AN EXPERIMENT IN HUMAN DIETARY NIGHT-BLINDNESS

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The earliest demonstrable symptom of vitamin A deficiency in man and other mammals is an impairment of visual capacities in dim light known as dietary night-blindness or hemeralopia.[2] This condition is associated with some failure in the dark adaptation processes which normally replace the retinal photopigments bleached in vision. The precise nature of the disturbance and of its dependence on vitamin A is still unknown, though all the components required for its investigation are now available. They include 1, the clarification of chemical and metabolic properties of vitamin A and its precursors among the plant carotenoids (Moore, 1933; Kuhn and Brockman, 1933; von Euler, Karrer and Zubrys, 1934; review by Zechmeister, 1937); 2, the demonstration that vitamin A participates with rhodopsin, the photosensitive pigment of the rods, in a retinal cycle (Wald, 1935–36); and 3, accurate information concerning the nature of human visual adaptation and its measurement (cf. Hecht, 1937; Hecht, Haig, and Chase, 1936–37; Wald and Clark, 1937–38). A thorough synthesis of this material should provide useful physiological information and a rational basis for the general clinical control of vitamin A deficiency.

The measurements presented below constitute an introductory attempt on the part of the authors to provide this information. They were performed by one of us (G. W.) at the Biological Laboratories of Harvard University, simultaneously with clinical and biochemical measurements by others to be reported elsewhere. H. J. initiated the deficiency experiment and supervised all dietary arrangements. J. A. served as subject throughout the research.

METHODS. Visual thresholds were measured with a specially designed adaptometer for which the instrument of Derby, Chandler and Sloan (1929) served as substrate. In this instrument the intensity of the test field may

[1] Aided in part by a grant from the Robert Dawson Evans Memorial, Boston.

[2] Clinical observations by Bloch (1921), Blegvad (1924), Birnbacher (1928), Aykroyd (1930); cf. review by Jeghers (1937a). Experimental studies on rats by Holm (1925), on cattle, sheep and pigs by Guilbert, Miller and Hughes (1937), and on a human subject by Jeghers (1937b).

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be varied accurately over a range of one to several hundred thousand by means of a pair of circular neutral wedges rotating in opposite directions so as to compensate each other. Provision is made for inserting neutral and color filters into the path of the test light. In certain of the experiments in which deep red illuminations were required, a Wratten number *70* "monochromatic" red filter, which transmits only wavelengths above about 650 mμ, was used. In all other cases the full white radiation from a tungsten-filament incandescent lamp was employed.

The test field is circular and subtends a retinal angle of 2.1°. Above it is situated a small "star" of red light; when this is fixated, the image of the test field falls 11.7° above the subject's fovea. The fixation point is visible at all times. The test field is exposed by the subject for short flashes by means of a camera shutter set at 1/50 second. All observations are monocular; the field and fixation point are exposed alternately to the right and left eyes by displacement of a sliding diaphragm.

For *light adaptation* the subject faces a large opal-glass screen, mounted on top of the adaptometer. This is illuminated from behind with a 1000-Watt lamp, which may be withdrawn to various distances along a calibrated track to furnish the desired intensities.

A *standard procedure* was employed throughout the experiments. The subject was light adapted for precisely 4 minutes to a brightness of 4200 millilamberts. At the close of this period the adapting light was snapped off. The subject, now in complete darkness, fixated his eye and proceeded to flash the test field at intervals of about 1 second. The field was set initially at a sub-threshold intensity. Between clicks of the shutter the operator raised the intensity by regular steps of about 0.02 log unit (about 5 per cent) until the subject reported seeing the flash. The operator noted time and wedge-reading. Following the initial light adaptation, such thresholds were determined periodically in darkness, regularly alternating the two eyes.

This type of procedure, in which the subject can report his response to each intensity of test-light before it is raised, precludes "over-shooting" the threshold, and eliminates any unconscious tendency on the part of the operator to influence the results. The short exposures which we employ permit measurements of threshold to be spaced as closely as three per minute without appreciably affecting the course of dark adaptation.

The *subject* (J. A.) is a fourth-year medical student, age 26, normal weight 135 pounds. Some time previous to the present research he had been found to be markedly hemeralopic due to vitamin A-deficiency (Jeghers, 1937b, case 1). This condition was corrected by addition of milk, butter, fresh fruits, vegetables and vitamin A supplement to his diet. When the present experiments were begun the subject possessed perfectly normal visual adaptation and was otherwise in good health.

