VITAMIN A DEFICIENCY AND NIGHT BLINDNESS

By John E. Dowling and George Wald*

BIOLOGICAL LABORATORIES OF HARVARD UNIVERSITY, CAMBRIDGE

Communicated May 16, 1958

One of the oldest diseases known to man is nutritional night blindness. Its descriptions go back to the ancient Egyptian medical papyri and are already accompanied by the correct prescription for its cure, the eating of liver. Toward the end of World War I the factor in liver which cures night blindness was identified with the then newly discovered vitamin A.[1]

Vitamin A is the precursor in the retina of the visual pigments of the rods and cones.[2] It seems reasonable to suppose that on a diet deficient in this factor the retina eventually synthesizes subnormal amounts of visual pigment, with the corresponding decline of visual sensitivity that constitutes night blindness.

Some of the first studies of experimental human night blindness seemed to reveal such a simple and direct relationship.[3] In two subjects deprived of vitamin A, the visual thresholds of both rods and cones began at once to rise, until a mild night blindness had been established.[4] On oral administration of vitamin A or carotene, the thresholds of both rod and cone vision returned to normal within 2–3 hours.

It looked for a time, therefore, as though this might be an exemplary instance of the origin and cure of a biochemical disease, all elements of which were well understood. Further studies, however, exposed two major discrepancies: (1) Though in some subjects placed on a vitamin A–deficient diet the visual threshold began at once to rise, in a larger number it remained unchanged for periods ranging from several months[5] to, in one instance, 2 years.[6] (2) Among the subjects who developed night blindness, some were completely cured within a few hours after receiving vitamin A, whereas others, though showing some immediate improvement, took months of vitamin A supplementation to return to normal.[7]

One might take a simple position with regard to the first of these discrepancies. The amounts of vitamin A stored in the livers of healthy human subjects are known to vary enormously.[8] In Britain, for example, Moore[6] found reserves in adults during 1941–44 ranging from about 7 to 750 μg/gm. If we take 1,500 gm. as the average weight of the adult liver and about 300 μg. (about 1,000 I.U.) as the daily drain upon stored vitamin A, the average Briton stores enough vitamin A in his liver—if used economically—to tide him over some 500 days of total deprival. An unusually well-supplied Briton—if we can disregard spoilage—might survive seven times as long, or almost 10 years! On the other hand, the most poorly supplied members of this group might have run through their stored vitamin A within 1–2 weeks. It is not difficult to understand, therefore, why most subjects taken from ordinary American or British environments fail to respond to vitamin A–deficient diets within months or even years. It is less clear why a fairly large proportion of them responded within a few days, even though in some instances highly supplemented with vitamin A for the preceding period.[3]

The second discrepancy—the great variability in the times required to cure night blindness—raises other issues. The visual pigments are composed of vitamin A

aldehyde (retinene) joined to specific proteins of the rods and cones called "opsins." The amounts of visual pigment that can be formed in the normal retina are limited, not by vitamin A, which is ordinarily present in excess, but by opsin. In thinking about night blindness, we have tended in the past to be too much preoccupied with vitamin A and have paid too little attention to the opsins.[2]

When one does consider the opsins, this at once suggests further relationships. The outer segment of a rod—and this must be true also of many cones—is composed in considerable part of visual pigment, that is, of opsin, since the retinene chromophore constitutes only about 1 per cent of these molecules. Opsin accounts for about 40 per cent of the dry weight of the outer segment of a frog rod and 14 per cent of that of a cattle rod.[2] It is an important structural constituent of the rods and probably of the cones; and any loss of this protein might be equivalent to the structural deterioration of the visual receptors.

Tansley[9] showed some years ago that in vitamin A–deficient rats and dogs, somewhat later than the decline in rhodopsin production that should have initiated night blindness, the outer segments of the rods deteriorated structurally. Johnson[10] confirmed and extended these observations in the rat; and recently similar changes have been observed in both rods and cones of the monkey.[11] According to Johnson, after 7–13 weeks of vitamin A deprivation in young rats, many outer segments have disappeared, and those that remain stain abnormally. As the deficiency progresses, the inner segments of the rods also degenerate, and then successively the external limiting membrane, the outer nuclear layer, and the inner nuclear layer. These changes occur sooner in central than in peripheral areas of the retina. The outer segments of rods which have deteriorated only slightly seem to repair considerably within 24 hours of feeding vitamin A. Even rods which have degenerated completely seem to be replaced within 10–18 weeks of vitamin A supplementation.

