Chapter 29

Historical aspects of the major neurological vitamin deficiency disorders: overview and fat-soluble vitamin A

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VITAMINS AND DIETARY DEFICIENCY DISEASES

Introduction
Vitamins are organic micronutrients that are essential to normal growth and metabolism, and are present in only minute amounts in natural foodstuffs. Historically, vitamin deficiency disorders have been major causes of neurological morbidity and mortality throughout the world, often affecting large segments of malnourished populations. The neurological presentations vary with the deficiencies involved, but included dementia, amnestic confabulatory states, delirium, acute psychosis, blindness, eye movement abnormalities, ataxia, myelopathy, polyneuropathy, and congenital neural tube defects.

Although diseases that are now recognizable as vitamin deficiency disorders have been known for millennia, the “vitamin doctrine” was not developed until the early 20th century (Hopkins, 1929/1965). Prior to this development, vitamin deficiency disorders were generally attributed to toxic or infectious causes, the most powerful pathophysiological paradigms of the late-19th and early-20th centuries.

Since the initial development of the vitamin doctrine, there has been an explosive growth in our understanding of cellular metabolism, including the coenzyme functions of many of the vitamins. In some cases we now possess a fairly complete pathophysiological understanding of the development of specific neurological vitamin deficiency disorders, are able to identify such disorders early, are able to treat and often cure people with such disorders when identified early using synthetic forms of the vitamins, and most importantly are able to prevent these disorders in the first place by targeted supplementation and food fortification. As a result, incidence, prevalence, case fatality, and mortality of neurological vitamin deficiency disorders have declined dramatically in developed countries since the middle of the 20th century. Endemic forms of these disorders have been either eliminated from or greatly curtailed in developed countries, and the relatively rare residual cases generally reflect individual predispositions because of altered intake (e.g., alcoholics, food faddists, total parenteral nutrition), malabsorption (e.g., pernicious anemia, chronic diarrhea, iatrogenic causes, alcoholism), and altered metabolism or abnormal utilization (e.g., medications, coincident disease). Unfortunately, high rates of many neurological vitamin deficiency disorders persist in developing countries and other populations besought with war, famine, and poverty.

This chapter begins a historical review of vitamin deficiency disorders causing neurological illness, and includes a general overview of the historical origins of the vitamin doctrine and an historical review of fat-soluble vitamin A. Study of the history of neurological vitamin deficiency disorders can be rewarding from several vantage points, and can augment understanding of normal neurochemistry and neuropathophysiology, the interrelationships between neurological and systemic illness, neurotherapeutics, neuroepidemiology, and neurologically-oriented social medicine and public health. Seldom in the case of neurological disorders is such a breadth and depth of medical understanding available to help prevention and treatment efforts.

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Components of a physiologically complete diet and Hopkins’ “accessory food factors”

In the late-19th century, a physiologically complete diet was believed to require only a sufficient amount of proteins, carbohydrates, fats, inorganic salts, and water. However, as early as 1880 and 1881, in studies for a doctoral thesis, Russian physician Nicolai I. Lunin (1853–1937) in Dorpat (now Tartu, Estonia) found that mice did not thrive or grow when fed on purified diets containing only these constituents, although he made no effort at identifying any missing essential factors in the inadequate diets (Hopkins, 1929/1965; Voss, 1956; Rosenfeld, 1997).

In 1905, Cornelius Pekelharing (1848–1922) in Utrecht performed similar experiments and achieved similar results, but found that animals that received milk instead of water thrived. Pekelharing suggested that there was an unrecognized substance in milk, present in very small amounts, which was necessary for the animals to adequately utilize the other dietary components—a clear statement of what would later be called the “vitamin doctrine” (Hopkins, 1929/1965; Rosenfeld, 1997).

Unfortunately, the early reports by Lunin, Pekelharing, and others attracted little attention until the work of British biochemist Frederick Hopkins (1861–1947) at Cambridge University was published in 1912. From 1906 to 1912 Hopkins conducted similar feeding experiments with young mice and rats (Hopkins, 1929/1965). Hopkins’ experiments were notable for providing careful observations under controlled laboratory conditions with “analytical control of materials, for the meticulous weighing and measurement of the quantities of food consumed, for the many days over which animals were studied . . . for consistent recording of the weight and growth of the rats, and for . . . independence from current dogma” (Weatherall, 1990, p. 123). Hopkins found that rats fed purified mixtures of protein (casein) or amino acids, carbohydrates (sucrose, starch), fats (butter or lard), mineral salts, and water failed to grow or even lost weight and died, unless the diet was supplemented with small amounts of milk (Hopkins, 1906, 1912, 1929/1965). Hopkins concluded that milk contained “accessory food factors” that are required in trace amounts for normal growth (Hopkins, 1912, 1929/1965). Although there were clearly others who anticipated this work, and although other investigators had difficulty reproducing and confirming Hopkins’ results, Hopkins shared the 1929 Nobel Prize in Physiology or Medicine for his contributions to the “discovery of the growth-stimulating vitamins” (Hopkins, 1929/1965).

