Coccinia indica as shown in Figure 1), which is rich in pro-vitamin A carotenoids, demonstrated that significant changes in dietary habits can be achieved in a relatively short time (6). A follow-up study of the social marketing project showed a sustained improvement in the consumption of ivy gourd and other vitamin A-rich foods as well as dietary fat, leading to a significant increase in serum retinol concentrations in preschool children (7).

Xerophthalmia in lower southern Thailand: a stepwise and combined approach

Whereas the problem in the north and northeast regions appears to be marginal, a hospital-based record in Yala province, southern Thailand, during 1988–1991 revealed alarming evidence of 31 cases of xerophthalmia among 3–15 months old infants (8). These infants were diagnosed with severe protein-energy malnutrition together with diarrhoea and pneumonia. They received little or no breast-feeding and instead were given non-fortified sweetened condensed milk. A survey in 1992 in the lower southern region confirmed the seriousness of the situation among preschool children (2–6 years old) as evidenced by keratomalacia, low serum retinol and depleted liver stores (9). The survey helped to identify priority areas for interventions. To counteract the problem, the Ministry of Public Health launched a stepwise and combined approach.

Supplementation

A universal distribution of high-dose vitamin A capsules was launched in 1992 for all children under five years of age residing in the three southernmost provinces of Yala, Pattani and Narathivat. The capsules were supplied by the Task Force SIGHT AND LIFE and the Royal Thai Government. Vitamin A capsules were distributed every six months and the supplementation program covered the period from 1992 to 1998. Besides health workers, traditional birth attendants were also trained to administer these supplements. In addition, the Ministry of Public Health strengthened supportive measures such as maternal and child care, immunisation, control of infection etc through working closely with the regional and local health officers. These combined efforts resulted in no new reported cases of xerophthalmia (10).

Fortification

Consumption of non-fortified sweetened condensed milk was a common practice and an important risk factor for infants in the endemic area, especially among the families of migrant rubber plantation workers. This product was attractive, due to its readily accessible nature, low cost and convenience. To effectively curb the problem, the Ministry of Public Health officers in collaboration with researchers at the Institute
of Nutrition, Mahidol University (INMU), compiled a case for vitamin A fortification of all brands of sweetened condensed milk. The major thrust behind this movement was the indication that xerophthalmia involving the cornea occurred when the mothers or guardians changed from a fortified to a non-fortified brand of milk because of the lower cost of the latter. The rationale to fortify sweetened condensed milk with vitamin A was to prevent any child from becoming blind as a result of being fed this food because of lack of knowledge. A meeting to discuss this matter with the manufacturers resulted in a willingness to cooperate in the fortification program. In 1993, the Committee under the Food and Drug Administration passed a regulation that sweetened condensed milk be fortified with vitamin A at a dose of 330 retinol equivalents (RE) per 100 g (11).

**Dietary diversification**

In 1995, a survey of vitamin A deficiency among preschool children in the lower southern region revealed no new cases of xerophthalmia. In addition, vitamin A status improved markedly as evidenced by serum retinol concentrations and liver stores of vitamin A estimated by the modified relative dose response test (12). The severity of the situation had declined. Therefore, the Ministry of Public Health in collaboration with the INMU research team decided to plan a dietary diversification program to promote consumption of local foods rich in vitamin A. Since the lower southern provinces are inhabited by a Thai Muslim population, a formative research was first conducted to collect data on potential sources, availability and consumption patterns of indigenous food sources. These data were confirmed with mothers (Figure 2), child caretakers and community elders. The food-based research (1996–1999) emphasised two activities: recommendation of vitamin A recipes for consumption by pregnant women and develop-
opment of vitamin A-rich snack (local fish chips enriched with chicken or beef liver) targeted at children (13). The outcomes of both research components was adopted for promotion by the Health Promotion Center in southern Thailand as part of a special public event (Vitamin A Day), regular counseling and education programs (an example of education material for local health officers is shown in Figure 3). A follow-up survey is being planned this year to assess the situation.

The challenges

While the vitamin A deficiency problem in lower southern Thailand seems to be under control, a new challenge emerged in the upper northern Thailand.

In 1998, a hospital-based record in the northern region reported cases of xerophthalmia among the Karen hill tribe preschool children (Figures 4, 5) in remote villages. These children were admitted to the hospital with symptoms of severe diarrhea and/or pneumonia together with a moderate degree of protein-energy malnutrition. Based on the previous experiences in the lower southern provinces, the Ministry of Public Health immediately launched a distribution of high-dose vitamin A capsules in endemic areas together with efforts to strengthen other supportive public health measures. In addition, a committee at a provincial level has been formed to integrate related activities among various sectors of health, agriculture, education and interior ministries. The efforts, so far, have resulted in no new reported cases of xerophthalmia in the area. Besides, the Ministry of Public Health has also supported an ongoing research program in collaboration with the INMU research team to explore ways to improve diet and nutritional status in this population.

It is apparent that the challenges facing Thailand are to effectively prevent the eruption of sporadic cases of vitamin A deficiency and to successfully improve vitamin A nutrition among vulnerable populations in a sustainable manner. An ongoing effort to develop a surveillance system to include vitamin A, an incorporation of vitamin A into a multiple micronutrient intervention program, a setup of program monitoring and evaluation components etc are parts of the tasks undertaken by the Ministry of Public Health in collaboration with academic institutes like INMU and other partners to bring the vitamin A deficiency problem under control, once and for all.

References


Figure 5. The girl is blind in one eye. She had a VAD problem in younger years.
Progress and challenges in controlling vitamin A deficiency in the Philippines

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The Philippines has made fairly good progress in the control of vitamin A deficiency (VAD) in the past 15 years, from 1982 to 1998. The Food and Nutrition Research Institute (FNRI) of the Department of Science and Technology (DOST) has been conducting National Nutrition Surveys (NNS) every five years, starting in 1975, followed in 1982, 1987, 1993 and 1998. The 1993 NNS revealed that the prevalence of clinical signs of VAD, i.e. a combination of night blindness (XN) and Bitot’s spots (X1B), among preschool children (6–60 months old) decreased from 3.5% in 1982 to 0.50% in 1993 (Figure 1) (1-3). This figure is slightly lower than the 1995 World Health Organization’s (WHO) estimated overall prevalence for developing countries, which is 0.6% among preschool children (4). The trend showed a 2.5 percentage point (pp)-reduction over a 10-year period (or a 78% reduction in 10 years), better than the WHO estimated trend change of 0.43 pp/10 years for developing countries (Table 1) (5).

Most deficiencies are “sub-clinical” based on serum retinol concentration. The FNRI national prevalence estimate of deficient concentration (<0.35 µmol/l) among preschool children showed a decrease in proportion from 10.4% in 1993 to 8.2% in 1998 (6). However, this figure is higher than the 5% cut-off that identifies VAD as a public health problem. The sub-clinical VAD prevalence of deficient and low concentration (<0.70 µmol/L) has increased from 35.8% in 1993 to 38.0% in 1998 (Figure 2).

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Table 1. Trends in the prevalence of clinical signs of VAD in the Philippines

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Prevalence 1982</th>
<th>Prevalence 1993</th>
<th>Trend% (pp/10 years)</th>
<th>Change per 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total X1B, XN</td>
<td>3.20</td>
<td>0.50</td>
<td>-2.50</td>
<td>-78.0</td>
</tr>
</tbody>
</table>

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Sub-clinical VAD (<0.70 µmol/l) among preschool children is present in all of the 16 regions in the country (Figure 3). Further, eight provinces and five municipalities in Metro Manila have been identified as areas of high-risk regions (7). On the other hand, prevalence of sub-clinical VAD among pregnant women increased from 1993 to 1998 while that among lactating women

showed no change (Figure 4). Maternal night blindness (XN) of 10% was also reported in the 1998 survey (8).

A general decrease in the consumption of various foods is observed from 915 g/day in 1982 to 803 g/day in 1993 (7). Specifically, vitamin A-rich food intake in rural areas was only 74% adequate, mostly coming from food of plant origin (9). In addition, precipitating events, particularly diarrhoea, respiratory disease, measles, and protein-energy malnutrition are still the leading morbidity among preschool children in the country. In spite of these contributory factors that are associated with the occurrence of VAD, including the political and economic upheaval the country was experiencing over the years, vitamin A status improvement has been encouraging.

The nationwide fight against VAD begun in 1974 upon the enactment of the “Nutrition Act of the Philippines”, which mandated the formulation of the Philippine Nutrition Program (PNP) with the specific objective of controlling and preventing protein-energy malnutrition, VAD, iron deficiency anemia and iodine deficiency disorders. The national government agencies in partnership with non-government organisations, implemented a combination of direct and indirect nutrition interventions.


Figure 3. Prevalence of sub-clinical VAD (serum retinol <0.70 µmol/l) among children, 6 months to 5 years old, by region, Philippines, 1998 (source: Philippine Nutrition Facts and Figures, FNRI, 2001).
Nutrition program devolved to local government units

The government renewed its commitment to fight malnutrition and developed the Philippine Plan of Action for Nutrition (PPAN) in 1993. In the same year, the power and authority of the national department of agriculture, welfare and health, including their nutrition program, were devolved to the local government units (LGUs) (10). This means more than 1000 local chief executives of the provincial, municipal and barangay (village) levels will plan and manage their nutrition programs to solve their malnutrition problems. To strengthen the delivery system, local chief executives employ barangay workers with specific tasks and service areas: midwives (1:5000 population), barangay nutrition scholars (1 per barangay) and barangay health workers (1:20–30 households) to effectively deliver vitamin A and other nutrition services.

The NCP, a private, non-profit organisation, launched an intensive retraining program for field personnel to strengthen the autonomous status of the LGUs in implementing effective nutrition programs. SIGHT AND LIFE supported NCP’s Short Course Training (SCT) for the planning and management of a Barangay Program of Action for Nutrition (BPAN), which includes vitamin A interventions (11). The main purpose of the training is to equip and develop local nutrition program managers and village health and nutrition workers with updated knowledge and skills for

![Figure 4. Prevalence of sub-clinical vitamin A deficiency among pregnant and lactating women 1993 and 1998 (source: Philippine Nutrition Facts and Figures, FNRI, 2001).]

![Figure 5. Short course on vitamin A and nutrition with information, education and communication as the centerpiece.]
Impressions from the “Short Course Training on nutrition and vitamin A”

A: Introducing the use of the Nutri-Guide handbook as take-home reference IEC materials after the Nutrition Education among the mothers of malnourished children

B: Governor Pablo Garcia of Cebu Province, Chairman of the Provincial Nutrition Council, together with his Nutrition Action Officer and Dr Florentino Solon reviewed and approved the Cebu Plan of Action for Nutrition (CPAN) and committed to support the devolved local government unit in his province for capacity-building in the management of their barangay nutrition programs

C: Advocacy on a public-private partnership for a better nutrition program

D: Container gardening (in urban poor areas) of carotene-rich vegetables in coconut husk promoting the Food Always In The Home (FAITH) approach. From left to right: mustard (Brassica juncea), spring onion, swamp cabbage (Ipomoea batatas aquatica), and malabar nightshade or “alugbati” (Basella alba)

E: Cooking demonstration using nutritious recipes taken from Nutri-Guide

F: Fun-learning activities (puzzle game) on vitamin A

G: “Pabasa sa Nutrisyon” or nutrition-reading session of Nutri-Guide as a means of communicating nutrition information to mothers of malnourished children in the community
effective delivery of vitamin A and other nutrition services (Figure 5). The SCT has reached 26 provinces and cities and trained 435 provincial and municipal nutrition managers and 1230 village health and nutrition workers. Twenty-nine more provinces are targeted this year for SCT.