These observations suggest that the time required to cure night blindness may depend on the extent to which vitamin A deficiency has altered the retinal structure. Simple lack of vitamin A, through lowering the concentrations of visual pigments, might induce a night blindness that is cured as rapidly as vitamin A re-enters the retina; but the structural deterioration of the retina, heralded perhaps by the loss of opsin, might take much longer to repair.

For these reasons it seemed worthwhile to map the entire course of vitamin A deficiency and its cure in the rat. In single groups of animals we have measured simultaneously the vitamin A in the liver and blood, the retinal content of rhodopsin and opsin, the electroretinographic threshold, and the ERG's obtained over a wide range of light intensities. In key instances we have also examined the retinal histology.

Not all these things were done for the first time. We have already discussed the histological studies of Tansley and Johnson and should mention particularly also Tansley's fine study of rhodopsin synthesis in normal and vitamin A–deficient rats[12] and the measurements of liver, blood, and retinal vitamin A in normal and deficient rats by Lewis, Bodansky, Falk, and McGuire.[13]

Plan of the Experiments.—A number of experiments were performed, all of which yielded substantially the same pattern of results. We shall describe primarily the last such experiment, because it brings together all the procedures and represents most completely and typically all our observations.

Male albino rats of the highly inbred Harvard colony, 22–24 days old and weighing 36-66 gm., were divided into two groups, one kept on the complete laboratory ration, the other placed on the standard USP vitamin A test diet. The animals on the deficient diet continued to gain weight for about 5 weeks, though more slowly than normal. At this time they weighed an average of 112 gm. as compared with the control weight of 215 gm. but were altogether normal in appearance. In the fifth to seventh weeks their weights plateaued and thereafter declined rapidly. At the same time—in the seventh and eighth weeks— the classic overt signs of vitamin A deficiency appeared, and by the end of the eighth week all the animals not sacrificed in the experiments had died.

For electroretinography, animals that had been dark-adapted overnight were anesthetized with nembutal. The eye was held open with threads drawn through the lids. Cotton-wick electrodes were used, moistened with Ringer solution, one touching the side of the cornea, the other a shaved area on the cheek. The response was recorded with a capacity-coupled Grass P4 preamplifier and a Dumont oscilloscope with camera attachment. The stimuli were $^1/_{50}$-second flashes of white light, the intensity of which was controlled with neutral filters and photographic wedges. The absolute threshold was measured by starting with the light well below threshold and flashing it every few seconds at gradually increasing intensities until a response could be detected on the oscilloscope. This procedure was repeated until constant readings were obtained. Then the ERG was recorded over a wide range of intensities.

After dark-adapting overnight, the same animals were used next morning for the biochemical measurements. They were again anesthetized, the body cavity was opened, and 5–10 ml. of blood were taken from the heart with an oxalated syringe. The entire liver was removed and also both eyes. One eye of each animal was used to measure rhodopsin, the other to measure opsin; but, since each of these determinations requires 2 retinas, animals were paired, usually on the basis of having yielded comparable electroretinograms.

To determine blood vitamin A, the oxalated blood from one animal was centrifuged, and the clear plasma was mixed with an equal volume of ethyl alcohol and extracted three times with petroleum ether. This extract was transferred to 0.3 ml. of chloroform, and a micro-antimony chloride test was performed by mixing 0.25 ml. of the extract with 0.50 ml. of antimony chloride reagent, recording the absorption spectrum at once in a Cary recording spectrophotometer.

The livers, weighing 4–12 gm., were ground with anhydrous sodium sulfate to a fine powder and extracted by shaking with diethyl ether. An aliquot of this extract was transferred to chloroform, and its vitamin A content determined by the antimony chloride procedure.

To measure rhodopsin, two retinas were hardened in 4 per cent alum solution for 15–20 minutes, then washed with distilled water and buffer, and extracted overnight with 0.2 ml. of 2 per cent digitonin solution. After centrifuging, 0.01 ml. of 1 *M* hydroxylamine was added to the extract, and the absorption spectra recorded before and after bleaching. The change in extinction at 500 mμ measured rhodopsin.

