The Experimental Induction of Vitamin A Deficiency in Humans

George Wolf

Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA 94720-3104

ABSTRACT This is an historical account of experiments that were undertaken to determine the daily amount of vitamin A or carotene (or both) required for health of humans, by deliberately (and in one case accidentally) causing them to become vitamin A deficient. The recommended daily allowance determined by all of the experiments described arrived at roughly the same range, although large differences were reported for the time of appearance and the severity of observed signs and symptoms of the deficiency. J. Nutr. 132: 1805–1811, 2002.

KEY WORDS: vitamin A • retinol • β-carotene • xerophthalmia • night blindness

Although vitamin A deficiency, with its manifestations of night blindness and eye lesions had been known since antiquity, the essential lipid dietary factor that prevented such conditions was discovered only in animal experiments, performed mainly by McCollum and Davis (1) and Osborne and Mendel (2) in 1913. Although liver or cod-liver oil had been used to treat night blindness, it was not known until the second decade of the 20th century whether the dietary lipid that prevented the deficiency diseases in experimental animals could also function in humans.

An unintended experiment: the nutritional cause of eye lesions in children

The maritime blockade of the continent of Europe during World War I led to many cases of malnutrition in several countries. It was therefore no coincidence that investigations of malnourished children were made by Harriette Chick and her associates (3) in Vienna in 1919, which led to a cure for rickets and ultimately to the identification of vitamin D, and by C. E. Bloch (4) in Copenhagen in 1916–1917, which established a connection between a nutritional factor and eye lesions in children, leading to the recognition of vitamin A as an essential nutrient.

Inflammation of the cornea and conjunctiva associated with night blindness had been ascribed to “defective nutrition” by Budd (5) in 1842. Similar observations were made by Wilde (6) during the famine in Ireland in 1851. He recommended cod-liver oil as an effective treatment. Bitor (7) in 1863 noted the connection between night blindness and foamy spots on the cornea due to shed keratinized epithelial cells (Bitot’s spots). In 1884, Kuschbert (8) recognized night blindness and xerosis (dryness) of the conjunctiva and cornea in children as a single disease entity. By observing that Bitor’s spots contained bacteria, he concluded that the disease must be of infectious origin. He argued that it could not be caused by defective nutrition, because the 25 night-blind children in the orphanage in Breslau (Germany) that he cared for consumed a better diet, in his opinion, than many poor children in the town who were not night blind. No information is given regarding the diet of the children, either in the orphanage or the town.

Carl E. Bloch (1872–1952) was the physician in charge of the pediatric clinic of the University of Copenhagen. By 1916, he had accumulated observations from 40 children with eye lesions in his clinic and suspected a connection to malnutrition. Bloch (9) described the progress of the eye disease as follows: the first symptom is dryness of the conjunctiva; later waxy spots (Bitot’s spots) appear on the conjunctiva. Then the dryness spreads over the cornea (xerophthalmia). Later, ulceration of the cornea sets in (keratomalacia), ending in blindness due to necrosis of the cornea. Bloch was aware of the work of Mori (10) published in 1904, who described an eye disease known in Japan as “hikan,” similar in appearance and progression to that observed by Bloch and, according to Mori, caused by lack of dietary fat and curable by cod-liver oil.

Bloch (4) carried out a nutritional experiment with malnourished children that was well controlled, with a reasonable number of subjects, defined diets, experimental and control groups, and objective criteria of outcome. However, the experiment was unintended and almost accidental. Of course, this was at a time when the concepts of sample size, randomization and statistical evaluation of data were not known. Bloch came to interpret the results he obtained by acute observation, over several years, to reveal the deficiency of an essential nutrient as the cause of the eye lesions in his patients.

In 1917, Bloch was in charge of a Children’s Home with 86 children, ages 0–2 y. The Home was divided into two departments, housed in separate buildings. In department A were newborns and sick children. Department B comprised healthy children >1 y old, and was subdivided into two wards, B1 and B2, with 16 children in each. All conditions in wards B1 and B2 were identical, i.e., ages and health of the children, their nursing care, and the arrangement and size of the rooms.
The only difference was in their diets. Because the children in department A were small and some were sickly, they were given a gruel made with whole milk. The children in department B, because they were older, received a more varied diet: for breakfast, oatmeal gruel with rusk made with fat-free (skimmed) milk or a traditional Danish beer-and-bread soup made with whole milk; for lunch, a gruel made with buttermilk or fruit soup or sometimes meat soup made with pearl barley, followed by boiled fish or minced meat and abundant mashed potato; in the afternoon, there was cocoa with bread and margarine; for supper, bread with margarine and gruel.