To support the LGUs' implementation of the nutrition program, heightened efforts were made on a nationwide scale for measles immunisation and vitamin A capsule (VAC) supplementation. A micronutrient day for VAC supplementation was observed in April and October of every year since 1993. The average coverage is about 87% or about 8 million preschool children each year.

The food fortification program was developed, which was started with the successful fortification of margarine with vitamin A (12). The manufacturer of the product was the first to receive the Department of Health’s seal of approval, locally called the Sangkap Pinoy Seal (SPS), for fortified food. The recently approved Food Fortification Law mandates the fortification of staple foods such as flour, cooking oil and sugar with vitamin A and encouraged fortification of processed foods. To date, there are 47 processed food products fortified with vitamin A with the SPS. However, there is a low level of awareness of fortified foods in general, and food products with SPS in particular (13).

Conclusion and recommendations

The prevalence of clinical VAD in the Philippines has decreased by 78% in 10 years (1982–1993). Sub-clinical VAD (<0.35 µmol/l) was reduced to 8.2% in 1998 from 10.4% in 1993. But the VAD situation in the country is still a threat to children’s health and survival. The challenge is to reduce the prevalence of sub-clinical VAD (<0.70 µmol/l) among preschool children to a level (≤15%) that will not constitute a problem of public health significance. The same challenge is true for the high prevalence of clinical and sub-clinical VAD among pregnant women.

It is recommended therefore that the coverage of the vitamin A supplementation among preschool children be intensified especially in high-risk provinces. The weekly administration of 2 low-dose vitamin A capsules (of 10,000 IU each) to pregnant women starting at the 4th month of pregnancy should be put in place. Likewise, an increase in the coverage of high-dose supplementation among lactating women is recommended.

Both government and private organisations should develop information and communication strategies that would increase public awareness and consciousness of fortified foods especially those with SPS. It is also recommended that modification of the diet to foods rich in vitamin A among infants, preschool children and women of reproductive age as well as promotion of breast-feeding be pursued vigorously at the local government level. Therefore, capacity-building efforts for local government nutrition workers should continue.

If political will to implement nutrition policy, plans and programs and legislative support continue to the next decade, virtual elimination of VAD is not far behind.

References


Successful vitamin A supplementation in Nicaragua

José O. Mora¹ and Josefina Bonilla², MOST, the USAID Micronutrient Program

Introduction

Even though clinically evident vitamin A deficiency (VAD) has not been identified as a significant public health problem in Latin America, sub-clinical VAD in children under 5 years of age (serum retinol <20 µg/dl) has been found to be a serious problem in a number of countries. In the late 1990s, the regional prevalence of sub-clinical VAD from national surveys amounted to about 25% in children (1). Estimated national prevalence rates in three countries exceeded the regional average: El Salvador (36%), Nicaragua (31%) and Peru (30%). High VAD prevalence in Central American countries was found since the mid-1960s, but it was not until the mid-to-late 1970s that some governments formally recognised VAD as a serious public health problem and began to address it. Universal or targeted supplementation, usually linked to immunisation activities (2), was adopted as a short-term response for at least a temporary improvement in vitamin A status while long-term interventions (e.g. food fortification) were implemented. The greatest advantage of adding vitamin A distribution to immunisation campaigns is that the additional cost is small, whereas the impact on child survival can be significant.

Nicaragua experience with vitamin A supplementation

Background

VAD was found a significant problem in Nicaragua since the mid-1960s but specific actions were not taken then. In 1993 the Nicaraguan Ministry of Health (MOH), with USAID assistance, carried out a national study to assess the prevalence of sub-clinical VAD in children and of anemia in women and children, and to estimate family and individual food consumption. The study revealed that about 60% of the children 12–59 months of age and 70% of the families consumed less than the recommended amounts of vitamin A per day, and 31% of the children had sub-clinical VAD. With these findings, the MOH nutrition group engaged in creating awareness on the health and development implications of micronutrient deficiencies, including the seriousness of VAD and the need to act. Sensitisation efforts targeted all levels of the public and private sector, academic institutions, politicians and the general population. This resulted in strong political commitment to address VAD as a priority problem.

Supplementation through National Health Campaigns (NHCs)

After contemplating different options, the MOH adopted supplementation as an emergency and temporary measure to control VAD while universal fortification of a staple food could be established³. Vitamin A supplements were officially included in the MOH list of essential medicines. A vertical supplementation program for vitamin A alone was not

1. Technical Advisor
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³. Reference number
seen warranted. The challenge was to secure high coverage of children 6–59 months twice a year. Despite some initial concerns about sustainability of the approach, it was decided that incorporation of vitamin A supplementation into the very successful National Immunisation Campaigns (NICs, “Jornadas Nacionales de Vacunación”) offered the best programmatic option.

NICs, carried out four times per year in the 1980s, were reduced to three times in the early 1990s. In order to secure high coverage twice per year, the scope of the NICs was expanded into an integrated package of preventive mother/child primary healthcare services to be implemented twice (rather than three times) per year. In addition to distribution of vitamin A and iron/folate supplements, the package includes routine immunisations, anti-helminthic medications, health education, oral rehydration salts, contraceptives, chloride for water treatment, and anti-louse medications. Therefore, twice a year National Health Campaigns – (NHCs, “Jornadas Nacionales de Salud”), spearheaded by immunisations, substituted for the NICs. This required establishing a semi-annual cycle of district activities improving facility usage for preventive services, as well as community outreach using schools and households of community leaders and/or “brigadistas” as delivery posts. The policy decision was made early in 1994 and later formalised in the 5-year National Micronutrient Plan 1996–2000. Technical guidelines were developed for

the services to be provided, including vitamin A supplementation targeted to children and post-partum women following WHO recommendations.

Under the MOH decentralisation process, the health sector in Nicaragua encompasses 17 Districts or Integrated Local Health Systems (“Sistemas Locales de Atención Integral en Salud, SILAIS”) which enjoy high management and budgetary autonomy. MOH central units provide technical guidance, training and supervision to districts. Implementation of the NHCs is a responsibility of the districts. MOH central units are responsible for setting the stage for coordinating and supporting NHCs implementation twice per year (in May and October) by securing sufficient supplies and providing training as needed to the districts and these, in turn, to the local health services. Media communications support is also provided to districts to timely sensitize and mobilise communities, and to enlist the long established cadre of community volunteers (“brigadistas”) in support of the NHCs.

During the NHCs, communities (particularly women and children) are massively mobilised by engaging media, municipal authorities, the church and other community groups, with very active participation of primary school teachers, secondary school and university health science students, community volunteers, traditional birth attendants, the military and non governmental organisations (NGOs). The need to take advantage of the variety of primary healthcare services provided at local health facilities is emphasised. Each NHC may take one week in urban areas and as many as four weeks in rural isolated areas where this is practically the only opportunity for people’s contact with the public health system.

Vitamin A supplementation is only one, albeit a very important one, of the services provided. It is targeted to children 6–59 months and post-partum women, although the latter are not covered by the NHCs. While most immunisation coverage is achieved in the first round of the year, the second round provides an opportunity for booster doses and for reaching children not covered in the first round with the full set of primary healthcare services. Each campaign is carefully planned jointly by the central MOH and the districts, and funded almost entirely from regular budgetary allocations. Up to 1998, the MOH procured vitamin A supplements using its own resources. Since then, supplements have been mostly donated by the Canadian government through the Micronutrient Initiative (MI), UNICEF, Wisconsin Lion’s Club, and the Japanese Government, in response to specific requests based on estimated needs prepared by the districts. In order to increase the coverage achieved through the NHCs, the districts are encouraged to tap any ongoing opportunities for contacts with mothers and children to ensure additional supplement delivery through routine health services.

A simple but effective supervision and monitoring system has been established which, in addition to oversee implementation, periodically provides information on
population coverage achieved by each district through both NHCs and additional routine distribution. Supplement delivery is registered on each child’s health card and recorded on immunisation tally sheets that are monthly compiled at health units and submitted to the district offices, and these, in turn, to the appropriate MOH central units. The Expanded Program of Immunisations (EPI) and the Department of Statistics enter and process the information to produce a monthly immunisation and vitamin A coverage report that is consolidated every six months. Semi-annual reports, with coverage rates by age group and district, are distributed and discussed at the central and district level in post-campaign evaluation meetings where the relative coverage ranking of each district is examined and options for improvement discussed. Public and professional recognition encourages health staff to achieve high rates of coverage.

**Population coverage**

Vitamin A supplementation coverage rates for children 6–59 months of age from 1994 to 2001, by year and round, are shown in Figure 1. Coverage has gradually increased in both rounds since 1994 and levels higher than 70% have been sustained since 1999, with levels above 80% in the last two years. The average coverage rate by round from 1997 to 2001 amounted to 79% in first rounds and 78% in second rounds. The latter is a remarkable achievement, as getting high second-round coverage rates has been a formidable challenge for many countries. Only 1–2% of the total coverage has been achieved through non-NHC routine health service distribution.

**Biological impact**

The ultimate biological impact of vitamin A supplementation would be expected as changes in serum retinol levels and, eventually, in infant and child mortality rates. A 2000 National Miconutrient Survey carried out about four months after the second NHC of 1999, with USAID/MOST technical and financial support, revealed a dramatic reduction (72%) in the prevalence of vitamin A deficiency (VAD) in children 12–59 months of age, from 31.1% in 1993 to 8.6% in 2000 (Figure 2). This significant improvement may be mostly attributed to the cumulative effect of vitamin A supplementation, as a result of the consistently high coverage rates in children over the six-year period preceding the survey, given the absence of other specific interventions in the same period. Successive rounds of supplementation may have gradually increased serum retinol levels over time. According to conventional knowledge, most of the effect of a large dose of vitamin A on serum retinol of children is expected to vanish after 3–4 months (6-7); however, studies on the long-term cumulative impact of repeated supplementation rounds have not been reported.

Alternative explanations, e.g. significant changes in the socio-economic conditions of the population and the possible impact of sugar fortification may be reasonably ruled out, as no evidence exists of improved social and economic conditions during the interim period and by the time of the survey in early 2000 the sugar fortification program was just starting. As shown in other countries where sugar consumption is practically universal, fortification would be expected to have a significant mid- to long-term impact on the vitamin A status of the
population. Once fortification is fully established, supplementation may need to be targeted only to the youngest children (e.g. under two years) who are less likely to benefit from fortification because of their low sugar intake.

Based on the results of experimental studies (8), a reduction in infant and/or child mortality would be expected as the ultimate biological impact of supplementation, particularly in countries with serious VAD and high levels of child mortality. Interpreting changes in infant/child mortality rates estimated from national surveys is complicated by methodological problems in estimating mortality rates and by the many interrelated factors that may influence child mortality in free living populations. Attribution of eventual changes to specific factors is particularly difficult in developing countries with a secular trend towards consistent decline in mortality rates. Recent trends in estimated infant and child mortality rates in Nicaragua (9) for five-year periods from 1973/78 to 1993/98 are shown in Figure 3. Both infant and child mortality consistently declined by 60–63% in the 20-year period (about 3% per year). There was a downward trend in the rates of decline by 5-year periods up to 1988–1993, e.g. from 30–33% (about 6% per year) between 1973–1978 and 1978–1983 to 4–9% (less than 1-2% per year) between 1983/88 and 1988/93. However, this trend reversed (back to higher rates: 20–22%, about 4% per year) in the period 1988/93 to 1993/98. Interestingly, this acceleration in the rate of mortality decline coincides with the implementation of vitamin A supplementation and, although there might be several potential explanations for such finding, a significant contribution of vitamin A supplementation seems highly plausible.

