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AN EXPERIMENT IN HUMAN VITAMIN A-DEFICIENCY

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The first observable symptom of vitamin A-deficiency in man and other mammals is a rise of visual threshold known as night-blindness. This response is based at least in part on the direct participation of vitamin A in a retinal cycle with the photosensitive pigment of the rods, rhodopsin.[2] With other conditions held constant, the sensitivity of the rods to light depends upon their rhodopsin content. As the level of vitamin A falls on a deficient diet, the concentrations of all components of the retinal cycle, including rhodopsin, decrease and the visual threshold reciprocally rises. Measurements of the rise and reversal of night-blindness therefore yield important information concerning the utilization of vitamin A *in vivo*. One set of such measurements upon a human subject has already been reported from this laboratory.[3] The present paper describes the results of a second experiment in which identical observational methods were employed.

The subject D. S. is 22 years of age, 5 feet and $10^1/_2$ inches in height and weighs 155 pounds. Throughout the course of the research he remained in excellent physical condition, ate very well and exercised regularly.

A standardized procedure was employed in all the measurements. Following 4 minutes' exposure to an intense light-adapting screen (4200 millilamberts), the absolute threshold of vision was measured periodically on alternate eyes during about 40 minutes in darkness. The test field for the threshold determinations is circular and subtends a visual angle of 2.1°. It is fixated 11.7° above the fovea, and is exposed by the subject for flashes of approximately $^1/_{50}$ second.

The experiment was begun with a period of vitamin A saturation, lasting 18 days, during which the normal diet was supplemented with 51,000 International Units of vitamin A daily, or from 10 to 25 times the accepted maintenance level.[4] Dark adaptation measurements during this period are shown as closed circles in figure 1. They are of characteristic form. The threshold falls rapidly to a first plateau, maintained until about the 11th

minute, then falls again to a second plateau, maintained thereafter. It has long been appreciated that the first segment is due to the dark adaptation of the cones, the second to that of the rods. The first plateau therefore represents the threshold of the completely dark adapted cones, the second that of the rods. The plateau intensities afford convenient indices for describing the course of the experiment. They are plotted separately in figure 2.

Following the saturation period the subject abruptly eliminated all dairy products, colored vegetables, liver, kidney and other selected foods from the diet, reducing its vitamin A content to 100–200 units per day.[4] Simultaneously the diet was supplemented with optimal amounts of vitamins B_1, B_2,

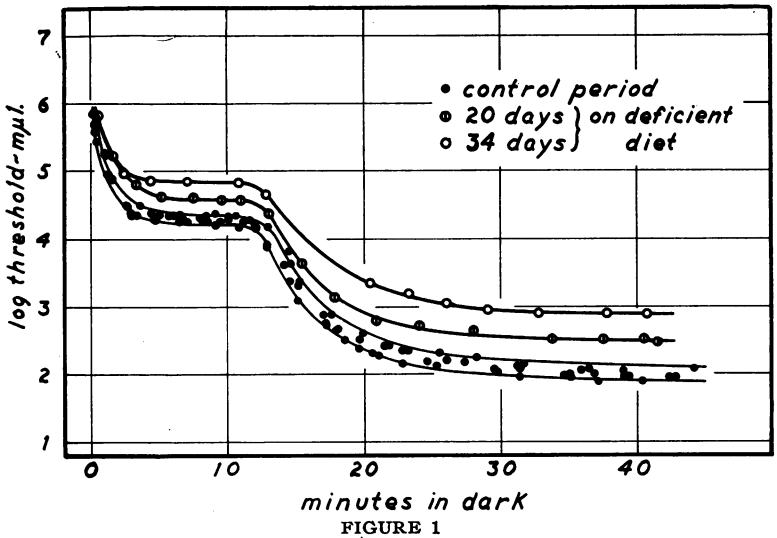

FIGURE 1

Dark adaptation during a period of vitamin A saturation (closed circles; 7 experiments), and at various intervals during vitamin A-deficiency (open circles). Each point represents a single determination of the absolute threshold of the right eye to white light. Threshold intensities in millimicrolamberts = 10^{-6} millilamberts.

C, D, calcium phosphate and ferrous sulphate.[5] Under these circumstances the diet probably is deficient in vitamin A alone.

On this diet the thresholds of both cones and rods rose regularly. The effects on the form of the dark adaptation function are shown in figure 1, and the changes in the plateaus in figure 2. The rise of log threshold is greater in the rods than in the cones, and greater in the plateaus than in the descending portions of either segment. The plateaus remain inside their normal ranges for the first 3 or 4 days on the deficient diet. Thereafter the log thresholds rise linearly in both cones and rods. It is an important fact that the lines which best fit the complete data for both rods and cones originate

on the first day of the deficiency. Apparently, following even a period of vitamin A saturation, the ascent into night-blindness on a deficient diet begins at once.