Only fat-free (skimmed) milk was used in the preparation of the gruel or cocoa. The margarine was made entirely of vegetable oils. Buttermilk, despite its name, was devoid of milk fat, being the residue from butter making.

The only difference between the diets in wards B1 and B2 was in the breakfasts: it so happened that at one time some of the children in ward B1 had a tendency toward loose or frequent stools. In consequence, the matron in charge “at her own discretion” (41) chose not to give them the traditional beer-and-bread soup made with whole milk, but instead the oatmeal gruel with rusk. This difference in the diets between wards B1 and B2 continued for many months.

When Bloch had collected all of his clinical observations from 1916–1917, he noted something that must have seemed startling; all cases of clearly diagnosed xerophthalmia had arisen in the same ward, B1. Eight of the 16 children in this ward had been so afflicted, all cases occurring almost simultaneously during May or June 1917. There were no cases in ward B2 or in department A. What was the difference? He examined their diet and realized that the B1 children had received no whole milk for many months with their oatmeal gruel and rusk breakfasts, whereas the B2 children had feasted on the beer-and-bread soup made with whole milk. The children in department A, of course, received gruel made with whole milk.

The conjunctival xerosis in all 8 cases in ward B1 was very marked. These children were initially photophbic, their eyes were red and he noted that in the early stages some of the children appeared to be night blind. In the later stages, he observed dryness of the conjunctiva, which spread to the cornea, and a viscous discharge from the eyes. In other respects, these children appeared at first sight to be normal; however, closer examination showed them to be apathetic, weak and without appetite.

All children were weighed daily. All department A children gained weight normally. Department B children gained weight more slowly. However, there were 15 among the 32 children in department B who lost weight; of these, 10 were in ward B1, and all 8 children with xerophthalmia were among the weight losers.

Following his diagnosis of xerophthalmia, Bloch continued the dietary regimen of all children exactly as before, except that the 8 xerophthalmic children received 10 g cod-liver oil twice daily. Their eye lesions began to disappear within days and were gone after 8 d. All 8 also began to gain weight. Bloch (4) emphasized that, because all conditions, including diets, had remained constant, therefore the cure must have been due to the cod-liver oil.

At the time when Bloch first published these findings in 1919, he discussed the work of Mori (10), who had suggested that the eye lesions he observed may have been caused by a lack of fat in their diet. This notion was supported by Monrad (11), who in 1917 noted the development of xerophthalmia in 5 infants in a clinic in Denmark, who had been given an almost fat-free diet, because they were suffering from gastroenteritis. Their eye lesions were rapidly cured by whole milk, suggesting that dietary fat prevented xerophthalmia. Bloch, however, argued that the 8 children with eye lesions in his care had received ample fat (e.g., margarine); therefore, the disease must be caused by lack of a component of fat present in whole milk, in his words, “by the absence of specific lipid substances.” Only later, after the war, when information on animal experiments from America became available to him, did Bloch realize that, as he explains: “There was close agreement between the experiments on animals and my previously published clinical observations.” He refers to the work of McCollum and Davis (1) and particularly Osborne and Mendel’s (2) studies on the development of xerophthalmia in young rats fed a diet of protein-free and fat-free milk, starch and pork fat. He concluded that the “specific lipid substance” in whole milk was identical to McCollum’s “fat-soluble accessory factor A”, later named fat-soluble vitamin A.

Bloch (9) comments further on the 40 cases of xerophthalmia that he had diagnosed in his pediatric clinic before those in the Children’s Home. He mentions the occurrence of night blindness as an early symptom of the disease, and that the first sign was inhibition of growth. It was often associated with antecedent infections and he correctly, as we now know, reports that xerophthalmia was sometimes found in cases of measles. Green and Mellanby (12) in 1928 were the first to describe vitamin A as the “anti-infective” vitamin (13). However, Bloch (9) had already pointed out that a deficiency of “fat-soluble A factor” increased susceptibility to infection and diminished immunity, a conclusion confirmed and much extended by the findings of Sommer et al. (14).