Key elements for success

A number of factors have been key to successful implementation of vitamin A supplementation in Nicaragua:

- Effective sensitisation at all levels of society, the health system and the community to generate awareness of vitamin A deficiency as a priority problem.
- Strong government political commitment expressed in policy and budgetary decisions and technical guidelines.
- Integration of the supplementation strategy into ongoing nutrition and health activities.
- Well trained and motivated staff who have the necessary knowledge and skills.
- Program ownership by health districts and local units.
- Skilled management of programs and timely supply of supplements.
- Building on a strong health infrastructure and community support.
- A supervision and monitoring system providing timely feedback to health services.
- Effective communication and behaviour change strategies.

Conclusions

Nicaragua provides a successful example of periodic, active, institutionalised, integrated distribution of vitamin A supplements with consistently high coverage. Integrating supplementation as part of a package of basic health services to be delivered twice a year through National Health Campaigns, Weeks or Days is a viable, affordable and effective option to facilitate achievement of consistently high coverage rates, and is more likely to be widely accepted and endorsed by health authorities than specific campaigns for vitamin A distribution alone. As national immunisation
days (NIDs) are scaled down or phased out in many countries, NHCs offer an effective alternative strategy to sustain the delivery of vitamin A to young children at the high coverage rates needed to realise its full potential to reduce mortality. Twice yearly delivery of vitamin A supplements through synchronised NHC distribution yields excellent results, as shown in Nicaragua. Vitamin A supplementation can be made a key component of an integrated package of preventive services designed to improve child survival by establishing a semi-annual cycle of district activities designed to improve facility usage for preventive services, thus high coverage of several key child survival interventions can be achieved all together.

The NHCs can be complemented by routine healthcare services to increase coverage, maintain staff motivation and strengthen long-term sustainability. All routine health service contacts offer opportunities to increase coverage with vitamin A supplements (post-natal clinic visits, immunisation, growth promotion, other Mother and Child Health clinic contacts, and sick child attendance). Although, in principle, integration of supplementation within regular health services is a desirable goal, particularly when the campaign approach is not feasible, this strategy alone has not proved effective in reaching consistently high coverage rates. Moreover, health facility attendance for preventive services tend to drastically decline for older children, making it difficult to achieve adequate coverage of preschool children. However, it offers a sound opportunity to enhance the coverage attained through NHCs and would increase the long-term sustainability of vitamin A supplementation.

References

Vitamin A deficiency in Micronesia*

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On 8–11 May 2002 in New York, the United Nations General Assembly held a Special Session for Children to review progress made since the World Summit for Children in 1990. The report by the UN Secretary-General Mr Kofi Annan, entitled “We the Children” (1), shows the significant progress made in the 1990s in large-scale vitamin A capsule distribution, which is estimated to have prevented 1 million child deaths between 1998 and 2000 alone. The report, however, also points to significant challenges ahead in continued distribution campaigns and it indicates that initiatives aimed at fortified food will be essential.

While in New York, Heads of State and Government were signing off on a vision and specific goals for social and economic development in the new Millennium (2), including the sustainable elimination of vitamin A deficiency (VAD) by 2010. Correction of the severe VAD problems in the Federated States of Micronesia (FSM) should receive high attention among the priorities following the renewed global commitment.

A clinic-based study in 1987 in Chuuk, one of four FSM states, found evidence of abnormal vitamin A status by conjunctival impression cytology among half of the 36–83 months old children attending the out-patient clinic (3). This initial exploration was followed by a state-wide study in 1988–89, resulting in similar findings (4). Then in 1992 in a study of children aged 18–24 months and 3–6 years, over 50% of the children were found to have VAD as defined by a serum retinol ≤ 0.70 µmol/l (20 µg/dl) (5). Similar results were found in a community-based study in Pohnpei with 362 children 24-47 months of age (6). In response, the FSM health service started periodic

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\text{Figure 1. Serum retinol distribution.}
\]
vitamin A supplementation among children up to 12 years of age in Chuuk and Pohnpei States.

Prior to extending the supplement policy nationwide, the FSM Department of Health, assisted by UNICEF and the Center for Disease Control, Atlanta, (CDC), surveyed the VAD situation among young children in Yap and Kosrae, the remaining two FSM states. Proportionate-to-population random sample surveys were carried out during January and February 2000 among 218 and 267 children aged 24–59 months in Yap and Kosrae, respectively. Body height and weight were measured to estimate wasting and stunting, using WHO/CDC references (Z-scores < -2 SD below the reference median). Venous blood was drawn for HemoCue estimation of haemoglobin, and the serum separated for retinol analysis at CDC by HPLC. The mean serum retinol was 0.712 µmol/l (95% C.I.: 0.691, 0.737); 0.628 µmol/l in Kosrae and 0.799 µmol/l in Yap (p<0.001), respectively. VAD among children was 48.8%, with a higher (p<0.001) prevalence in Kosrae (63.3%) than Yap (33.8%) (7).

The retinol frequency distributions found of FSM children are compared in Figure 1 with the serum retinols among 48–71 months old, apparently healthy US children (NHANES III, 1988–94). Although the respective locations on the serum retinol scale differ somewhat, all the distributions among FSM children show a marked downward shift compared to US children, illustrating an associated risk of morbidity and mortality among the FSM child populations (8). The prevalence of wasting (3.8%), stunting (16.6%) and anaemia (11.2%) among children aged 2–4 years in Kosrae and Yap did not differ significantly between the two States, nor were they much different from prevalences reported among children of Chuuk and Pohnpei in 1992–93. In contrast to cross-sectional surveys in other countries with severe vitamin A deficiency (9,10) the poor vitamin A status in FSM children does not co-occur with similar high prevalences of other malnutrition indicators.

As already instituted for children of Chuuk and Pohnpei, universal vitamin A capsule distribution 2–3 times annually is a most practical response for the children of Yap and Kosrae. However, in view of the scale and commonness of VAD in each State among the young children – the most vulnerable group often used as proxy for the whole population – a more comprehensive approach may be indicated for FSM to address the widespread problem. Fine-tuned to local conditions in each State, a national policy should devise a thoughtful combination of public health measures, promotion of more nutritious diets – including local vitamin A-rich foods – for all age groups, and vitamin A fortification of commonly eaten foods, to complement the ongoing capsule distribution among young children.

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The contribution of the “carotenoid world”

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In a way it feels strange to find myself among all the other correspondents, most of whom have devoted their entire working lives to the cause of fighting vitamin A deficiency. I have come to it late after years of working in the field of carotenoid chemistry and biochemistry. My interest in the problem of vitamin A deficiency was kindled by Delia Rodriguez-Amaya during some highly enjoyable visits to Brazil. Then, like so many others, I came under the influence of the vision and inspiration of Jim Olson during time we spent together at the Latin-American Congress on Food Carotenoids organised by Delia in Campinas in 1998. This led me to my first, and so far only, IVACG meeting in Durban in 1999, where I had the privilege of taking part in Jim’s wonderful little special interest workshop on provitamin A carotenoids in food.

In this first encounter with the world of vitamin A deficiency (VAD), my immediate impression was of two strongly held and forcefully promoted views. One group of people felt strongly that increasing the intake of provitamin A carotenoids from food can make a major contribution to improving vitamin A sufficiency in populations, whereas another group believed that this approach can never be effective; the only effective solution lies in supplementation or fortification with vitamin A or even β-carotene. With the benefit of inexperience, it was easy to take the simple view that any and every approach that can contribute to improving the vitamin A status of an individual or a population is valuable and should be used. Since then neither time nor thought nor reading nor discussion have changed this view. It seems logical that the basic vitamin A status of a population can be maintained and improved by increasing consumption of β-carotene-containing vegetables and fruit, with supplementation and fortification then being used when required, to give a rapid improvement in populations seen to be at risk.

In addition to this, in the West, a diet rich in fruit and vegetables is recommended because it provides many other vitamins and micronutrients, and is associated with reduced risk of cancer, heart disease and other degenerative diseases, as well as maintenance and stimulation of the immune system. Surely everyone deserves the benefits that increased consumption of fruit and vegetables would bring.

Can the carotenoid world and carotenoid research make a

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The photographs of animals and plants coloured through carotenoids were given by courtesy of Mr Jonathan Taylor, Roche Vitamins, Basel.
practical contribution to the fight against vitamin A deficiency? The expertise that is available for the analysis of carotenoids has been used and could be used in a number of ways. Possibilities include (i) Helping to optimise and standardise analytical methods for general use; (ii) Establishing a “pyramid structure” with simple quantitative analysis in the field as the base and progressing through local or regional analytical centres to national or international centres where sophisticated equipment is available and complex problems, including structure elucidation, can be solved; (iii) Using this to compile a comprehensive data base of carotenoid occurrence and concentrations in local plants in all regions. This would have possible benefits in two directions, first by identifying new provitamin A sources (new species, varieties) in relation to the fight against VAD and second by revealing potential sources for commercial production that would increase general commercial activity and standard of living through local enterprises; (iv) Providing a sound analytical basis for selecting the best varieties for cultivation, evaluating growth conditions for optimum yield and carotenoid content, and for determining and understanding the effects of storing, processing and cooking on carotenoid stability and bioavailability.

In pursuing this work, we must be reasonable and realistic about what is actually needed and useful. With the HPLC methods now available, it is easy to obtain very precise data for the carotenoid content and composition of foods. The practical value of the precise figures is questionable, however. The numerical values come from the analysis of particular samples of a given variety, collected in one place, having been grown under unspecified or optimised conditions and stored in some way for an unspecified time. Tabulated data should be seen as a useful indicator of what may or may not be good sources of carotene, but it cannot be assumed that the figures given are even close to those for a real food sample produced under often difficult local conditions. This emphasises the need for carotenoid researchers to be aware that not only environmental and climatic conditions but also local traditions, customs and culture must be taken into account when trying to find practical solutions. There is no substitute for experience in the field. With this in mind, it would be particularly useful, for example, to develop a simple method to give a rapid, reliable and sufficiently precise indication of provitamin A carotene content of the food materials that are actually being consumed.
It will also be very beneficial if carotenoid researchers can be encouraged to keep VAD in mind when evaluating other carotenoid studies, and to extract from the mass of data available the information that is really useful and relevant in the context of VAD. People who are working in the field and trying to encourage increased production and consumption of provitamin A carotenoids, for example via cottage gardens, are not going to read the primary literature or assimilate details of analytical procedures and tables of precise figures. They should be able to rely on “experts” to do this for them in an unbiased way and then to give them guidance that they can act upon in practice.

Sadly, apart from visionaries such as Jim Olson, few carotenoid researchers outside the regions affected have given more than a passing thought to the global problem of vitamin A deficiency. As the carotenoid field has diversified, new aspects have aroused public interest, been seen as exciting and challenging, and generated commercial activity, especially in response to the ever-increasing reports that carotenoids may have many health benefits. As far as VAD is concerned, a typical view might be that “It is well known that β-carotene is a major dietary source of vitamin A, it is converted into vitamin A by cleavage, it is present in fruit and vegetables, and it is easy to determine precisely how much is present in the various sources. We know the answers; it’s up to them to get on with it. We’ve got more interesting and challenging problems to tackle”.