Opsin was determined by measuring the capacity of retinas to regenerate rhodopsin when incubated with neo-*b* (11-*cis*) retinene.[14] We found that rat rhodopsin

in digitonin solution regenerates very little when bleached and incubated with neo-*b* retinene. For this reason whole retinas were exposed to bright light until wholly bleached. Then a large excess of neo-*b* retinene, dissolved in 0.025 ml. acetone, was added to the retinas suspended in buffer solution, the mixture was stirred periodically during 4–6 hours at room temperature and then left at 5° C. overnight. The rhodopsin which had formed was extracted and measured as described above. Control measurements showed that 70–80 per cent of rhodopsin originally present in a retina was regenerated and recovered by this procedure.

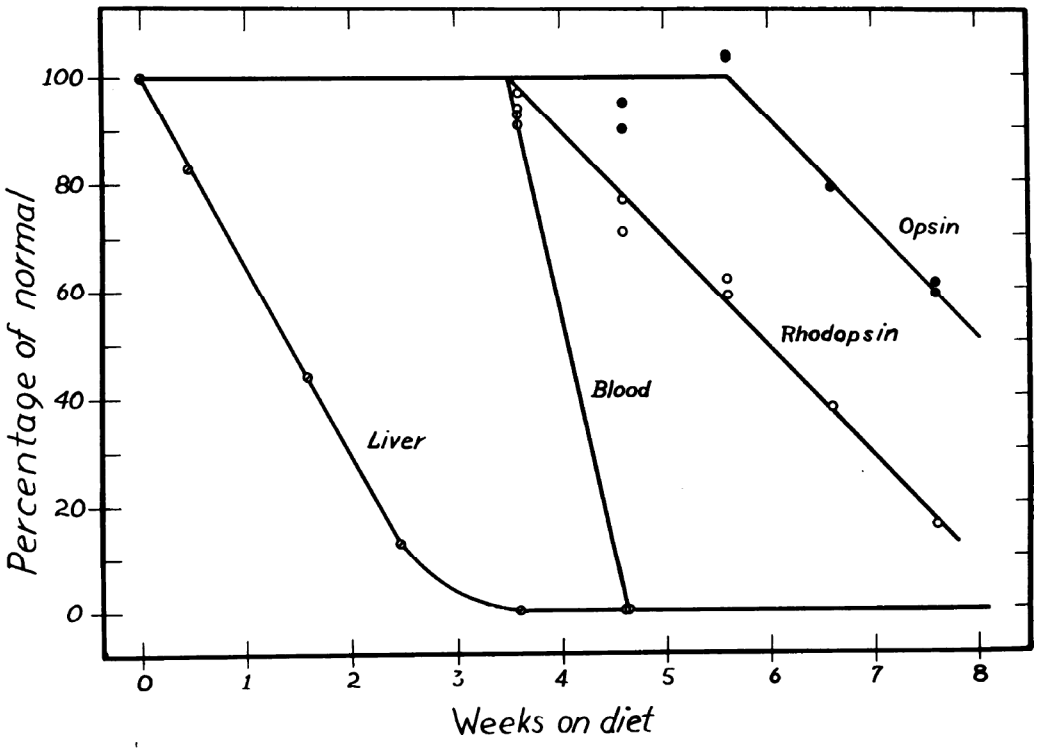

FIG. 1.—Biochemical changes in a group of white rats on a vitamin A–deficient diet. The animals were 22–24 days old when the diet was begun. The liver vitamin A began to fall at once and within 3 weeks had reached low values. Then within a week the blood level fell from normal to zero. With this, the rhodopsin content of the retina declined, marking the onset of night blindness. Later the opsin also declined, marking the beginning of the histological deterioration of the retina.

Vitamin A of Liver and Blood; Rhodopsin and Opsin.—Figure 1 shows in one group of animals the effects of the deficient diet on the vitamin A content of the liver, the vitamin A concentration in the blood, and the rhodopsin and opsin of the retina. The values are expressed as percentages of normal. For the liver this means the percentage remaining of the vitamin A present in control animals at the time the diet was begun. The blood vitamin A, rhodopsin, and opsin are expressed as percentages of the values found in control animals of the same age.