The seasonality of the incidence of xerophthalmia, as mentioned above, was linked by Bloch (4) to an investigation by Malling-Hansen (15) published in 1886, who determined that children in Denmark had three periods of growth during the year, i.e., a minimum growth rate from August to November; an intermediate growth rate from November to March and a maximum growth rate (2.5 times the minimum) during March, April and May. All 8 cases of xerophthalmia in ward B1 in the Children’s Home were diagnosed with xerophthalmia in the 3 mo of maximum growth. Bloch emphasized that they had consumed the same diet throughout the year and ascribed the seasonal incidence of the disease to the growth period (Fig. 1). He quite reasonably postulated that during the growth spurt in March, April and May, a “specific lipid factor” is used up more rapidly, perhaps thereby precipitating the eye disease. This thought is in accord with much later observations. Arroyave (16) reported that protein-malnourished children showed no signs of vitamin A deficiency because of growth depression. As soon as they obtained protein and began to grow, the vitamin deficiency symptoms were precipitated. In animal experiments, Rechcigl et al. (17) observed a precise linear relationship between growth rate and vitamin A utilization. The possibility that the seasonal appearance of the eye disease may be related to seasonal changes in the fat-soluble factor A content in cow’s milk was dismissed by Bloch (4) on the grounds that the children in ward B1 had received only fat-free milk throughout the year they were under observation.

Before world war I, xerophthalmia was not uncommon in Denmark, but it rose precipitously after 1914, because of war-related scarcities (Fig. 2). The biggest rise was in 1917. The German submarine blockade of Denmark began in February 1917 and stopped importation of cattle fodder. Consequently, the price of butter became exorbitant and with it, the incidence of xerophthalmia increased. Bloch asks (9), “why, then, while the blockade continued, was there a drop to no more than one case admitted to my clinic in 1918?” (Fig. 2). The answer was that the Danish government introduced ra-
tioning of butter in December 1917. Every person, adult or child, could obtain 250 g per week (containing 8000 IU vitamin A, a little less than a child’s weekly requirement) at a price everyone could afford, and xerophthalmia virtually ceased to exist in Denmark.

Experimental vitamin A deficiency in humans: attempts to determine the daily requirement

Vitamin A deficiency was induced in humans by several researchers with the important aim to determine the daily requirement of the vitamin for healthy adults. This proved to be unexpectedly difficult because of the large but variable reserves of the vitamin stored in the livers of healthy persons. Moore (18), by analysis of livers of accident victims in England, found a median value of liver reserves of 324 IU/g for a healthy British adult, with the enormous variability of 10 IU to 1500 IU/g liver. The aim of the human experiments were generally two-fold: 1) to maintain the subjects on a vitamin A and carotene-free diet to determine the time taken until liver stores were exhausted, when plasma vitamin A would decline, and night blindness would set in; 2) to determine the daily intake required to restore normal plasma levels and vision by administration of the vitamin in different doses over varying times.

In 1936, Edmund and Clemmesen (19) attempted to determine an average daily requirement of vitamin A by observing young men in a state prison in Denmark who showed defective dark adaptation when eating the prison diet that was estimated to provide 1225 IU of the vitamin per day. When 0.5 L of milk was provided daily for 3 mo, their vision became normal. The authors determined that the milk provided an additional 200–800 IU/d. However, their method for determination of dark adaptation was severely criticized by Nylund and With (20).

A better-controlled experiment was performed by Booher et al. (21) in 1939. A specially prepared vitamin A–limited diet was given to five healthy adults for 6 mo. The low fat milk included in the diet was estimated by bioassay with rats to contain some vitamin A, so that the totally unavoidable daily vitamin A intake was 90–100 IU. Other vitamins were provided. Dark adaptation was measured by first adapting the subjects to light, then placing them in the dark. The subjects were then shown a faintly illuminated pattern. They informed the experimenter of the time it took them to see the pattern. The process was repeated with a less and less illuminated pattern. This generated a time vs. lighting curve, which was biphasic (Fig. 3), the first phase representing cone vision, the second rod vision. Impaired dark adaptation was observed after a surprisingly short period of between 16 and 39 d when subjects consumed the depleted diet. When subjects showed 1.0 log unit or more over their normal threshold values of light sensitivity (Fig. 3), they were given cod-liver oil containing 3000 IU vitamin A/g, as determined in biological assays in rats, with crystalline -carotene as a reference standard (0.6 g equivalent to 1 IU). After the first dose of cod-liver oil, dark adaptation improved within 2–3 h, but declined again after 24 h, suggesting that the depleted subject first repletes the eye, a phenomenon observed again by Sauberlich et al. (22) (described below). Dark adaptation became normal after 2–3 wk.
of cod-liver oil treatment; the doses required varied greatly among subjects, from 1900 to 3900 IU/d. This value is not far from the current (2002) recommended daily allowance (RDA) of 3000 IU for adult men, 2333 for adult women. The requirement of β-carotene, also very variable, was found to be about twice that for vitamin A.