Can this attitude be changed? I hope so and I believe so, and I would like to think that a key role can be played in this by the relatively newly formed International Carotenoid Society, of which I had the honour to be the first President. The International Carotenoid Society was established to fill the need for a focus for research, education and other activities in the broad carotenoid field and, especially, to provide a forum for contact and communication between carotenoid researchers from different disci-
plines and with complementary expertise, and from different parts of the world. A major aspect of this which is now being promoted strongly, is the establishment of regional branches of the Society, to encourage contact and interaction between people in the region with interest in carotenoids, and to stimulate communication between workers from different regions who have similar interests, so that all can benefit from dialogue and progress. Branches have been set up, or are being set up, in Latin-America (contact person Delia Rodriguez-Amaya, UNICAMP, Brazil), South-East Asia and the Pacific (contact person Pongtorn Sungpuag, Mahidol University, Thailand), and the SAARC countries of the Indian sub-continent (contact person Umesh Goswami, Gauhati University, India). We are open to approaches from carotenoid researchers in other regions, to explore the possibility of additional branches or alliances.

Regional meetings, such as the Latin-American Congress and the South-East Asia and Pacific Regional Meeting have brought together many interested people from all over the region and have been very successful catalysts for the foundation of regional branches and activities. Practical workshops have proved particularly useful; more are projected in the near future.

The International Carotenoid Society as a whole and through its branch activities will not ignore the problem of VAD. I will be very pleased to hear at any time from anyone who has questions about carotenoids and the Society, ideas for activities or suggestions of how the ‘carotenoid world’ could get involved and contribute to the valuable work that is being done in the fight against VAD.
Carotenes as dietary precursors of vitamin A: their past and their future*

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James Allen Olson became a guiding light of the Carotenoid Research Interaction Group (CARIG). Hence, it is more than appropriate that the CARIG should institute a permanent memorial in his name on the academic platform of Experimental Biology. As for me, as a professional, and for the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) in Guatemala, we were attracted to CARIG not because of the emerging issues of carotenoids as prophylaxis for chronic diseases, but rather because of their role as precursors of vitamin A. This was also a concern of Jim Olson, and it is a special honour to be invited to give the first James Allen Olson Memorial Perspectives on Carotenoids, lecture.

Jim Olson was a special spirit. In an obituary article I published in the European Journal of Clinical Nutrition after his untimely death in September 2000, I wrote, “James Allen Olson was a unique giant – a gentle giant – in our nutrition community. His interests bridged and spanned the chemistry, biology and public health interests of both retinoids and carotenoids, dominated both topics, and assumed leadership.” (1). In this spirit of dual concern, Jim Olson created a cocktail reception called VARIG (Vitamin A Research Interaction Group) at FASEB, and eventually this has merged to the CARIG-VARIG reception, mingling those whose primary professional concern was retinoids with those with a carotenoid bent.

Finally, Present Knowledge in Nutrition, published by the ILSI Press, is perhaps the second most important of the comprehensive general-nutrition textbooks. Last year, I completed the task requested of me by another founding father of CARIG, Rob Russell. As co-editor of the Present Knowledge in Nutrition, VIII, he invited me to write the chapter on “Vitamin A and Carotenoids” (2). This was the first time that the chapter had combined both entities under one roof, and Jim Olson had been the perennial author of the vitamin A chapter for this textbook. After proclaiming my unworthiness for the task, I was convinced by Dr. Russell to undertake it, but was confronted with an organisational quandary. How do I separate and streamline the discussion of carotenoids as provitamin A precursors of retinoids and that of intact, absorbed carotenoids as bioactive agents? I may not have gotten it right, but once again I was counselled by the simple, but profound, distinction that was promoted by Jim Olson with regard to food constituents, that of functions and actions. Functions are: “an essential role played by the nutrient in growth, development, and maturation.” Actions are: “demonstrable effects in various biologic systems that may or may not have general physiologic significance.” (3). Here, we are going to be talking about function.

What is past is prologue

The chemical identification of dietary vitamin A

The year of 1913 was a landmark year for vitamin A research, as it was the date when the retinol form of the vitamin, a colourless, lipid-soluble compound, was discovered simultaneously in two laboratories by McCollum and Davis (4) and by Osborne and

* This was presented in April in New Orleans in the mini-symposium on carotenoids at the annual meeting of the American Society for Nutritional Sciences at the Experimental Biology 2002 meeting as the first James Allen Olson Memorial Perspectives on Carotenoids Lecture, sponsored by the Carotenoid Research Interaction Group (CARIG) of which Prof. Olson had been a motive force and founding member.
Mendel (5). In 1919, Steenbock suggested a linkage between a yellow pigment in plants and the retinoid compound known as vitamin A. It was not until 1930 that two distinct investigators – Capper (6, 7) and Moore (8) – confirmed the existence of provitamin A activity in the carotenoids of plants.

1965 was another key year as two groups identified the 15-15' dioxygenase enzyme, which apparently introduced molecular oxygen into the centre of the symmetrical β-carotene molecule to produce two molecules of retinal (retinaldehyde) (9, 10). More recent insights include confirmation in vitro (11) and in vivo (12) that the conversion is typically stoichiometric. A diagrammatic representation of the chemical scheme is shown in Figure 1 (13).

The most recent advance, bringing us to the present, has been the cloning of the dioxygenase (14, 15). With this has come evidence of the regulation of the dioxygenation, consistent with any assumption of homeostatic maintenance of total-body vitamin A status (13).

Eccentric cleavage to apocarotenals by oxidation or enzymatic mechanisms can produce retinoic acid (Figure 1). An enzyme specific for 9,10 cleavage by inserting molecular oxygen has been characterised (16, 17). We must accept that the operation of this pathway must yield a maximum of one retinoid moiety for each parent molecule, but that it must be regulated so as not to produce any risk of excessive vitamin A accumulation.

For the two other common provitamin A moieties, α-carotene and cryptoxanthin, and for minor contributors as well, we have little knowledge of the bioconversion process. Once again, however, we must accept that the operation of this pathway can yield only a maximum of one vitamin A moiety from each provitamin A molecule, and that it must be regulated so as not to produce any risk of excessive vitamin A accumulation.

The evolutionary significance of carotenoids for plants and animals

Most naturalists believe that photosynthesis by the chloroplasts of green plants is the single most essential evolutionary landmark to allow for life as we know it on Earth for both the Plant Kingdom and the Animal Kingdom. It produced a renewable origin and source of calories, trapping the energy of the sun into the food chain. At the same time, it released oxygen, which would allow for efficient metabolism of energy. Solar radiation and oxygen, however, were double-edged swords. They permit efficiency energy trapping in plants, but they stimulate the formation of damaging free-radicals and reactive oxygen species. Carotenoids are nature’s sunscreen, providing the antioxidant protection for photosynthesis.

All retinoids in nature and in the food chain originally come from plant carotenoids. Photosynthesizer (green) plants are autonomous and self-sufficient. Herbivores eat green plants (plant-based nutrients). Terrestrial carnivores generally eat herbivores, whereas sharks (elasmobranchs), higher marine fish, and marine mammals consume other carnivores. Omnivores eat plants, herbivores, carnivores and other omnivores.

Humans are the classical omnivores in evolution. The interesting question arises as to whether humans would adapt to the dietary vitamin A problem in the spirit of an herbivore (i.e. a highly efficient converter of plant precursors) or the spirit of a carnivore (i.e. largely dependent on preformed vitamin A).

Malnutrition and nutrient overload as evolutionary dysadaptation

If we consider evolution to be synonymous with adaptation, we would expect that evolution would always be tending towards a situation in which the nutrient requirements are adequately met – but not exceeded – by the habitual diet. As such, in the equilibrium evolutionary state, no species should be subject to malnutrition or nutrient overload. That is, by consuming enough eucalyptus leaves, and only eucalyptus leaves, a koala should be nutritionally replete; similarly, a queen bee should have all her energy and micronutrient needs satisfied by an ad libitum diet of royal jelly and honey.

In nature, temporarily imbalances can occur that put pressure on the nutritional stability. Seasonality of food availability can produce cyclical nutrient deficiencies, but presumably this does not endanger the survival of a species. There can, however, be a prolonged scarcity of the traditional dietary elements due to plagues among the food specie(s), competition for the foods by other consumers in the ecological niche, or overpopula-
tion by the index species, itself, leading to over-hunting or over-grazing. Alternatively, abrupt change can occur in the nutrient composition of food. These phenomena produce a kind of “famine” situation which can be resolved rapidly by recovery of the food supply, or migration to areas of richer sources. If neither of these is an option, natural selection can proceed; those members of the herds with lower requirements will demonstrate increasing reproductive fitness, shifting the genetic pool toward lower requirements.

Similarly, a species in evolution might confront the situation of a drift of increasing nutrient concentration in their food(s) of choice, leading to a situation of nutrient overload bordering on toxicity. Again, genetic selection can favour reproductive fitness among those with the higher tolerances, and lead eventually to a genetic shift to a population with tolerance to the new dietary levels.

**For humans, cultural and technological advances trump the natural evolutionary order**

Following the aforementioned logic, human diets should not produce entrenched micronutrient deficiencies, but the existence of xerophthalmia is evidence that they do. Anthropologists and paleonutritionists assure us that the evolved traditional hunter-gatherers of prehistory were generally well-nourished (18, 19). The nutrient requirements of the evolving hunter-gatherers were adapted to their dietary selection when all traditional foods were abundant, although seasonality effects were likely. As humans evolved first to the pastoralist and then to agrarian societies, distortions in dietary selection disrupted the harmony of their evolved nutrient requirements (19). The micronutrient deficiencies we combat today are a consequence of the agrarian revolution, which produced primary staples (plant foods such as cereal grains and tubers) of low nutrient density.
Humans cultural dysadaptation can also produce nutritional excess

On the other side of human nutritional imbalance is a propensity to have nutrient excess evolve in the agricultural and technological eras. Obesity is an example of excess energy storage. Hypervitaminosis A is the nutrient excess of relevance to this discussion. Preformed vitamin A (retinoids) can be toxic. Carnivores, especially those at the top of the marine food chain such as sharks, walruses, narwhals, and polar bears, are most resistant to any toxic effects of retinoids. Omnivores are intermediate in tolerance, as a large percentage of their vitamin A can come from plant sources. Herbivores are most sensitive to retinoid toxicity, as preformed vitamin A is never part of a plant-based diet.

A series of adverse consequences of excessive exposure to vitamin A

With intakes of >500 mg of preformed retinyl esters, as occurred when European Arctic explorers consumed liver of marine mammals, a severe and often fatal acute hypervitaminosis A syndrome will result. With self-medication or unwise prescription of 7500 RE (retinol equivalent) preformed vitamin A daily, chronic hypervitaminosis with neurological and hepatic consequences can occur. Rothman et al. (20) have estimated that daily intake of 3000 RE of retinol is sufficient to produce teratogenesis. Finally, the paradox of why the highest rates of osteoporosis are seen in countries with the highest per capita consumptions of calcium was resolved in Sweden by the suggestion that intake of the vitamin A in the calcium sources, milk, dairy products and fish, above the level of 1500 RE daily begins to produce significant bone demineralization (21, 22). On the other side of the ledger, precursors of vitamin A (carotenoids) are regulated homeostatically in their conversion to vitamin A and cannot produce hypervitaminosis A (23).