From Funk’s “vitamine” to vitamin

In 1911 and 1912 Polish chemist Casimir Funk (1884–1967), then working at the Lister Institute for Preventive Medicine in London, proposed that the active dietary factor that was effective in the treatment of animal models of beriberi was a specific organic substance present in trace amounts—one of several trace dietary factors that were essential for life and which, when deficient, resulted in such diseases as beriberi, scurvy, rickets, and pellagra (Funk, 1911, 1912, 1922; Harrow, 1955). Funk had isolated a concentrate from rice polishings that seemed to be curative for polyneuritis in pigeons, and that his chemical analyses suggested was probably an amine. Because this substance appeared to be vital for life, Funk named it “vitamine” for “vital amine” (Funk, 1912).

Funk supposed that vitamines would all belong to the same chemical class, just as amino acids as the constituents of proteins are chemically related. However, Funk’s concentrates were primarily nicotinic acid, which was contaminated with small amounts of the anti-beriberi factor (thiamin). Nevertheless, Funk’s term was widely adopted and applied to a series of food substances, regardless of their chemical structures. Indeed, the introduction of the term and its popularization led to further research efforts internationally, as these dietary substances became recognized as clinically important beyond the prevention of some distant tropical diseases. In 1920, Jack Drummond (1891–1952) proposed that the “e” be dropped from “vitamine,” because there was no evidence that the essential dietary factors are amines; the revised term “vitamin” would then conform to a standard nomenclature convention, one in which substances of undefined composition end with the suffix “-in” (Drummond, 1920; Rosenfeld, 1997).

VITAMIN A DEFICIENCY: NIGHT BLINDNESS AND KERATOMALACIA

Introduction

Vitamin A is integral to sensory transduction and specifically the transduction of light for visual perception. Vitamin A is the precursor of the visual pigments within the rods and cones of the retina. In particular, a derivative of vitamin A, 11-cis retinal, is the chromophore within the G protein-coupled photoreceptor protein, rhodopsin, which is localized to the outer segments of rod cells in the retina. This transmembrane detector undergoes a conformational change in response to light, which activates an intracellular G-protein-coupled transduction cascade, and ultimately cellular responses that lead to visual perception. The
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study of this physiological process has not only greatly expanded knowledge of sensory transduction, visual perception, and dark adaptation, but has provided a prototypical model and tremendous insights into the functions of a large class of structurally related G protein-coupled cell receptors involved, for example, in detecting odorants, neurotransmitters, and hormones (Hargrave and McDowell, 1992; Filmore, 2004).

Because rhodopsin is available in greater quantities than any other G protein-coupled receptor, it has been studied in much greater detail than its fellow G protein-linked receptors (Hargrave and McDowell, 1992). Drugs targeting members of this integral membrane protein family now represent nearly half of all prescription pharmaceuticals and are a major focus of current drug development (Filmore, 2004).

The neurological disorder associated with vitamin A deficiency is night blindness, which has plagued malnourished populations for millennia, and remains a major public health problem in many countries around the world.

Description of night blindness and keratomalacia

Night blindness was recognized by the ancient Egyptians and the ancient Greeks (Dowling and Wald, 1958; Wolf, 1996). During the Roman Era, Galen (ca. 129–199 A.D.) clearly described “nyktalopia” as night blindness and recommended eating raw beef liver (now known to contain high concentrations of vitamin A) to correct the condition (Wolf, 1996). Chinese medical texts also described night blindness: Sun-szu-mo (7th century AD) recommended pig liver as a treatment (Wolf, 1996). Later, during European colonial expansion, several physicians—including Jacobus Bontius (1592–1631), the first European physician in the Dutch East Indies, and Willem Piso (1611–1678) in Brazil—described night blindness: Sun-szu-mo (7th century AD) recommended pig liver as a treatment (Wolf, 1996). In 1754, German physician C. A. von Bergen described widespread xerophthalmia (corneal dryness), and corneal epithelial defects (“silver scales on the cornea”), among Japanese children subsisting mostly on rice and barley, and further reported that liver and cod-liver oil were curative (Mori, 1904; Wolf, 1996).