A very similar series of experiments was carried out in 1942 by Wald et al. (23), except that they supplemented the normal diet consumed by their five subjects before the experiment with vitamin A, likely intending to diminish the great variability in requirement observed by Booser et al. (21) by saturating the livers of his subjects at the start of the experiment. They found that after 6 mo of consuming the vitamin A-deficient diet, only two subjects showed a very modest increase in their visual threshold. Similarly, in 1943, Brenner and Roberts (24) did not detect any changes in dark adaptation or in the plasma level of vitamin A among six volunteers subsisting on a vitamin A–depleted diet for 6 mo.

In 1942, Nylund and With (25) examined 118 free-living healthy persons and their diets in Finland. Their food was rationed because of war; thus, their intake of vitamin A and carotene could be estimated from existing food composition data. These authors divided the group into subgroups consuming different amounts of vitamin A and carotene and found that even in the subgroup (n = 36) eating the highest level (600 IU vitamin A and 2000 IU carotene/d), six persons developed night blindness. They concluded that 1730–2800 IU vitamin A or 1.5–2 times as much carotene were the required daily allowance.

Wagner (26), in 1940 in Leipzig (Germany), undertook an extensive and elaborate investigation to determine not only the daily requirement of vitamin A, but also to study ophthalmologic and hematologic consequences of vitamin A deficiency in otherwise healthy adults. The author stated that the 10 subjects (ages between 23 and 45 y, except for one aged 57, gender not indicated) consented voluntarily to eat the vitamin A– and carotene-free diet for a prolonged period in complete isolation, as a group. Further, the author explicitly stated that the subjects were in a “good nutritional state,” i.e., well nourished and healthy. Although he reported analyses showing the very low amounts of β-carotene in various components of the diet (sausages, peas, lentils), he does not provide an average unavoidable total carotene intake, as did Booser et al. (21).

From the sample menus he provided, one can estimate that on a typical day, a subject would consume ∼80–160 IU of carotene. Thiamin was estimated to be adequate and ascorbic acid was given as a supplement. Energy density was kept high, to prevent weight loss due to lack of appetite and for psycho-energetic reasons. Indeed, for the first 3.5 mo, all subjects gained weight. After that time, for the following 2.5 mo, all 10 subjects simultaneously lost amazing large amounts of weight, e.g., two persons lost 10 kg of their maximal weights (79 kg and 59 kg). The author interprets this weight loss to be a consequence of the exhaustion of the subjects’ vitamin A reserves, and compares it to the weight loss observable in vitamin A–deficient rats. This is not, however, a legitimate comparison because the rat experiments were usually done with young, growing animals. In the absence of measured food intake, one would think that the severe weight loss was caused by lack of appetite. After 6.5 mo, β-carotene (5000 IU/d) or a vitamin A concentrate (2500 IU/d) were administered for 3.5 mo, with very rapid weight gains (average gain: 5.4 kg) in each case.

After 2.5 mo, the first signs of night blindness appeared in the subjects subsisting on the depletion diet. After 5 mo, the investigator found that, for the same light intensity, the depleted persons were on average 1/28 as sensitive as control subjects (20 well-nourished students). There was also a decline in the sensitivity to color vision, especially regarding the color yellow. Hematologic studies showed a decline in leukocytes and a 70–85% drop in the level of blood platelets, after only 4 wk of the depleted diet.

Upon repletion, 5000 IU/d over 1.5 mo of β-carotene supplementation was required to reach 60% of normal dark adaptation; the same goal was attained with only 2500 IU vitamin A. No vitamin A was detectable in the blood until 60 d after dosing with vitamin A, whereas β-carotene appeared in only 45 d of supplementation. Although only traces of vitamin A were recovered in feces and none in urine, as much as 44–54% of the administered β-carotene was found in feces.