Rating the diet for its vitamin A content:

Conversion factors for dietary vitamin A

If one wants to know the total amount of vitamin A available in a diet, one has to perform calculations with certain assumptions. This was done by estimating the foods consumed and assigning a value to the chemical constituents of the foods. In foods of animal origin the predominant form of vitamin A is the retinoid form (retinyl esters), although eggs, viscera, animal fat, and milk contain important amounts of provitamin A. Plant foods, have only pigment sources, with the carotenoids β-carotene, α-carotene, and β-cryptoxanthin being the most common members of this family with the capacity to be precursors of vitamin A.

Retinol equivalents

Initially, dietary vitamin A was expressed in terms of the weight of the compounds of interest in foodstuffs. This soon moved, however, to the expression of International Units (IU). The realisation that bioconversion of dietary provitamin A carotenoids was neither stoichiometric nor equivalent across different chemistries led to a rationalisation of the quantitative expression of food vitamin A value. In 1967, an expert panel of the United Nations' agencies devised a system based on the retinol equivalent (24). By this convention, one RE was defined as equal to 1 µg of all-trans-retinol, to 6 µg of all-trans-β-carotene, and to 12 µg of other provitamin A carotenes. At the level of description of population nutrient consumption, these assumptions went without major challenge for 25 years. However, when it came to population action in the renewed public health battle against hypovitaminosis A that arose from the findings of Sommer and colleagues (25), these conversion factors began to receive scrutiny.

Critique of the assumptions of retinol equivalency of provitamin A sources

The 1967 retinol equivalents system has been used to interpret the vitamin A in the food supply. The estimates from U.N. sources, reproduced by McLaren and Frigg (26) (Figure 2), are based on retinol equivalents. Common sense questions begin to emerge when one takes note of the small difference in the daily vitamin A supply for affluent countries and the African region, and juxtaposes this with the fact of far higher rates of hypovitaminosis A and xerophthalmia in the latter area. My colleague, Jesus Bulux and I pondered a series of observations that led us, in 1993, to challenge the conventional bioconversion factors (27). These included the fact that the range of hepatic vitamin A stores was lower in herbivores than in carnivores. Among hu-
mans, hepatic liver reserves were lower in vegans than in omnivores. Hypovitaminosis A was most common in populations subsisting on primarily plant-based diets. In the most vulnerable population (preschoolers), getting acceptance and consumption of dark green leafy vegetables (DGLV) is problematic, and it would be more difficult if programs called upon youngsters to consume even more. In our review, we said: “Evidence from feeding studies shows an almost universally poorer intake of intact carotenoids from plant sources as opposed to pure, chemical sources. With notable exceptions, the bioconversion of plant carotenoids to preformed vitamin A also seems to be inefficient.” We were overestimating vitamin A availability, underestimating risk, and overselling the promise of plants to protect a population from hypovitaminosis A and its consequences.

A team of investigators from the Wageningen Agricultural University placed it a bit more bluntly in the title of a paper that appeared two years later in *The Lancet*. “Lack of improvement of vitamin A status with increased consumption of dark green leafy vegetables” (28). In their conclusions, they assert: “There is little evidence to support the general assumption that dietary carotenoids can improve vitamin A status. Our findings do not support the long-standing assumption that vitamin A deficiency can be combated by increasing the intake of dark green leafy vegetables.”

Resistance to this notion of inaccuracy in the retinol equivalent conversion factors came from a particular source, namely the academic community of the nation of India. For a people committed to the Hindu faith, any notion of an obligatory necessity for animals to become part of the diet was anathema. The evidence base for the conclusion of high bioconversion, however, were largely from metabolic balance techniques. Naturally, if there were colonic destruction of unabsorbed carotenoids, this would tend to augment the apparent absorption value from such studies (29). This led to sometimes strident confrontations between those who sought to reform the conversion factors for provitamin A from plant-based diets and those who sought to conserve their validity.

**Prelude to retinol activity equivalents**

If a population is in equilibrium, plant provitamin A can maintain stability of nutritional status (30), but it is not a robust approach for interventions to remedy a population’s status. On the plant matrix side, research has shown that matrix factors in plant tissues can explain the reason for lower availability of plant provitamin A compounds for vitamin A in humans (31, 32), confirming the arguments of Solomons and Bulux (27) and de Pee et al. (28).

The prospects of efficient utilisation from provitamin A, however, were not entirely bleak. On the β-carotene-in-oil side, classical metabolic studies had examined functional responses to restoration of vitamin A status using β-carotene dissolved in an oily matrix (29). The reported bioconversion efficiency had
ranged from 2 to 25%. Recent stable-isotopic studies employing isotopic techniques have found bioefficacies of from 5 (33) to 2.6 \( \mu g \) (34) of \( \beta \)-carotene needed to produce 1 \( \mu g \) of retinol.

At the conclusion of this round of research, it became clear that not all provitamin A sources in nature were created equal. It became apparent to everyone in the field that the 1967 REs were an over-valued currency in terms of making legitimate exchanges for vitamin A activity from plant-based diets. Thus, in January 2001, in the United States and Canada we initiated a new era of quantifying dietary vitamin A with the designation of the Retinol Activity Equivalents (RAE) (35). Influenced by the research in the previous decade, the U.S. Food and Nutrition Board (FNB) basically halved the official estimate for bioconversion efficiency of provitamin A compounds in most plant foods. The RAE is equivalent to 1 \( \mu g \) of all-\( \text{trans} \)-\( \beta \)-carotene, to 12 \( \mu g \) of all-\( \text{trans} \)-\( \beta \)-carotene, and to 24 \( \mu g \) of other provitamin A carotenones. On the other hand, the FNB spoke on the bioefficacy of \( \beta \)-carotene in oil. They stated: “The carotene:retinol equivalence ratio (\( \mu g: \mu g \)) of a low dose (less than 2 mg) of purified \( \beta \)-carotene in oil is approximately 2:1...” (35). With higher dosages, the efficiency decreases in a manner related to the total amount of oil-based provitamin A sources presented.

**The policy and program perspective**

The late food scientist Kenneth Simpson commented almost two decades ago: “Recent statistics show that carotene from vegeta-
bles contributes 68% of dietary vitamin A on a world-wide basis and 82% in developing countries. In spite of the abundance of carotenoids in the world, vitamin A deficiency is still a very serious problem.” (36). By applying the RAE retrospectively to these global estimates, it would be clear that the contribution from preformed vitamin A, 32% and 18%, respectively, would remain constant in the recalculation while that from plants is cut in half. Hence, by this revision, the true vitamin A intakes would have been only 66% of what we thought it was for the world at large, and 59% of that estimated for developing countries.

**Present and future shock**

At last we are armed with conclusive and quantitative evidence, and almost a full consensus regarding the issues of the expected bioefficacy of oral provitamin A compounds in their distinct food formats. The recent past guides us in the present to avoid excessive enthusiasm; overly enthusiastic expectations can bring unintended consequences and damage the reputation of applied research and intervention programs as illustrated in two case examples below.

**Damaged reputations**

Sometimes there is a cost in excess mortality and public relations to applying too high a dosage of chemicals for nutritional “chemoprevention”. Both the carotenoid and the vitamin A communities have suffered uproars regarding alleged damage done to recipients of high doses of the respective substance in interventions. With respect to carotenoids, we have the cancer prevention trials with \( \beta \)-carotene provided daily or every two days in multi-milligram doses of \( \beta \)-carotene (37, 38) in subjects considered to be at risk of lung cancer by virtue of a history of tobacco smoking or asbestos exposure. A narrative interpretation of these studies has been provided by Cooper (39). As we now know, the net effect was a slightly higher mortality rate among those in the \( \beta \)-carotene intervention group. A number of explanations for this paradoxical effect have been entertained (39, 40). The implications of such unintended consequences is producing a “Bad name for a perfectly good nutrient” (41). Since the context in these trials was the isolated, chemical form in high doses and an unphysiological format, it should have no implications for natural provitamin A compounds in their food forms (41).

More recently, in 2001 we have the case of the Assam, India, and the vitamin A prophylaxis pulse campaign with retinyl palmitate (see article by V.Reddy in this volume, ed.). This provides another example of the potential erosion of confidence in nutritional interventions and damage to the reputation of vitamin A. In this instance, it was the safety of mass prophylaxis with preformed vitamin A coming under scrutiny and frontal attack. The facts are in dispute (42). What is clear is that periodic high-dose supplement pulse campaign programs, in which all eligible preschoolers in a province are to be dosed on a
given day with imported capsules, have been conducted in India using a nationally prepared liquid elixir. It was generally administered as 2 ml volumes on a spoon, but acceptability issues forced a change to small medicine cups of 5 ml capacity. The dose was still to be 2 ml containing 200,000 IU (60,000 RE). Whether or not some centres actually filled the cups producing a 2.5-fold overdose or not, the claim was made in the aftermath of the campaign that 14 children had died as a direct result of the vitamin A-dosing and multiple others had suffered adverse side effects (42). The existence of a scandal facts notwithstanding has produced official reticence regarding the pursuit of periodic supplement dosing. A rapid retreat from the policies and programs of vitamin A supplementation without an effective alternative would pose potential danger to the vitamin A nutriture of 80% of the world’s population.

Golden rice and other gilded grains

Dietary staples are so named because they are the underpinnings of cultural foodways; everyone in the society would consume quantities of the dietary staple. Hence, if one wants to assure that everyone in the society receives a given nutrient, it should be added to a staple food. Rice is the staple grain throughout the southern reaches of Asia, and may represent the staple for one-half of humankind. In recent years, and primarily with financing from the Rockefeller Foundation, an effort in genetic biotechnology has been applied to inserting into the rice (Oryza sativa) the genetic machinery that enables marigolds and daffodils to make β-carotene. One or another of these approaches to making golden rice has been reported from the Institute for Plant Science of the Swiss Federal Institute of Technology in Zurich and the Center for Applied Biosciences of the University of Freiburg, Germany (43, 44). This has been much commented upon as a paradigm of applied genetic biotechnology (45-47).

This approach has generated its skeptics (48). Legitimate concerns exist that the plant will only express a fraction of the provitamin A needed. Persons would have to consume more than usual rations of rice to meet even 50% of their vitamin A recommendation. Bioefficacy of provitamin A from a rice matrix is unknown. The efficiency of bioconversion is likely to be low. The anthropological factor that may be critical is the acceptability of a rice that is “off-white” in colour. Asian populations often put a high premium on the whiteness of their rice, and the yellow colour may prove to be a detractor in terms of long-term acceptance.

The line of investigation is worthy of pursuit. What the approach of golden rice – or any similar strategies with staple products – requires is careful stewardship by the professional communities involved to avoid the pitfalls that could lay in wait. This is an area for important continuing research. Other staples from white potatoes and other tubers (cassava, yams) to other grains can be explored (49). The influence of the lipid content of the diet is important in the assessment of effectiveness potential at the public health level.

One issue of safety would be to assure, if not so much for human consumption (about which there is little question), but with regard to the environment isolation of its genetic characteristics from other rice crops where not desired. The other would be in terms of efficacy. Learning the lesson from green and orange vegetables, we must not raise expectations for these genetically altered crops beyond conservative predictions, and not leave populations unprotected if their needs exceed the delivery capacity of golden rice.