On the 34th day of the deficiency the cone plateau had risen 3.4 times, the rod plateau 9.1 times above their average normal levels. This mild state of night-blindness was completely reversed with a single administration of carotene. The course of the reversal experiment is shown in figure 3. A preliminary standard light and dark adaptation showed the subject

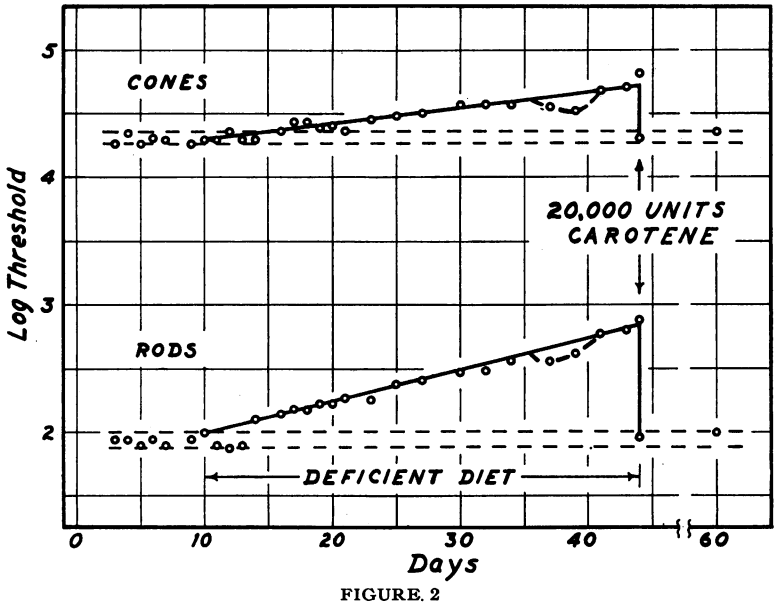

FIGURE 2

Thresholds of the completely dark adapted cones and rods (cone and rod "plateaus"), during 34 days on a vitamin A-low diet, and during previous and subsequent normal periods. The normal ranges of variation for cones and rods are enclosed within broken lines. The depression of thresholds on the 35th to 41st days of the experiment is due to a slight break in the diet on the 35th day. On the 34th day of the deficiency the night-blindness was reversed with carotene; the details of this experiment are shown in figure 3.

to be night-blind. Had he been normal the cone plateau would have lain within the upper pair of broken lines in figure 3, the rod plateau within the lower pair. After dark adaptation was complete, the subject was given 2 gelatine capsules of carotene in cottonseed oil (Smaco; about 20,000 units) orally with water. For 12 to 14 minutes the threshold remained constant. This is the "latent period" noted previously,[3] and presumably associated with the dissolution of the capsules and passage of the oil to the gut. Then the rod threshold fell rapidly, entered the normal range within about 90

minutes after the oil had been taken and became stable within about 100 minutes. Immediate repetition of the standard light and dark adaptation procedure showed both cone and rod plateaus to have entered their normal ranges.

The results of this research differ from our previous experiment[3] only in time relations. The rise in log threshold per day of the deficiency—the slopes of the lines in figure 2—is in the present experiment 0.012 in the cones and 0.025 in the rods. Corresponding values for the previous subject had been about twice as great: 0.022 in the cones and 0.054 in the rods. In the present instance the "latent period" following ingestion of carotene is about half as long, and the subsequent descent of the night-blind threshold to normal about twice as rapid as in the previous subject.

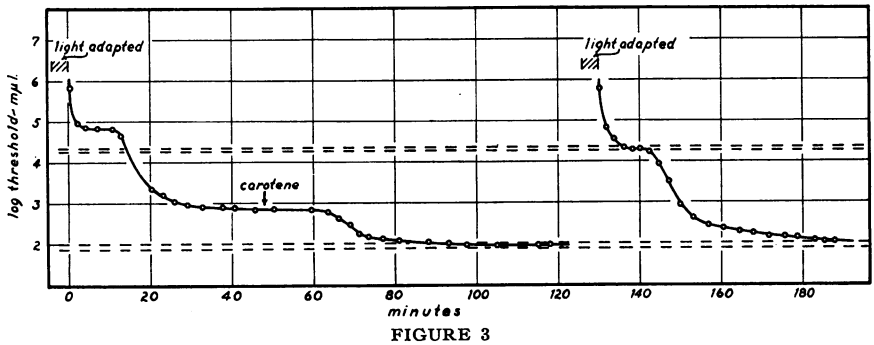

FIGURE 3

Reversal of night-blindness with carotene. Following a standard light adaptation, the measurement of dark adaptation shows both cone and rod plateaus to lie above their normal ranges (shown by broken lines). After dark adaptation is complete, 20,000 units of carotene are administered in gelatine capsules orally. For a "latent period" of 12–14 minutes the rod threshold remains constant. Then it falls rapidly to normal. Immediate repetition of the standard adaptation procedure shows both cone and rod plateaus to have entered their normal ranges.