The author calculated that in 76 d of supplementation, the subjects received a total of 142,000 IU or an average of 1870 IU vitamin A/d, an amount apparently required to saturate the specific vitamin A storage organs. This amount then was taken as the lowest daily requirement and he suggested 2500 IU as a good “recommended daily allowance” for vitamin A and 5000 IU for β-carotene.

The Sheffield Experiment

A most extensive and elaborate human experiment was carried out by Hume and Krebs (27) at the height of the Second World War in Sheffield, England from 1942 to 1944, at the request of the Ministry of Food, to provide information on the human requirement of vitamin A or carotene “more accurate than was then available.” As subjects, 20 men and 3 women were recruited, aged between 19 and 34 y. They were conscientious objectors to military service and, therefore, highly motivated to cooperate as experimental subjects. Sixteen subjects received a vitamin A and carotene-deficient diet, analyzed for adequacy in nitrogen, fat, carbohydrate, calcium, phosphorus, iron, thiamin, riboflavin, nicotinic acid and ascorbic acid. Vitamin D was given as a supplement. Analyses of 12 different foods and of quantities of each food consumed in 2 sample weeks were carried out in five laboratories specializing in vitamins and nutrition (Universities of Cambridge, Oxford, Liverpool, Reading and Sheffield). In particular, total carotenoids and α- and β-carotene and vitamin A were determined in the deficient diet. Unavoidable total carotene was found to be ∼70 IU/d consumed. Even this was suppressed during 4 mo in 1943, when all fruits and vegetables were withheld. The deficient diet was tested in rats to ensure that it was biologically deficient in vitamin A activity. Plasma concentrations of carotene and vitamin A were measured simultaneously in two laboratories and checked against each other.

As controls, 2 subjects received the deficient diet with 2500 IU vitamin A/d (as esters) in oil; 5 subjects received 5000 IU of β-carotene, either as pure carotene in oil, or in previously analyzed carrots or spinach per day.

The first observed change was a rapid drop in the plasma level of carotene after 3 mo from 1500 IU to 120–400 IU/L. Vitamin A began a slow decline after 8 mo but decreased to ≤300 IU/L in only 2 of the 17 subjects by 1 y and one other after 1.5 y. Dark adaptation began to deteriorate slightly after 5 mo, with an increase in the transition time from cone to rod vision (Fig. 4), although a substantial impairment in dark adaptation occurred in only three subjects, preceded by the drop in plasma vitamin A level to 300 IU/L in 1–1.5 y. The three other subjects of the six remaining after 1.5 y showed no ill effects except a slight decline in plasma vitamin A.

Audiometry tests showed a loss of hearing in three of the deprived subjects and an improvement after dosing with the
vitamin. They also exhibited slight hyperkeratosis of hair follicles. Hematologic tests could not confirm the steep decline of blood platelets reported by Wagner (26).

A curious seasonal oscillation was observed in dark adaptation, which deteriorated after 5 mo in the subjects fed the deficient diet (Fig. 5), coinciding with the winter months (November to February), and showed recovery during the following summer months. This seasonal effect, which was observed again during a second winter, could not have been caused by seasonal changes in carotene content of the diet because that was rigorously controlled. Furthermore, the deterioration was not accelerated when fruit and vegetables were completely eliminated from the depleted diet for four summer months. Hume and Krebs (27) suggested that the effect may have been due to changes in temperature (Fig. 5), although why cold should lower plasma vitamin A level remains unexplained.

These seasonal changes are reminiscent of the observations of Bloch (4) referred to above, reporting that all children in the Children’s Home consuming the fat-free milk diet came down with xerophthalmia during March, April and May. Of course, these months differ from those in the Sheffiel d experiment. Perhaps xerophthalmia follows a seasonal decline of plasma vitamin A in winter by a few months.

Therapeutic tests were undertaken in the Sheffiel d study with the three subjects who showed the significant impairment in dark adaptation. One subject was given vitamin A orally as esters in oil (1300 IU/d) and showed the first improvement in dark adaptation in 2 wk. Two subjects were dosed with 2500 IU of β-carotene in oil. One recovered normal vision within 3 wk; complete recovery took 5.5 mo for the other. Subjects consuming cooked vegetables were found to excrete 59–76% of the food’s carotene in their feces. The investigators found that, among the control volunteers who ate the depleted diet together with different amounts of carotene supplements, the

dose of 5000 IU/d taken from various sources over 14–17 mo was just sufficient to maintain health and vision, although blood levels of vitamin A were somewhat lower than normal.