The association between night blindness with corneal epithelial defects was recognized in the late-19th and early-20th centuries. In 1860, V. von Hubbenet first reported the association between night blindness and corneal epithelial defects (“silver scales on the cornea”), attributed this to an inadequate diet, and found it treatable with beef liver (Wolf, 1996). In 1862, Bitot reported foamy white spots (“Bitot spots”) on the corneas of children with night blindness (Wolf, 1996), and by 1863 concluded that night blindness and xerophthalmia are both manifestations of the same disorder (Bitot, 1863; Wolf, 1996, 2001). Similarly, in 1913, S. Ishihara recognized the association of night blindness and keratomalacia in malnourished children (Ishihara, 1913; Wolf, 1996).

In 1919, during World War I, E. Bloch studied malnourished Danish children with night blindness and keratomalacia, who had subsisted on fat-free milk, oatmeal, and barley soup (Bloch, 1919; Wolf, 1996). In a critical experiment, Bloch prospectively studied 32 institutionalized toddlers (aged 1–4 years), half of whom received animal fat (whole milk and butter),
while the others received vegetable fat (margarine). The animal fat group remained healthy, whereas 50% of the vegetable fat group developed corneal xerosis (Bloch, 1919; Wolf, 1996, 2001). All of the xerosis cases were rapidly cured with cod-liver oil. Bloch concluded that whole milk, butter, and cod-liver oil contain a fat-soluble substance that protects against xerophthalmia.

**Boll’s discovery of the visual pigment**

In 1876 and 1877, Franz Boll (1849–1879) observed a relationship between retinal color and light exposure: (1) the frog retina is paler after light exposure and can become completely colorless in direct sunlight; and (2) excised animal retinas that had been exposed to light are colorless, but the purple color is restored if animals are kept in the dark for a period of time after exposure to light before they are killed (Boll, 1876, 1877, 1877/1977; Baumann, 1977; Hubbard, 1977; Kühne, 1879/1977; Wolf, 2001). Boll concluded that light causes bleaching of the retinal pigment, and also suggested that the outer segments of the rods contain a substance that conveys an impression of light to the brain by a photochemical process.

**Kühne’s experiments with retinal preparations and rhodopsin**

Willy Kühne (1837–1900), professor of physiology at the University of Heidelberg, stimulated by Boll’s observations, began experimental studies of the retina in 1877, and continued these over a productive 5-year period, resulting in a series of 22 important papers (Kühne, Ewald and Kühne, 1877; Crescitielli, 1977). By the time he began his retinal studies, Kühne was already a renowned physiologist who had previously isolated and named the digestive protease trypsin from the pancreas, coined the word “enzyme,” and contributed greatly to the biochemistry of protein digestion (Wolf, 2001). Kühne pursued his retinal studies with similar zeal and with arguably even more important results.

In frogs, Kühne confirmed that visual purple (Sehpurpur) was bleached by light, but maintained its color in the dark, even after death. In a darkroom illuminated by red light, Kühne perfected a technique for isolating frog retinas, which remained purple in the dark but became colorless when exposed to sunlight (Kühne, 1879/1977; Wolf, 2001). In contrast to previous opinions (including Boll’s) that the retinal pigment is red, Kühne was adamantly that the rod pigment is in fact purple and named it visual purple. This bleaching process progressed through several different color stages (from purple to orange to yellow to buff and then to colorless), which Kühne correctly interpreted as indicating chemical transformations, because of the changing absorption spectra and the changing fluorescence of the different stages in ultraviolet light (Kühne, 1879/1977; Wolf, 2001). It is now known that rhodopsin (11-cis-retinal plus the protein opsin) is purple with blue fluorescence, an intermediate stage is orange with contributions of purple and yellow, all-trans-retinal-opsin is yellow, and the end-product of the light-bleaching process, free all-trans-retinol, is colorless with green fluorescence (Wolf, 2001).

The rate of photo-bleaching is dependent on temperature, as well as on the intensity and wavelength of light. Kühne correctly concluded that photo-bleaching is a photochemical process, and not a strictly thermal process, because infrared light is invisible and does not bleach the retinal preparations.