Carotene-pigmented oils

As reviewed above, when the localisation of a provitamin A in a food is not in its tissues but in the oil, a distinct set of circumstances comes into play (33, 34) and the dietary vitamin A value can be considered full equivalent.
to preformed vitamin A on a weight basis (35). Red palm oil (RPO) from the palm fruit (*Elaeis guineensis*) is a source of oil-emulsified \(\alpha\)- and \(\beta\)-carotene in abundant concentrations (50, 51). Crude RPO contains from 500 to 700 ppm of carotenoids, of which 50% is \(\beta\)- and 37% is \(\alpha\)-carotene. Some varieties have up to 4000 ppm (50). A commercial product from Malaysia contains 45.5 mg of provitamin A carotenoids per 100 g, in a ratio of 28:18 in favour of \(\alpha\)-carotene, which has conventionally been quantified as providing 6000 RE per 100 g.

In a comparative study of high-dose vitamin A supplementation in the Orissa Province of eastern India, periodic dosing with RPO produced the same degree of vitamin A status prophylaxis as did the more traditional administration of 200,000 IU of retinyl palmitate (52). This led me to comment on that finding: “It is now time to explore \(\beta\)-carotene in foods in a matrix-free context, such as the purified pigment and that in RPO, as the key to a safe and effective vitamin A supplement, fortificant, and food-based solution all wrapped up in one” (53).

A much less well known, but potentially even richer source of natural provitamin A compounds in oil is to be found in the previously obscure “gac” fruit (*Momordica cochinchinensis*), native to Vietnam (54). Its content of provitamin A is greater than that of the palm fruit, and the potential of feeding gac for improving vitamin A and carotene status in malnourished Vietnamese children has been studied (54).

### Bioefficacious provitamin A for the needs of infants

During the first year of life, issues of vitamin A adequacy are paramount. Two strategies – not mutually exclusive and both employing retinyl palmitate supplements – are currently in vogue for protection of the infant. One is the direct supplementation of the infant with dosages at 7600 RE (25,000 IU) of vitamin A. The other is the recommendation to provide the mother with one to two 200,000 IU dosages within seven weeks of delivery. Should the stigma associated with high-dose preformed vitamin A extend to these two settings, the alternatives with provitamin A are uncertain.

With respect to the direct feeding of provitamin A in oils to infants, the lipase and bile salt secretion of infants requires development and maturation. Their digestive capacity is suited primarily to human milk and its fat content and composition. It would take about 125 ml of RPO to provide 7500 RE (25,000 IU) of vitamin A activity, or 250 ml to provide 15,000 RE (50,000 IU), using the conventional RE assumptions. How infants would tolerate and utilise this vehicle in these dosages remains to be studied.

The International Research on Infant Supplementation (IRIS) group sponsored a four-nations intervention trial with a compacted tablet as the vehicle (55). In a new multicenter initiative (IRIS III) to test the potential of the fat-based, ready-to-use food in the form of a spread (56) to serve as the vehicle for food-based multi-micronutrient intervention (57, 58), the dual source of vitamin A and E is being derived from RPO.

More is known about the indirect approach of routing the vitamin A, as precursor provitamins, to the infant via the mother and her milk. This has been studied in Honduras (59, 60) and in Tanzania (61, 62). An improvement in the vitamin A status of both mother and offspring has been demonstrated. Data from Lietz et al. (63) suggest that the ratio of circulating to breast milk \(\beta\)-carotene is roughly 10:1, independent of the absolute levels, and much lower than the 5:1 ratio for lutein.

### Bioefficacious provitamin A answering the call for affluent populations?

There may be too much of a good thing (vitamin A), i.e. when that good thing can also be a bad thing. Hence, the utility of provitamin A as principal vitamin A sources for developed countries also has merit. The issues of teratogenesis risk for fertile-age women (20) and life-long retinyl-ester intakes for bone health of the mature adult (21, 22) exemplify the downside aspects of an increasingly vitamin A-rich diet. So provitamin A as a fortificant might be just what the epidemiologist ordered for the issue of willy-nilly fortification and self-supplementation in the U.S.

### An oily supplement cocktail for mature adults

Much is being learned about functions and actions of the four fat-soluble vitamins and the carotenoids, specifically for the health of the elderly. Arguments can be made for at least assuring adequate nutrition, if not providing supraphysiological supplies, of three of the fat-soluble vitamins. For vitamin D, recom-
Recommendations for vitamin D five to ten times that which is specified in the recent DRI-RDAs have been advanced (64, 65). For vitamin K, Booth et al. (66) have cited evidence for its role in bone health and mineralisation, important to counteract the osteoporotic process of aging. The benefits of intakes of vitamin E in preventing coronary heart disease have been documented (67), and doses of the vitamin well in excess of the RDA or that could ever be achieved from dietary sources are immuno-regulatory in the elderly (68). To this constellation, we can add a series of observations on the health promotional values of lycopene and the oxo-carotenoids, lutein and zeaxanthin (69, 70).

Since the full efficiency of fat assimilation by the intestine requires a stimulation of lipase and bile salt secretion and intestinal cell lipoprotein packaging, the absorption of fat-soluble nutrients, taken fasting on an empty stomach, is even more variable and limited. Promotion of the supplementation of mature adults with fat-soluble nutrients might entail their separation from water-soluble vitamins and minerals, and their combination in a fat-based elixir or snack. Only vitamin A among the fat-soluble nutrients must be used sparingly and with caution in older individuals (71). The resolution might come in using provitamin A carotenoids as the vitamin A source, thus taking advantage of individual regulation mechanisms to adjust hepatic vitamin A reserves. In such an oil base, all of the mentioned compounds should have maximal bioefficacy.

The challenge for the carotenoid research community

Extend the use of stable isotope research into developing countries

The issues related to the true efficiency of bioconversion of plant provitamin A languished in confusion for decades in part because of inappropriate design and interpretation of research (29). Policy-makers relied on calculations of “apparent absorption” of carotenoids from metabolic balance studies without realising that colonic degradation produced a systematic overestimation of the absorption values. The view that the poverty in developing countries relegates the scientific effort to the most rudimentary technology has to be challenged (71). In fact, Jim Olson was an enthusiastic advocate of the application of stable isotopes to study vitamin A metabolism in the Third World (72, 73). In recent years, his vision has been exemplified (74), but continued investment in higher technology, guided by the nature of the scientific question (not that of the geographic setting), is required to examine the efficacy of provitamin A-based solutions for developing country hypovitaminosis A.

Is the “low-responder” status a result of genetic polymorphism?

Effective public health measures require not that a population is covered “on average”, but that a suitable level of protection is achieved by each and every vulnerable subject. A potential fly in the ointment of substituting oil-based provitamin A for preformed vitamins in supplementation and fortification is the unresolved issue of “low responders”. These are a subsegment of the population that seems to represent a second (lower) distribution for the ability to effect bioconversion. Now that the enzymatic and molecular basis of β-carotene cleavage has been so well reviewed (13), mutations and polymorphism emerge as viable explanations for heterogeneity in bioconversion capacity. Wyss (13) states: “A considerable part of the population is known as ‘low responders’, these people have unusual high β-carotene plasma levels. They only cleave a small amount of the absorbed β-carotene to form vitamin A. It would be interesting to see if mutations in the coding sequence of the dioxygenases or polymorphisms in the promoter region are responsible for the difference seen in β-carotene cleavage among the population.” In terms of public policy, this has a practical context, as such individuals might have to be identified and selectively assigned to provitamin A therapy in any shift over to general reliance on provitamin A.

Carotenoid actions in developing countries

James Allen Olson was a man for all seasons. His paradigm of “function” and “action” (3) sets the tone for the professional community. The challenge for the carotenoid research community is to institutionalise the dual vision of “function” and “action” when it comes to the investigative agenda for the next two decades.
For the 20% of the world’s population living in privileged conditions, abundant preformed vitamin A from animal products and food fortification in spreads and breakfast cereals protects most of the consumers (75). For the remaining 80%, this is still a matter of sight and life, or blindness and death. The ironic novelty, however, is that for this 80% of the population, issues of chronic disease prevention are beginning to rise (76). The actions of carotenoids take on a new meaning while the functions of provitamin A remain eternal.

In a series of studies performed in Tanzania, morbidity and mortality in children were inversely related to the calculated vitamin A intake of the habitual diet, although they were unaffected by large-dose supplements of preformed vitamin A (77, 78). Thus, either total food vitamin A activity is a marker for bioactive constituents of the diet or the substances accounting for vitamin A activity are playing a role aside from their provitamin A role.

Gastric cancers have long been the primary malignancy for adult populations of low-income countries. Abundant intakes of non-provitamin A carotenoids have been associated with lower incidences of stomach malignancy (2, 69).

Finally, the health and antioxidant protection of the ocular retina is important in preterm birth. Lutein and zeaxanthin are important to this aspect of preterm nutrition (79). As survival of smaller and smaller infants proceeds, this issue will loom larger in both developed and developing countries.

The professional community must continue to see its research and food technology in terms of the dual mission of carotenoids as dietary precursors of vitamin A (function) and carotenoids for their bioactive properties in preservation of physiology and prevention of illness (actions). An inspiration for this mission will forever be the life and works of James Allen Olson who clarified important biological and conceptual points and cleared the channels for decades of consolidation investigations to proceed.

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**A Digest of Recent Literature**

Donald S. McLaren*

**Introduction**

During the period of more than a quarter of a century that I have been producing a regular digest, first for the Xerophthalmia Club Bulletin, and now SIGHT AND LIFE Newsletter I have tried not only to produce a faithful abstract of a paper but also to make personal comments or criticisms where these seemed to be appropriate.

It is interesting to note how the content of the digest has tended to change over this period. In the 1970s and 80s there was still a strong emphasis on xerophthalmia and various aspects of the effects of vitamin A deficiency on the eyes. Since then increasingly attention has been paid to advances in knowledge of the adverse effects of vitamin A deficiency on other systems of the body, its relationships to various infections and to its important role in childhood mortality and morbidity, and more recently maternal mortality. From about the same time the fundamental role of retinoic acid with nuclear receptors in gene transcription and other aspects at the molecular biology level demanded attention. It would clearly be inappropriate for someone untutored in this field to be so bold as to make critical comments, but among our readership there are surely those who have this competence and such contributions would be welcomed.

Some further remarks might be made on the subject of comments and criticism. Most scientific and medical journals offer their readership an opportunity to contribute appropriate correspondence, as does this newsletter, and the topics covered in this digest surely offer a great opportunity to have your views broadcast widely. To a close observer it should be evident that in the VAD field insufficient attention is being paid to some of the criteria and standards that have been formally adopted by the scientific community working in this field. Two examples, one clinical and one biochemical, come readily to mind and will serve to illustrate the point that is being made here.

The clinical example relates to the use of the various eye signs indicative of xerophthalmia. These have been fully described and their use commented upon in detail, originally in the two technical reports of WHO (1976 and 1982) but frequently more recently in other authoritative documents. The criteria and standards set, although to some extent arbitrary, are based on solid science and are universally agreed to have stood up to the test of time. Nevertheless papers continue to be approved by referees, accepted by editors, and assimilated by interested scientists that flagrantly flout in all kinds of ways the approved methodology. Editors and referees are to blame, but it is highly unlikely that these guilty parties will be reading this!