In their final summary, Hume and Krebs (27) recommended 2500 IU/d of vitamin A as the daily requirement. They considered this to be “an estimate . . . to cover individual variations and to leave a margin of safety.” For carotene, taking account of individual variation, Hume and Krebs proposed 3000 IU daily as a “minimum effective dose.” However, if supplied in cooked vegetables, because of losses in feces, they recommended 7500 IU/d “as being a figure roughly representative of the different sources of carotene in the diet.”

A human experiment in the modern era

An experiment with human volunteers was undertaken in 1974 by Sauberlich et al. (22) to determine the requirement of vitamin A and carotene and in part to replicate the Sheffiel d experiment with more modern methods, including the use of labeled vitamin A and β-carotene to determine body pools and turnover. It will most likely remain the last such study because of current, more stringent restrictions than 30 y ago on the use of human subjects in experiments. Unfortunately, the results of the Sauberlich study were published only as a review (22); experimental details were never reported. No reason is given why the vitamin A–deficient diets (casein-based or soy protein–based or the modified Sheffiel d diet) consumed by the subjects were changed in the course of the experiment.

 Intravenous administration of radioactive vitamin A (retinyl-15-14C acetate) revealed a threefold difference in body pool (1 × 106 to 3 × 106 IU)3 among 4 subjects and an exponential depletion rate dependent on initial reserves, with a half-life of between 75 and 241 d, showing the enormous variability in the reserves of the vitamin among the subjects.

In 5 of the 8 subjects who consumed the various deficient diets, plasma vitamin A dropped rapidly during the first 6 mo, from 950–1300 IU/L to 330–530 IU/L; after 1 y, 6 of the 8 subjects had very low levels of ~160 IU/L. It should be noted that in the Sheffiel d study, only 3 of 17 subjects reached such a low level after 1.5–2 y.

Dark adaptation became impaired in all 8 subjects after 8–15 mo of eating the vitamin A–depleted diet, and became

3 Sauberlich et al. (22) expressed their data in micrograms. These have here been recalculated in terms of IU.

---

**FIGURE 4** Dark adaptation response to vitamin A deprivation. Dark adaptation measured at different times after of administration of vitamin A-depleted diet for one subject. Dates of measurements shown. Note increased cone-rod transition times on January 17, 1944 and February 16, 1944. The starting date was July 30, 1942 (adapted from Ref. 27).

**FIGURE 5** Seasonal variation of dark adaptation. Monthly averages of final rod thresholds in dark adaptation measurements for seven vitamin A–deprived subjects and monthly averages of daily minimum temperatures (adapted from Ref. 27).
serious when plasma levels declined below 1000 IU/L. All subjects described by Sauberlich et al. (22) exhibited follicular hyperkeratosis. Electroretinograms became abnormal before there was an impairment of dark adaptation. The depleted subjects also reported abnormalities in the senses of taste and smell, and there were changes in vestibular function and in cerebrospinal fluid pressure (no details reported).

After 1–2 y of the study (depending on the severity of deficiency signs), subjects were repleted with vitamin A. No effect on plasma vitamin A was detectable when 500 IU/d of vitamin A were provided for 2.5 mo, some increase with 1000 IU/d over 1.5 mo and a rapid rise to normal with 2000 IU/d given over 1 mo. For β-carotene, as much as 4000 IU/d was needed to restore normal vision, even though plasma vitamin A remained low; presumably the depleted retina would be the first tissue to be replenished.

On the basis of their experiments, Sauberlich et al. (22) recommended 4000 IU of vitamin A to be consumed per day for optimal health. For β-carotene, they also recommended 4000 IU/d, an amount much lower than that proposed by Hume and Krebs in 1949 (27). Most likely, the reason for this discrepancy is that Sauberlich et al. gave the carotene dissolved in corn oil, resulting in optimal absorption.