The photo-bleaching process was also found to be reversible and dependent of the retinal pigment epithelium (Kühne, 1879/1977; Wolf, 2001). An excised frog eyeball could be fully bleached after being kept in sunlight for 30 min, but the purple color still reappears in the dark, independent of the circulation of the blood. In contrast, an isolated, bleached retinal preparation separated from the retinal pigment epithelium is unable to regenerate the purple color. However, if the isolated bleached retinal preparation is placed onto an isolated retinal pigment epithelium, the purple color does regenerate, demonstrating unequivocally the essential role of the retinal pigment epithelium in pigment regeneration within the retina.

The process of regeneration of the visual pigment was puzzling though, as it was “extraordinarily slow” (too slow by far to account for the rapidity of changing visual sensation), and because it seemed to occur by two processes: one slow process different than a simple reverse of the bleaching process (which Kühne named neogenesis), and a relatively rapid process that could reverse the intermediaries (but not the final product) back to visual purple (which Kühne named anagenesis).

Kühne localized visual purple (rhodopsin) to the outer segments of the rods within “platelets” (now called “disks”), and observed that bile or bile salts dissolve the rods, bringing rhodopsin into solution where it could be further studied chemically (Kühne, 1879/1977; Wolf, 2001). Kühne further surmised that rhodopsin included a protein moiety, because it was a large molecule that, when in solution, did not diffuse through a semipermeable membrane and could be precipitated with ammonium sulfate (Kühne, 1879/1977; Wolf, 2001).

As early as 1877, Kühne likened vision to a repetitive photographic process (Wald, 1950). Kühne developed this idea further when he found he was able to see images bleached onto the retina. After having a
frog stare into a flame for 14 h, he isolated its retina and observed a bleached area in the shape of an inverted flame. Kühne found he could create other retinal images, which he called “optograms,” after having frogs or rabbits stare at a window for several minutes. Kühne’s optograms stimulated widespread speculation that such images could be used forensically to determine the guilty party from the retinal image depicting someone just murdered. Kühne initially dismissed such speculation; however, by 1880, when a young man was beheaded by guillotine in the nearby town of Bruscal, Kühne apparently had a different viewpoint and immediately retrieved the corpse, extracted the eyes in a dimly lit room screened with red and yellow glass, and within 10 min of the decapitation viewed one of the few reported human optograms (Wald, 1950).

Kühne’s work concluded with his proposed “optochemical hypothesis,” which attributed vision to a photochemical change in visual purple (rhodopsin), such that the chemical products or some process related to the chemical change is responsible for stimulating the visual cells and thereby conveying a visual image. Kühne’s prescient concept of photochemical transduction would later be shown to be largely correct, particularly with the important work of Wald and colleagues beginning in the 1930s.

**McCollum, Osborne, and Mendel, and the discovery of fat-soluble vitamin A**

In 1913, Elmer Verner McCollum, PhD (1879–1967) at the University of Wisconsin in Madison, with his volunteer assistant Marguerite Davis, discovered a fat-soluble accessory food factor, present only in certain fats and distinct from the water-soluble anti-beriberi factor (McCollum and Davis, 1913, 1914; McCollum, 1964; Day, 1997; University of Wisconsin College of Agricultural and Life Sciences). Rats fed on a diet of presumably pure protein (casein), carbohydrates, and salt grew well for several months, but then stabilized or lost weight, unless the diet was supplemented with certain “lipins” that were extractable with ether and were present in butter fat and eggs. McCollum felt that the lapse of growth after several months was due to the exhaustion of body stores of some unrecognized organic growth factor that was normally present in certain fats. Similar work was presented almost simultaneously by Lafayette Mendel (1872–1935) of the Sheffield Scientific School (affiliated with Yale University) and Thomas Osborne (1859–1929) of the Connecticut Agricultural Station in New Haven, who together also reported the high potency of cod liver oil in supporting growth under these conditions (Osborne and Mendel, 1913, 1914).

In 1913, Gowland Hopkins and A. Neville suggested that both American groups had been able to obtain growth with the diets of casein and lactose, because these substances had been incompletely purified and were contaminated with a water-soluble growth factor (Hopkins and Neville, 1913). In further studies, McCollum and Simmonds (1916) confirmed Hopkins’ suspicion and concluded that rats need both a water-soluble factor and a fat-soluble factor for growth. In 1916, McCollum and his graduate student Cornelia Kennedy labeled these “fat-soluble A” and “water-soluble B” (McCollum and Kennedy, 1916; Day, 1997).