The liver vitamin A begins to fall as soon as the diet is begun and within 3 weeks has reached a very low value. This depletion proceeded at the average rate of 2–2.5 μg. daily, the withdrawal rate for animals of this age and weight. Meanwhile, the control animals on the complete diet increased their liver stores at an average rate of 45 μg. daily. It is this that makes the age at which the diet is

begun decisive for the course of the deficiency. Our control animals when 53 days old had livers weighing, on the average, 16 gm. and containing 1,360 μg. vitamin A; withdrawn at a daily rate of even 5 μg., this might have tided them over 9 months of a deficient diet.

The blood maintains its normal concentration of vitamin A (10.4 μg. per cent in the deprived animals, 11.2 μg. per cent in the controls) until the liver has been

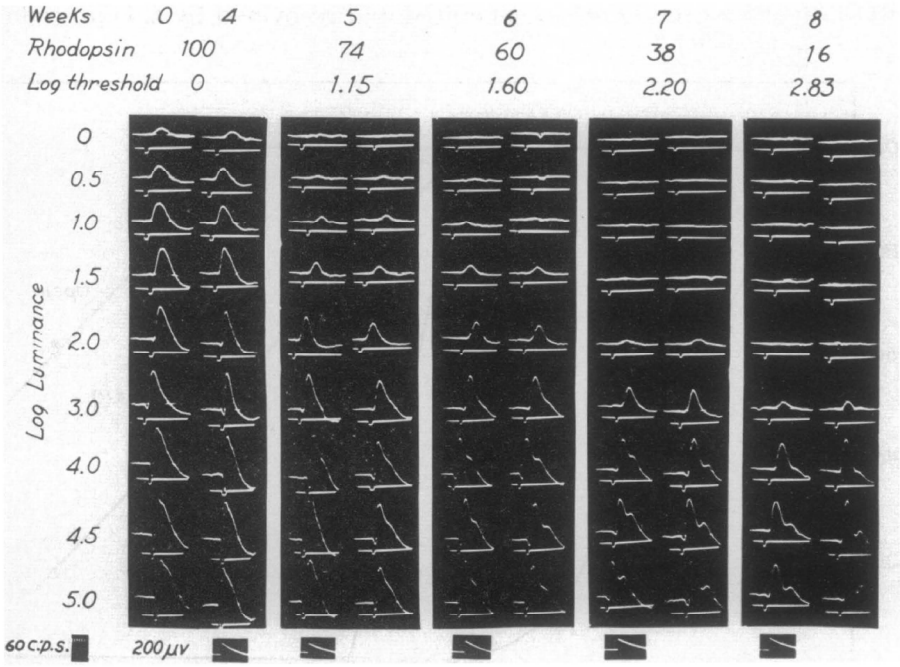

FIG. 2.—Effects of vitamin A deficiency on the electroretinogram (ERG). The top three lines show the number of weeks on the deficient diet, the rhodopsin content of the retinas as percentages of normal; and the logarithm of the luminance of light needed to evoke a just perceptible ERG, the average threshold of normal animals being set arbitrarily at 1 (log threshold = 0). ERG's are shown in response to a range of luminances of 5 log units, i.e., 1 to 100,000. Below each ERG, a marker shows the $^1/_{50}$-second flash. The small rectangles at the bottom show a trace of the 60-cycle A.C. to indicate the time scale, and weekly calibrations of the oscillographic response to a pulse of 200 microvolts. The first two vertical rows of ERG's show responses from a rat about to begin the diet and those from another after 4 weeks, when the vision is still normal. Thereafter, records are shown from a pair of animals each week. As the rhodopsin declines, the ERG threshold rises (night blindness), and the ERG displays characteristic changes: (*a*) the *b*-wave at each level of luminance declines; (*b*) the *a*-wave declines still more rapidly; and (*c*) an inflection on the downward sweep of the *b*-wave is delayed longer and longer until it appears as a separate positive wave.

emptied. Then in the space of a few days the blood vitamin A falls precipitately to zero.

Up to this time the rhodopsin content of the retina remains normal. The extract of two retinas in 0.21-ml. solution possesses an extinction at 500 mμ of 0.280, corresponding to a rhodopsin content of 7.24×10^{-4} μmoles per retina. This is equivalent to a vitamin A content of 0.21 μg. per retina. (Lewis *et al.*[13] found only one-fifth to one-third as much vitamin A in the rat retina; the description of their preparative procedure suggests that it may have involved large losses of rod outer segments.)

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.