The biochemical example relates to a phenomenon that was first observed more than half a century ago. This is the fact that during what is known as the “acute phase response” (APR) retinol and some other plasma constituents diminish, often dramatically. This renders it in these circumstances unreliable as an indicator of vitamin A status. Many young children in developing countries who are being investigated for suspected subclinical vitamin A deficiency will undoubtedly be subject to various infections and infestations, minor trauma and other ailments that will activate in them to some degree the APR. The criticism raised here applies, of course, not only to serum retinol concentration per se but also to any test in which it is used; i.e. relative dose response (RDR) and modified relative dose response (MRDR). Retinol-binding protein (RBP) which can serve as an appropriate surrogate for retinol may not be so affected (see paper by Semba et al below). As far as I am aware the effect of the APR on an indicator of nutritional status has only been investigated in the case of retinol and albumin (for protein status). What about other constituents of plasma like cholesterol, triglycerides and other lipids, those employed to assess iron status, other vitamins and essential elements? Perhaps some helpful readers can shed some light!

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Blindness


This editorial comments for its audience of ophthalmologists on the United Nations VISION 2020 programme. Worldwide approximately 45 million persons are blind and 135 million visually impaired - so that they cannot read newsprint with either eye even with the best possible spectacles. In two thirds the impairment is avoidable, with three quarters being due to cataract. It is estimated that the number of blind and visually impaired will double by 2020 unless concerted action is taken.

Vitamin A deficiency is included among the causes of “preventable blindness” and considered to have “largely a public health solution”. VISION 2020 focuses on three main areas: control of major causes of blindness; human resource development; and infrastructure and appropriate technology development. Vitamin A deficiency along with other causes of childhood blindness is included as a major cause of blindness to receive priority. The other major causes are cataract, trachoma, onchocerciasis, and refractive errors and low vision. (DSM, the editorial concludes - “ophthalmologists throughout the world have a role of leadership and responsibility in implementation of VISION 2020”. This was true 30 years or so ago, but here there is no recognition of the changes that have occurred since then. Xerophthalmia as a cause of childhood blindness has been greatly reduced over this period. However, recognition has taken place as to how subclinical vitamin A deficiency greatly increases the risk of young child and maternal mortality and morbidity. This outdated and outdated approach to this serious and widespread health problem, which incidentally is reflected in major medical textbooks (see Newsletters 2/2001 p 10; 3/2001 p 43) can only impede the implementation of measures for its control.


Comparison of national blindness surveys conducted in the Gambia in 1986 and 1996 showed an increase in blindness and visual impairment from non-trachomatous opacity. This study aimed to investigate the causes and gives details of the total of 154 patients (39 bilateral and 115 unilateral opacities), out of 13046 people examined. It is stated in the Methods section that “aetiology of corneal opacity was ascribed on the basis of history and examination”. No further details are given and no mention is made of the WHO Technical Reports on Xerophthalmia (see Introduction above) or of any other guidelines for its detection. Throughout the combined diagnosis of measles/vitamin A deficiency was used. “Of the aetiological categories that were identified, the most common was corneal infection, followed by trauma, then measles/vitamin A deficiency, and then harmful traditional practices including coughing. Vitamin A deficiency and/or measles was thought to be responsible for corneal opacity in an estimated 1200 patients. Vitamin A-rich foods such as mango, papaya, and red palm oil are readily available throughout Gambia and nutritional education together with a measles immunisation program which already reaches 97% of the population should see this problem decreasing in the future”. (DSM - There are several disappointing features to this report. It comes from a reputable institution in the UK and it so happens that I was responsible for teaching the subject of nutritional blindness there for a number of years spanning the 1996 survey. On no occasion was I consulted about the vitamin A aspects of the studies. Xerophthalmia and measles are not inevitably associated and these two diseases have their own distinctive characteristics with regard to both eye examination and history. It is evident that none of these features was taken into account in coming to a diagnosis. Other aetiologies may also accompany xerophthalmia, especially traditional practices and corneal infection, but the possibility of these concurrences is not allowed for. Another feature that causes serious doubts as to the value of the study for making conclusions with regard to past trends, the present situation, and recommending future action is the fact that the single most common category for both bilateral (56%) and monocular (34%) cases was “unknown”).

“Environmental risk factors in congenital malformations of the eye” by Hornby SJ, Ward SJ,
Gilbert CE, Dandona L, Foster A, Jones RB. Ann Trop Paediatr 2002, 22: 67-77 (Dept Epidemiology/Int Eye Hlth, Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK). Coloboma was selected as a common congenital malformation of the eye. It consists of a defect in a tissue, in this case the iris, which extends inferiorly and results from the failure of part of the fetal fissure to close. There is a suggestion that they are more common in India than elsewhere. Information on the possible role of environmental factors, birth order, night blindness, drug use, maternal illness in pregnancy, rubella antibodies and exposure to agricultural chemicals was recorded. Through hospital records and community-based rehabilitation programs in Andhra Pradesh children with colobomata were recruited from schools for the blind. 83 mothers of children with colobomata were interviewed. 43% of parents were consanguineous, 19% had a family history and the frequency of coloboma was highest in second-born children. 16% of mothers had a history of night blindness while pregnant with the affected child, 8% took medication during the first trimester, 13% reported exposure to agricultural chemicals. (It is very difficult to draw firm conclusions from studies of this nature. The number of cases is small and by the nature of the sources of cases it is clear that they are not representative of any larger grouping, posing difficulties for the making of general conclusions. There were no controls with which to compare the data from the study group. It is not clear why schools for the blind should house children with colobomata. Unless they are accompanied by other more serious ocular defects colobomata do not cause blindness and may only slightly impair vision in one eye).

Community studies

“Activating the community for nutritional improvement” by Devadas RJ. Food Nutr Bull 2002, 23: 119-132 (The author died on March 17, 2002. For several decades she was one of the leading figures in the field of food and nutrition in India. This issue carries a fitting tribute. This article summarises the community aspects of her valuable contributions).

Among the many studies carried out and described here is a section dealing with vitamin A deficiency. 500 households and the local market were surveyed and a seasonal calendar for locally available vitamin A-rich foods was developed. This calendar, containing information on vitamin A value and cost, is used in educational programs. Another study showed that the introduction of crude red palm oil markedly reduced clinical signs of vitamin A deficiency in young children. The encouragement of development of backyard gardens had a similar beneficial effect.


This paper reports on the activities in Vietnam over the period from 1985 - 1998 to control a serious public health problem of VAD. Nationwide there was instituted universal vitamin A capsule supplementation according to WHO criteria, dietary improvement through promotion of food production and consumption at the family level, and development of community nutrition education activities. Success is claimed for these measures but the data provided do not warrant this conclusion. (DSM: 1) in the 1985-88 survey neither XN nor X1B exceeded the WHO criteria for a public health problem. Although X2/3 and XS did, these criteria for complex reasons cannot on their own be relied upon for a community diagnosis. 2) Several tables of hospital data on “xerophthalmia”, without any definition with regard to different stages, are presented as evidence of improvement. Such data are notoriously unrepresentative of the community at large. 3) Recent data (1995 -8) on serum retinol of children < 5 years show more than 10% have low levels (<0.70 µmol/l). 4) Similar data for breast milk retinol show low levels (<1.05 µmol/l) in 40% (1995), 49% (1997), and 59% (1998) - suggesting the continuing presence of a VAD problem and possibly deteriorating. 5) Finally, as so often happens, no account is taken of the likely influence, for good or bad, of many other factors, all uncontrolled and many unknown.)

A cross-sectional study of 128 healthy and 230 malnourished (PEM) preschool children included serum retinol and CIC-T. Low vitamin A status (serum retinol <10 µg/dl) and abnormal CIC-T were similar (7.3%) and (6.2%) in all children. VAD was present in 6.3% of healthy and 7.8% of malnourished children. Children aged 3 years accounted for 70% of VAD cases. Measles, persistent diarrhoea, and wasting predisposed to VAD. CIC-T was predictive of serum retinol of 10 µg/dl with sensitivity of 83.3% and specificity of 73.3%, suggesting its value as a screening tool.


This study tested the value of RBP as a surrogate for serum retinol and whether it was influenced by factors such as inflammation and protein status (see Introduction above). In 236 preschool children the Spearman correlation coefficient between plasma RBP and retinol concentrations was 0.55 (p < 0.0001). By linear regression 0.70 µmol/l retinol was equivalent to 0.69 µmol/l RBP. With these cut-offs for defining VAD RBP had a sensitivity and specificity of 75% and 63.2% respectively. The correlation between RBP and retinol was not affected by inflammation markers or serum albumin (marker for PEM), suggesting RBP is a suitable retinol surrogate.


This was a meta-analysis including 492 children, aged 6 months to 13 years treated with vitamin A and 536 given placebo, in 6 trials. There was no significant reduction in the incidence of pneumonia or diarrhoea, but a 47% reduction in group in those given vitamin A. A significant decrease in duration of diarrhoea, pneumonia, hospital stay and fever was reported. Vitamin A should be given to all hospitalised cases of measles.

"Randomized double-blind trial of the effect of vitamin A supplementation of Indonesian pregnant women on morbidity and growth of their infants during the first year of life" by Schmidt MK, Mussulumi S, Schultink W, West CE, Hautvast JGAJ. Eur J Clin Nutr 2002, 56: 338-46 (Division of Human Nutrition, Wageningen University PO Box 8129, 6700 EV Wageningen, Netherlands).

Supplementation comprised 120 mg iron and 500 µg folic acid with or without 4800 RE vitamin A. Additional vitamin A had no beneficial effect on infant growth over 1 year, immunisation coverage, feeding mode or morbidity. Infants with serum retinol >0.70 µmol/l had greater growth than those with lower levels. Serum retinol was not associated with morbidity.

"Effect of vitamin A administration at expanded program on immunization contacts on antibody response to oral polio vaccine" by Bahl R, Bhandari N, Kant S, Molbak K, Ostergaard E, Bhan MK. Eur J Clin Nutr 2002, 56: 321-5 (Department of Pediatrics, All India Institute of Medical Sciences, Center for Diarrheal Disease/Nutrition Research, Ansari Nagar, New Delhi 110029, India).

Vitamin A given to mothers in the postpartum period and their infants with oral polio vaccine did not interfere with the antibody response to any of the three polioviruses given and enhanced the response to polivirus type 1.

"Effect of vitamin A supplementation on measles-specific antibody levels in Guinea-Bissau" by Benn CS, Balde A, George E, Kidd M, Whittle H, Lisse IM, Aaby P. Lancet 2002, 359: 1313-4 (Danish Epidemiology Science Centre, Statens Serum Institute, Copenhagen, Denmark).

This group previously reported vitamin A with measles immunisation at 9 months increased measles-specific antibody concentrations at age 18 mo. Reexamination at 6-8 years showed fewer vitamin A supplemented children had non-protective antibody concentrations (µ = 0.0095) and among those with protective antibody levels they tended to have higher geometric mean antibody titres (µ = 0.09).

"Vitamin A supplementation and human immunodeficiency virus type 1 shedding in women: results of a randomized clinical trial" by Baeten J, McClelland RS, Overbaugh J et al. J Infect Dis 2002, 185: 1187-
91 (University of Washington, 325 Ninth Avenue, Box 359909, Seattle, WA 98104-2499, USA). Using a variety of tests no evidence was found for any effect of supplementation on infectivity of women infected with HIV-1.