McCollum initially believed that “fat-soluble A” was a single vitamin capable of treating both xerophthalmia and rickets (McCollum and Kennedy, 1916); however, in 1922, McCollum and colleagues demonstrated that cod-liver oil could be treated (by aeration at 100°C for at least 12 h) so as to eliminate its efficacy against xerophthalmia, while maintaining its antirachitic activity in rats (McCollum et al., 1922). Ultimately the anti-xerophthalmia factor was named vitamin A and the anti-rachitic factor was named vitamin D.

**Linking visual manifestations to vitamin A deficiency**

In 1913, Ishihara proposed that a “fatty substance” in blood is necessary for synthesis of both rhodopsin and the surface layer of the cornea, and that night blindness and keratomalacia develop when this substance is deficient. Shortly thereafter, Osborne and Mendel showed that, in the absence of the dietary supplementation with certain fats, rats developed weight loss, night blindness, and corneal ulcers, thus illustrating the most important physiological functions of vitamin A (i.e., support of vision, epithelial differentiation, and growth), and also providing an experimental model of human night blindness and keratomalacia (Osborne and Mendel, 1914; Wolf, 1996). In 1925, Fridericia and Holm directly linked vitamin A to night blindness in animal experiments: vitamin A-deficient rats, when light adapted (i.e., with light-“bleached” retinas) and placed in the dark, formed rhodopsin at a slower rate than did normal rats (Fridericia and Holm, 1925; Wolf, 1996). In 1929, Holm demonstrated the presence of vitamin A in retinal tissue.

In the late 1930s, Wald and colleagues began studies of experimentally induced human vitamin A deficiency and demonstrated a progressive rise in the visual threshold over a month-long period on a vitamin A-deficient diet, and subsequent rapid resolution of the deficit over 90 min upon ingestion of carotene (i.e., provitamin A) (Wald and Steven, 1939). A number of subsequent studies in animals and man further...
corroborated the association between vitamin A deficiency and night blindness and keratomalacia (Dowling and Wald, 1958; Wolf, 2002).

The isolation, chemical structure, and chemical synthesis of vitamin A

In attempts to isolate the active growth-promoting factor in fats, Osborne and Mendel (1914) obtained an active yellow oil from butter, egg yolks, and cod-liver oil, but not from lard or olive oil. Steenbock (1919) subsequently noted that active growth-promoting extracts from butter, egg yolks, or carrots are yellow, while active extracts from liver or kidney are white. In 1920, Steenbock and Gross suggested that fat-soluble factor A is associated with a yellow pigment and is converted in vivo to an active colorless form (Steenbock and Gross, 1920; Wolf, 1996). However, around the same time, Palmer and Kempster (1919) fed chickens a diet free of yellow pigments (i.e., white corn, skimmed milk, bone meal, and small amounts of pork liver) and discovered that the chickens nevertheless grew normally and laid eggs that (despite colorless liver) and discovered that the chickens nevertheless grew normally and laid eggs that (despite colorless egg yolks) produced normal chicks.

These confusing results were not resolved until 1930, when Moore, in experiments with rats, showed that the active yellow pigment extracted from plants, butter fat, or egg yolks (β-carotene) is a precursor (i.e., provitamin) that is indeed converted to an active colorless factor (vitamin A or retinol) in vivo and accumulates within the liver (Moore, 1930; Wolf, 1996). Later studies found that the enzymatic conversion of β-carotene to retinol occurs in the intestinal mucosa (Wolf, 1996).

In the 1930s, Paul Karrer (1889–1971) and colleagues at the University of Zurich isolated β-carotene (the main dietary precursor of vitamin A) and retinol, and determined their chemical structures (Karrer et al., 1931; Karrer, 1937/1966). Karrer shared the 1937 Nobel Prize in Physiology or Medicine “for his investigations on carotenoids, flavins and vitamins A and B2” (Karrer, 1937/1966). In 1947, Isler and colleagues completed the full chemical synthesis of vitamin A. The availability of vitamin A through food fortification and medicinal supplements virtually eliminated ocular vitamin A deficiency from developed countries by the second half of the 20th century (Underwood, 2004).