The speed of rise of night-blindness in this type of experiment may vary much more widely than these two researches indicate. Our more recent work has shown that on an identical régime and within comparable periods about half of our subjects develop no night-blindness at all. The basis for this sharp variation in response is still unknown. It seems to have little to do with the vitamin A reserve since all our subjects have been "saturated" with vitamin A before beginning the deficient diet.

The parallelism in response of cones and rods to vitamin A-deficiency has been noted previously.[6, 3, 7] One obvious inference to be drawn from it is that vitamin A is the precursor of cone photopigments in addition to rhodopsin. But this is not the only plausible possibility, and in default of direct chemical information the significance of the cone changes remains uncertain.

The linearity of rise of log threshold during the deficiency appears to possess the following significance. Hecht[8] has shown that the photodecomposition of rhodopsin is first-order, and is governed by an equation of the form, $-dr/dt = kIr$, in which k is a constant, and r, t and I are respectively the rhodopsin concentration, time and light intensity. If it be assumed that at the threshold the flash of constant duration, dt, decomposes a constant quantity of rhodopsin, dr, the expression reduces to $Ir = constant$. That is, the threshold intensity is inversely proportional to rhodopsin concentration. A linear rise of log threshold therefore corresponds to a simple logarithmic decrease in the concentration of rhodopsin, and this in turn to a logarithmic decrease in the available vitamin A, with which rhodopsin is in retinal equilibrium. This is almost the simplest result that could have been anticipated. It means that each day of the deficient diet a constant fraction of what rhodopsin and vitamin A remain is lost.

In human vitamin A-deficiency experiments recently reported by Hecht and Mandelbaum,[7] though the rise of night-blindness was much as we have described it, its reversal on vitamin A administration occupied about two months. This is in striking disagreement, not only with our own results, but with clinical reports that severe night-blindness is reversed within periods of 24 to 48 hours.[9] The basic deficiency diets which all of us have employed are almost certainly low in other dietary essentials than vitamin A. Hecht and Mandelbaum's procedure differed significantly from ours in omitting supplements of these factors. It may be that in this case conditions other than simple lack of vitamin A contribute to the rise of night-blindness, and produce effects which are only slowly reversed by vitamin A administration and the return to a normal diet.

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[5] Daily supplements were taken of about 280 International Units of vitamin B_1 and 160 Sherman Units of B_2 in brewer's yeast tablets, 50 mgm. ascorbic acid, about 1700 International Units of vitamin D, 2 grams dicalcium phosphate and 9 grains ferrous sulphate. We are much indebted to the Abbott Laboratories of North Chicago, Ill., for supplying these materials and the halibut liver oil used as the source of vitamin A.

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69 (1936)) have reported complete cures of night-blindness within 7 to 10 minutes following intramuscular injection of vitamin A concentrates; but this work requires confirmation with more accurate procedures (cf. Groth-Petersen, E., *Acta Med. Scand.*, **95**, 110 (1938)).

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*THE STEADY POTENTIAL OF THE HUMAN EYE IN SUBJECTS WITH UNILATERAL ENUCLEATION**

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In some previous communications[1, 2] a method was described for the direct study of the polarity potential in the human eye *in situ*, and the reliability of the measurements, typical results and individual differences were reported. The subjects in these former studies were all normal people in the respect that each had a pair of functioning eyes free from obvious pathology and exhibiting conjugate motility. By different electrode leads the eyes were measured separately and in combination when rotated laterally 30° to either side of the primary line of regard; and the relationships between the potentials found for these different leads were examined. Since each eyeball appears to constitute a separate and independent source of biological potential and the distance between their centers is ordinarily about 62 mm., it seems probable that the area of electrification that extends from each eye partially overlaps that of the other. The potential of one eye therefore cannot be measured independent of some influence from its mate. The desirability of investigating eye potentials in some people who each possess only one active eye naturally suggested itself.

The present study concerns data secured on a group of subjects each of whom has suffered unilateral enucleation and in consequence wears one artificial eye. Eye potential measurements were taken in the standard manner on a total of thirteen people with enucleation who coöperated in this investigation; but because of complicating pathologic conditions in the active eye the records for four cases could not be used in the final comparison. Results are therefore limited to five cases of right-eye and four cases of left-eye enucleation, a total of nine cases.

All subjects, whether belonging to the enucleation group or to the normal comparison group, fulfilled the same measurement routine, at least so far as they were able. This routine included certain physiological tests such as pulse rate, blood pressure and several visual tests as well as some physical measurements and queries about health and physiological state. Four small metal foil electrodes (as illustrated in a previous communica-