“Vitamin A deficiency and genital viral burden in women infected with HIV-1” by French AL, Cohen MH, Gange SJ et al. Lancet 2002, 359: 1210-2 (Division of Infectious Diseases, Durand 115, Cook County Hospital, Chicago IL 60612, USA). In 301 women infected with HIV virus no association was found between retinol status and genital HIV-1 load. This lends support to other studies that report no association between retinol deficiency and perinatal HIV-1 transmission.

“Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years : Randomised controlled trial in an urban slum” by Bhandari N, Bahl R, Taneja S et al. Brit med J 2002, 324: 1358-61 (Dr MK Bhan, Department of Paediatrics, AIIMS, Ansari Nagar, New Delhi 110029, India). 2482 New Delhi slum children aged 6 to 30 months participated. Both groups received vitamin A, one received additional zinc. In this latter group the incidence of pneumonia was substantially lower.

Basic studies

“Vitamin A supplementation ameliorates butyric acid-induced intestinal mucosal injury in newborn rats” by Nafday SM, Green RS, Chauvin SN et al. J Perinat Med 2002, 30: 121-7 (Dr J Lin, Lack/Lucy Clark Department of Pediatrics, Division of newborn Medicine Box 1508, One Gustave L Levy Place, New York, NY 10029-6574, USA). The lesion induced simulated neonatal necrotizing enterocolitis. Supplementation improved daily weight gain, and at sacrifice colon wet weight was heavier and histology injury scores for ileum and proximal colon were better.

“Mechanism of protection induced by vitamin A in falciparum malaria” by Serghides L, Kain KC. Lancet 2002; 359: 1404-6 (Tropical Disease Unit, Division of Infectious Diseases, Department of medicine, Toronto General Hospital, Ontario M5G 2C4, Canada). Supplementation with vitamin A potentiates host resistance to malaria, but the underlying mechanism is unknown. The effects of 9-cis-retinoic acid were tested on various aspects of the immune process. These included CD36 expression (a mediator of the phagocytosis of non-opsonised parasitised erythrocytes), non-opsonic phagocytic clearance of parasitised erythrocytes, and TNFalpha (tissue necrosis factor) production in human monocytes and macrophages. This study found reduced secretion of TNFalpha, upregulated CD36 expression, and increased phagocytosis of Plasmodium falciparum-parasitised erythrocytes. The resulting enhanced parasite clearance and inhibition of excessive proinflammatory responses might partly explain the beneficial effects of vitamin A supplementation in malaria.

“Oxidative stress-independent depletion of epidermal vitamin A by UVA” by Sorg O, Tran C, Carraux P, Didierjean L, Falson F, Saurat J-H. J Invest Dermatol 2002, 118: 513-8 (Department of Dermatology, University Hospital, Geneva, Switzerland). In hairless mice epidermal vitamin A is markedly decreased fol-
lowing a single exposure to UVB. In this study the effect of UVA exposure was similarly studied. UVA exposure induced lipid peroxidation as well as reducing vitamin A content, not by oxidative stress but probably by a photochemical reaction in which UV radiations at about 325 nm are involved.

“Prevention of vitamin A teratogenesis by phytol or phytanic acid results from reduced metabolism of retinol to the teratogenic metabolite, all-trans retinoic acid” by Arnhold T, Elmazar MMA, Nau H. Toxicol Sci 2002, 66: 274-282 (Zentrum Lebensmittelwissenschaften, Tierärztliche Hochschule Hannover, Germany). Phytol and phytanic acid, ineffective when administered alone, did not potentiate the teratogenicity induced by retinol or retinoic acid (RA). In fact they effectively blocked the teratogenic effects of retinol by markedly reducing the metabolic production of RA. It is suggested that phytol and phytanic acid may be useful for the prevention of vitamin A teratogenicity.

“Expression of cellular retinol- and retinoic acid-binding proteins in normal and pathologic human parathyroid glands” by Melhus H, Li Q, Nordlinder H, Farnebo LO, Grimelius L. Endocr Pathol 2001, 112: 423-7 (Department of Medical Sciences, Uppsala University Hospital, S 751 85 Uppsala, Sweden). The group previously reported human parathyroid gland as a target organ for vitamin A. Stellate cells were identified in this study. CRABP 1 (cellular retinoic acid binding protein) was present in cytoplasm, cell membranes, and nuclear membranes of normal glands, but only exceptionally in nuclear membranes of abnormal glands. Since RA inhibits the secretion of parathyroid hormone and CRABP 1 is thought to play a key role in regulating the amount of RA available to interact with specific nuclear receptors, these results suggest impaired transport of RA to cell nuclei as a possible cause of hyperparathyroidism.

“Expression studies of key adipogenic transcriptional factors reveal that the anti-adipogenic properties of retinol in primary culture human preadipocytes are due to retinol per se” by Machinal-Quelin F, Dieudonne MN, Paquin P, Subirade M. Int J Cosmet Sci 2001, 23: 299-308 (Service de Biochimie, Centre Hospitalier Intercommunal, 78303 Poissy Cedex, France). This study was designed to investigate the mechanisms of the anti-adipogenic effect of retinol previously reported by this group. It was shown that retinol per se inhibits the adipo-conversion of human preadipocytes and it is suggested that at least in part inhibition of C/EBP (alpha) transcriptional activity is involved.

“Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: application for the protection of retinol” by Beaulieu L, Savoie L, Paquin P, Subirade M. Biomacromolecules 2002, 3:239-248 (STELA Dairy Research Centre, Faculty of Agricultural Science and Alimentation, University of Laval, Quebec, G1K 7P4, Canada). Full technical details are given of a new method of producing whey protein beads to protect sensitive molecules like retinol.

“Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway” by Stephensen CB, Rasooly R, Jiang X, Ceddia MA, Weaver CT, Chandraratna RAS, Bucy RP. J Immunol 2002, 168: 4495-503 (US Department of Agriculture Western Human Nutritional Research Center and Nutrition Department, University of California, Davis, CA 95616, USA). It has been shown that vitamin A deficiency diminishes Th2-mediated Ab responses and that treatment with RA or high level dietary vitamin A enhances such responses. This study showed that stimulation of the RXR pathway enhances Th2 development, perhaps by affecting the relative expression of pertinent transcription factors, cytokines, and cytokine receptors.

“Vitamin A deficiency promotes bronchial hyperreactivity in rats by altering muscarinic M(2) receptor function” by McGowan SE, Smith J, Holmes AJ, Smith LA, Businga TR, Madsen MT, Kopp UC, Kline JN. Am J Physiol Lung Cell Mol Physiol 2002, 282 : 1031-9 (Department of Veterans Affairs Research Service, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA). This study was undertaken in an attempt to contribute to the understanding of lung infections in developing countries. VAD led to bronchial constriction developing at lower concentrations of inhaled methacholine. The function and abundance of muscarinic receptors were reduced in VAD rats. Retinol and its congeners
may be required to regulate bronchial responsiveness in addition to maintaining a normal bronchial epithelium.


It has been shown that retinoids modulate nephrogenesis in a dose-dependent manner in vitro and in vivo. Midkine (MK) is a retinoic acid-responsive gene for a heparin-binding growth factor. This study showed that MK is implicated in the regulation of kidney development by retinoids. It is also suggested that MK plays an important role in the molecular cascade of the epithelial conversion of the metanephric blastema.


The study showed that thyroid hormones and vitamin A are coregulators of the UGT1 family expression, without affecting the UGT2 family. By modifying activity and expression of the bilirubin isofom, a member of the UGT1 family, thyroid hormone reduced the glucuronidation of T4 and rT3.


Vitamin A is important for immune function and deficiency is associated with adverse pregnancy outcome. This study showed that maternal vitamin A deficiency is associated with abnormal placental apoptosis induced by neutrophil derived TNF-(alpha) acting through the TNFR1 (p55) and/or a change in the bcl-2/bax ratio in the trophoblast giant cells. These changes may underlie the effects of vitamin A deficiency on fetal development.

**Carotenoids**


A detailed profile, not including cis-isomers, of carotenoids by HPLC is given. The total vitamin A value is 432 µg RE/100g fresh sample. (In the Sight and Life Manual page 11 another sample of cucurbita contained 862 µg and dark green leafy vegetables were also higher at 685 µg).


The vitamin A activity of β-carotene is variable and surprisingly low in women. The activity in men is still uncertain. This study used a double-tracer test-retest method in 11 healthy men. Only 6 men had sufficient plasma concentrations of D6 β-carotene and D3 retinol that could be measured. The authors conclude that the activity, even when measured under controlled conditions, was surprisingly low and variable.

“Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit Momordica cochinchinensis (gac)” by Vuong Le T, Dueker SR, Murphy SP. Am J Clin Nutr 2002, 75: 872-9 (Department of Nutrition, University of California, Davis, CA 95616, USA).

This fruit is commonly used in Vietnam with rice (xoi gac). Of local sources it has the highest known vitamin A activity. 185 preschool children with low haemoglobin were assigned to one of three groups - 1) fruit group received xoi gac that contained 3.5 mg β-carotene per serving; 2) powder group received rice mixed with 5.0 mg synthetic β-carotene powder; 3) control group received rice without fortification. Groups 1 and 2 had significantly higher plasma β-carotene than the control group. The plasma retinol was significantly
higher in group 1 than in groups 2 or 3. In children with Hb < 110 g/l group 1 had significantly higher increase in Hb than control but not different from group 2. Vitamin A and Hb status were both improved by gac supplementation of children’s diet.


Dietary fat is required for the absorption of β-carotene and the minimum has been reported to be as little as 3-5 g. However, necessary long-term studies have not yet been undertaken. Body stores need to be measured and this can now be done by the use of stable isotope dilution methods. Animal studies have shown that higher liver vitamin A concentrations have resulted when higher dietary fat accompanied the carotenoid. Other factors also need to be investigated, such as type of fat ingested, physico-chemical properties of carotenoid source, amount of carotene ingested, whether fat and carotene sources are provided in the same meal, the presence of helminth infestations, age and vitamin A status. (Clearly much work is required before these important questions can be answered).

SIGHT AND LIFE homepage on CD, update June 2002

Slides and presentations

SIGHT AND LIFE has prepared different slide sets and presentations. The standard slide set (S-slides, 57 slides) was prepared along with the manual while the P-slides (54 slides) were collected to complete presentations as given by the three examples P1, P2 and P3. In order to allow most flexible use for the composition of presentations slides are given in the formats JPG, PDF and PowerPoint (PPT). Also the whole sets as well as the individual slides are given.

Besides making available all slides as PPT we also have adapted the format for presentation digitally. However, we still have hardcopies of both series (P-slides only a limited number) available.
The Task Force SIGHT AND LIFE is a humanitarian initiative by F. Hoffmann-La Roche Ltd to help combat nutritional blindness and all forms of vitamin A deficiency. A low intake of vitamin A, termed subclinical deficiency, is impairing the health of children in numerous developing countries. Increased health risk with susceptibility to infections and increased child mortality are the consequences.

SIGHT AND LIFE has supported numerous locally and internationally active organisations. Blindness prevention, research and application projects in many countries in Africa, Asia and Latin America have been sponsored. Vitamin A, mostly in the form of capsules, grants, information and educational materials, such as books, posters, reprints etc, are contributed. Furthermore, SIGHT AND LIFE gives technical assistance, if necessary, and promotion of training and education aims to increase local knowledge and expertise in order to work towards sustainable improvement of nutrition.

SIGHT AND LIFE publishes educational materials as well as a Newsletter to disseminate knowledge on vitamin A and nutrition and to give relevant information on programs and scientific news.