Wald and the visual cycle of vitamin A


Wald began work in the laboratory of biochemist Otto Warburg (1883–1970) at the Kaiser-Wilhelm-Institut (now the Max-Planck-Institut) für Biologie in Berlin-Dahlem, Germany, where he dissected animal retinas to obtain the light-sensitive compound rhodopsin (Dowling, 2002). Based on a chemical test and the absorption spectrum, Wald tentatively concluded that the retina contains vitamin A, a finding that he subsequently confirmed in the laboratory of Paul Karrer (1889–1971) at the University of Zurich (Warburg had suggested the transfer because Karrer had elucidated the structure of vitamin A in 1931) (Karrer, 1937/1966). After this, Wald moved briefly to the cellular metabolism laboratory of Otto Meyerhof (1884–1951) at the Kaiser Wilhelm Institute for Medical Research in Heidelberg, Germany, where Wald discovered the visual cycle of vitamin A (Wald, 1935a,b; Wolf, 2001).

Wald’s former mentor Hecht had proposed, based on the relationship between photosensitivity and time during dark adaptation, that dark adaptation “follows the course of a bimolecular reaction...[and that] visual reception in dim light is conditioned by a reversible photochemical reaction involving a photosensitive substance and its two products of decomposition” (Hecht, 1919, pp. 516–517). Wald confirmed Hecht’s prediction and showed that visual purple (rhodopsin) is decomposed by light into a compound he called “retinene” and a protein (later called “opsin”) (Wald, 1935a,b; Karrer, 1937/1966; Wolf, 1996). Retinene was subsequently shown by R. A. Morton and T. W. Goodwin to be the aldehyde of vitamin A, “retinaldehyde,” now known as “retinal” (Morton and Goodwin, 1944; Ball et al., 1946). Retinal could either recombine with opsin to reform rhodopsin, or could instead be converted to retinol (vitamin A). Because “vitamin A is the precursor of visual purple [rhodopsin], as well as the product of its decomposition,” Wald proposed that the “visual processes therefore constitute a cycle” (Wald, 1935b, p. 368).

During the 1930s, Wald also initiated studies of cone vision and was able to extract a red-sensitive pigment from chicken retinas that he called iodopsin (Dowling, 2000; Kresge et al., 2005). In the mid-1950s, Wald and colleagues showed that iodopsin bleaches to form retinal and a protein, which is different from the protein opsin in rhodopsin. Hence, Wald proposed the terms “scotopsin” for the opsin of rods, and “photopsins” for the opsins of cones (Wald et al., 1955b; Dowling, 2000).
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Wald’s group subsequently elaborated the enzymatic conversions of various elements in the rhodopsin system (Wald, 1948a; Wald and Hubbard, 1949, 1950; Wald and Brown, 1950; Hubbard and Wald, 1951; Hubbard, 1956; Dowling, 2000). By the mid-1950s, Wald, Ruth Hubbard (Wald’s second wife), and Paul Brown, with the assistance of several organic chemists, determined that the rhodopsin system is dependent upon an isomerization of retinal (a conformational change in the molecule), and specifically that the 11-cis isomer of retinal was the precursor of all visual pigments (Wald and Hubbard, 1950, 1955a; Hubbard and Wald, 1952; Hubbard et al., 1953; Brown and Wald, 1956; Hubbard, 1956, 1966; Oroshnik et al., 1956). 11-cis retinal is twisted and sterically hindered and stable only in the dark. Light causes isomerization to the all-trans form of retinal, which in turn must eventually be re-isomerized for the visual cycle to continue (Hubbard and Kropf, 1958; Hubbard, 1966). By slowing the chemical processes with liquid nitrogen, Wald’s group also demonstrated that rhodopsin goes through a series of very transient molecular transformations, and that one of these intermediaries (meta-rhodopsin II) triggers excitation of the photoreceptor before retinal is ultimately hydrolyzed from opsin (Matthews et al., 1963).

In 1942, Hecht and colleagues had demonstrated that a single photon might be enough to trigger excitation in a rod. In 1965, Wald suggested that a large chemical amplification must occur for a single photon to be able to trigger such excitation of the rod, and by analogy with the blood clotting system this amplification could potentially occur by a cascade of enzymatic reactions. Later studies showed that rhodopsin is a transmembrane protein consisting of seven membrane-spanning helices, that are interconnected by extracellular and intracellular loops, and that form a binding pocket for its ligand, 11-cis retinal. Absorption of light by 11-cis retinal causes isomerization to all-trans retinal and propagation of a conformational change in the protein to the cytoplasmic surface. The meta-rhodopsin II intermediary then interacts with transducin, a G-protein, to activate phosphodiesterases that control cyclic GMP levels, which in turn modulate release of neurotransmitter from the rod cell (Stryer, 1986; Hargrave and McDowell, 1992).