A case of self-induced vitamin A deficiency was reported in 1969 by Sharman (28). A 20-year-old man, who had been suffering from epilepsy from age 12 became convinced from reading the literature, that vitamin A caused his epileptic fits. He deliberately eliminated all vitamin A and carotenoids from his diet. His blood retinol dropped to 130 IU/L (normal, about 2000 IU/L) and the blood carotenoid level became extremely low. Subsisting on this diet for 4 years resulted in corneal xerosis and severe retinal damage (29). He reported that his epilepsy was much ameliorated; he had suffered only one fit during that period. Upon repletion with vitamin A, he recovered his ocular health. No information is available regarding his epilepsy subsequently. In order to investigate further a possible connection between vitamin A and epilepsy, Sharman (30) then studied 8 epileptic patients given a vitamin A-depleted diet for 2 years. No cases of night blindness were observed. When their plasma retinol level had reached 230 IU/L, they were given a vitamin A supplement. Although the patients reported fewer fits during the depletion period, there was no increase in the fits during repletion. The author (30) called his experiment a “dietary contretemps.”

**A comparison of the human experiments involving induction of vitamin A deficiency**

The daily allowances of vitamin A recommended by Booher et al. (21), Wagner (26), Hume and Krebs (27) and Sauberlich et al. (22) on the basis of their experiments were roughly in the same range and similar to the current recommended allowance. However, differences were observed by the different investigators in the response of their subjects to the developing vitamin A deficiency. A striking difference between the experiment of Hume and Krebs and that of Wagner was the severe almost simultaneous weight loss of all 10 volunteers in the Wagner study after only 3.5 mo of consuming the deficient diet, and no weight loss in the former study. Furthermore, severe impairment of dark adaptation in all 10 subjects was observed by Wagner after 5 mo. In the study of Hume and Krebs, only 3 of the 17 subjects showed significant deterioration of dark adaptation after 1.5–2 y. What could be the explanation for these extreme discrepancies? Hume and Krebs (27) note in a “personal communication” received from Wagner after the war (in 1947), that Wagner’s subjects had been “under detention in an institute” (perhaps meaning “institution”) “where they had been receiving a diet nearly devoid of vitamin A for periods of up to two years before beginning the experiment,” even though Wagner (1940) in his report explicitly states that all his subjects had freely consented (“freiwillig”) to be subjects and that they had been in a “well-nourished” state.

In this light, the experiment of Booher et al. (21) should be mentioned; those authors observed a deterioration of dark adaptation among all five subjects who consumed the deficient diet for only 16–39 d. Hume and Krebs, in their discussion of this work, suggested that “perhaps they were selected as likely to be deficient in vitamin A.”

The study of Sauberlich et al. (22) comes somewhere in between Wagner’s work and the Sheffield experiments. All eight of Sauberlich’s volunteers developed clear signs of vitamin A deficiency after ~13 mo of consuming the depleted diet, compared with 3 of 17 subjects in the study of Hume and Krebs within about the same time. This difference reveals the well-known great variability in vitamin A reserves in the population, although one wonders why the Sauberlich volunteers all appeared to have lower reserves than most of those in Sheffield. Sauberlich et al. (22) state that their subjects were “healthy male volunteers.” The possibility should be considered that they were volunteers from prisons because the same group of investigators (31) state in a previous report that they employed volunteers from the Iowa State Penitentiary in their work on the human requirement for ascorbic acid. It would not be far-fetched to suppose that a prison diet, although not in any sense deficient, may have been low in protein and the vegetables required to supply enough carotene to build up a good vitamin A reserve.

In conclusion, human experiments carried out to determine the RDA for vitamin A and carotene were beset with difficulties because of the large storage capacity for vitamin A in liver. The amount stored is dependent on the subject’s diet before the experiment and can therefore lead to great variability in the development of the deficient state. This was not the case for other vitamins. For instance, in a human experiment reported by Ziporin et al. (32) to determine the RDA for thiamin, variability was extremely low. Eight young adults were given a thiamin-restricted diet: all subjects ceased to excrete thiamin in urine on 18 of the experiment and all subjects continued to excrete thiamin metabolites at almost identical rates. From these data, the investigators could readily calculate the minimum daily requirement for this vitamin.

Nonetheless, approximate agreement was achieved for the human requirement for vitamin A, Booher et al. (21) reported that between 1900 and 3900 IU/d given over 2–3 wk cured human vitamin A deficiency. Wagner (26) restored normal health to his depleted subjects with 1870 IU/d given over 2.5 mo, and recommended 2500 IU/d of vitamin A and 5000 IU β-carotene for good health maintenance. Hume and Krebs (27) recommended as a daily requirement 2500 IU vitamin A or 7500 IU for β-carotene as consumed in vegetables. These amounts compare well with today’s RDA, expressed as vitamin A activity, of 3000 IU/d for men and 2300 IU for women.

**LITERATURE CITED**