In 1967, Wald was awarded a Nobel Prize in Physiology or Medicine for “discoveries concerning the primary physiological and chemical visual processes in the eye” (Wald, 1967/1972).

Hormone-like actions of vitamin A

In 1960, Dowling and Wald showed that almost all of the non-visual roles of vitamin A are carried out by retinoic acid, and further that retinoic acid could not be metabolized to retinol (the form in which vitamin A is transported), or to retinyl esters (the form in which vitamin A is stored), or to retinal (the aldehyde needed for synthesis of visual pigments) (Dowling and Wald, 1960). As a result, their rats maintained with retinoic acid (but not vitamin A or its various provitamins, i.e., dietary carotenoids) grew normally but became extremely night blind and eventually permanently blind. As rhodopsin concentrations declined, visual thresholds rose, followed by loss of opsin, disintegration of the outer segments of the rods, and severe dropout of visual cells. Because retinoic acid is not stored in the body and cannot be metabolized to a storage form, the animals stopped growing within a few days of deprivation of retinoic acid and developed severe systemic symptoms within 1–2 weeks.

Subsequently retinoic acid was found to act in a hormone-like fashion to regulate gene expression (Ross and Ternus, 1993), a role for vitamin A that Wolf and De Luca had proposed as early as 1970 (Wolf and De Luca, 1970; Wolf, 1996). In 1987, P. Chambon and colleagues in Strasbourg, France, and R. M. Evans and colleagues in San Diego, simultaneously discovered retinoic acid receptors in cell nuclei, which bind with retinoic acid to modulate gene expression, thereby influencing embryonic development, cellular differentiation (including that of the cornea), and growth (Giguère et al., 1987; Chambon, 1996; Wolf, 1996).

Public health interventions to address vitamin A deficiency in developing countries

The first global survey of xerophthalmia conducted by the World Health Organization in the early 1960s suggested a significant prevalence of the disorder in many countries (Ooman et al., 1964), but was later found to have seriously underestimated the global burden of vitamin A deficiency (Sommmer et al., 1981; Reddy, 2002; Underwood, 2004). Subsequently, various intervention trials were conducted, including controlled trials in Jordan and India in the late 1960s, which demonstrated the feasibility of periodic vitamin A dosing to prevent vitamin A deficiency disorders (Reddy, 2002). In 1975, under the auspices of the US Agency for International Development (USAID) and the World Health Organization (WHO), the International Vitamin A Consultative Group was established at a meeting of the United Nations International Children’s Emergency Fund (UNICEF) in New York, to coordinate and facilitate activities to combat vitamin A deficiency disorders worldwide (Reddy, 2002; Underwood, 2004).

By the 1980s, global estimates suggested 13 million children suffered from xerophthalmia and another
40–80 million children were at risk (Somer et al., 1981; Underwood, 2004). But within 20 years these estimates were again found to underestimate the burden of illness resulting from vitamin A deficiency, with recent estimates suggesting that 3 million preschool children have clinical signs of vitamin A deficiency annually, and that another 140–250 million preschool children are at risk based on serum vitamin A levels (Underwood, 2004).

In the 1980s and 1990s, large randomized, double-blind, placebo-controlled clinical trials were conducted in developing countries. They demonstrated that vitamin A supplementation could reduce childhood mortality by approximately one quarter to one third, even by giving concentrated vitamin A supplements at 6-month intervals (Somer et al., 1986; Beaton et al., 1993; Semba, 1999; Underwood, 2004).

Vitamin A supplementation of malnourished children is now considered one of the most cost-effective health interventions known (World Bank, 1993; Semba, 1999). Although consistent periodic distribution of vitamin A supplements can control vitamin A deficiency disorders, sustained control by this means is fragile, especially in countries with economic decline and civil unrest (Underwood, 1999, 2004). As was the case with eradication of endemic pellagra in the United States in the early-20th century (Lanska, 2002), other measures are also needed to ensure adequate diets and improve socio-economic conditions before vitamin A deficiency disorders can be truly controlled (Underwood, 1998, 2004; Underwood and Smitasiri, 1999).

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