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MEASUREMENTS. *Control period.* The research was begun with a control period lasting 48 days. During this interval the subject consumed a normal varied diet, supplemented with about 70,000 units daily of vitamin A.[3,4] About three times per week his dark adaptation was measured

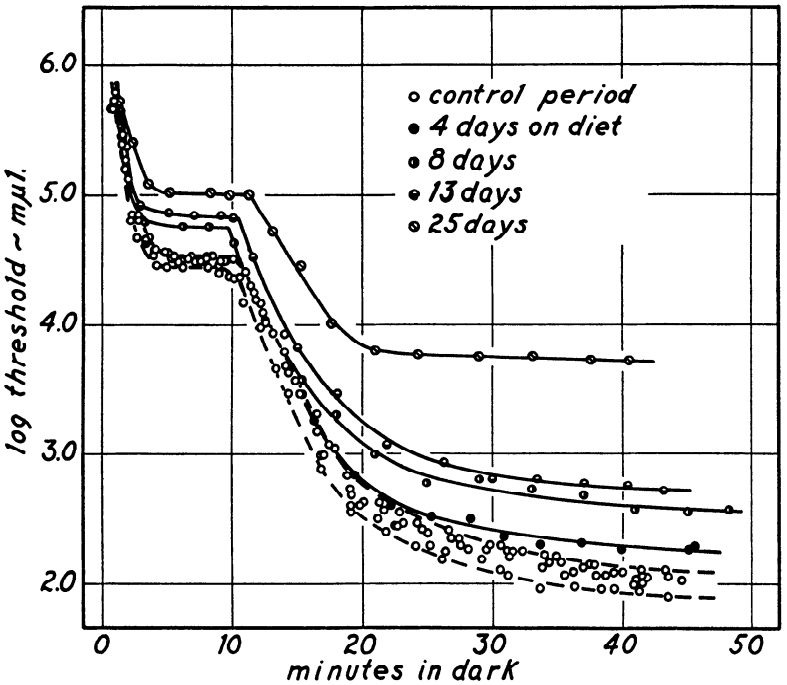

Fig. 1. Dark adaptation during a control period of optimal vitamin A nutrition (open circles; 6 experiments), and after various intervals on a diet deficient in vitamin A (closed and modified circles). Each point is a single determination of the threshold of the left eye to white light. The unit of threshold intensity is the milli-microlambert = 10^{-6} millilambert. Only abnormal portions of the data obtained on the fourth and eighth days of the deficient diet are shown.

with the standard procedure. The further course of the research can be evaluated only with close reference to these control data.

At the moment the adapting light is extinguished, the eye begins to

[3] Vitamin A and carotene quantities are expressed throughout this paper in the U. S. P. XI unit, equivalent to one International Unit (I.U.), or about 0.6γ of pure vitamin A or carotene.

[4] We wish to thank Parke-Davis & Co., Detroit, for supplying the halibut liver oil concentrates used as a source of vitamin A; the S. M. A. Corp., Cleveland, for carotene-in-oil and colloidal carotene preparations; and the Abbott Laboratories, North Chicago, for the brewer's yeast tablets, ascorbic acid, viosterol and calcium phosphate tablets used in these experiments.

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increase in sensitivity, i.e., its threshold falls. When the logarithm of the threshold is plotted as a function of time in darkness, this change follows a characteristic course (fig. 1, open circles). There is an initial rapid fall to a plateau maintained from about the fifth to the tenth minute, then, following a sharp break, a second descent to a final plateau attained in about 40 minutes. It has been appreciated for some time that the initial segment is due to cones, the later portion to rods (cf. Hecht, 1937). The initial plateau therefore represents the threshold of the dark adapted cones, the final plateau that of the rods.

Cone and rod adaptations may be more completely separated by the use of deep red test lights, to which the rods are relatively insensitive (Hecht, 1937; Kohlrausch, 1931). In a number of experiments the standard light adaptation was followed with measurements of thresholds to "monochro-

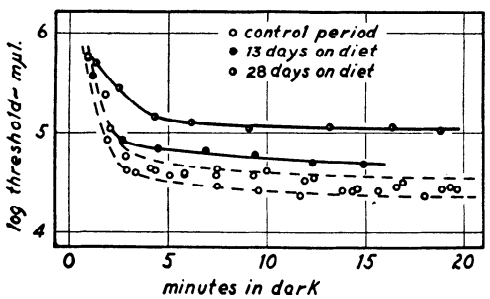

Fig. 2. Dark adaptation of the subject's left eye, measured with deep red test illuminations to which the rods are relatively insensitive. The data therefore describe the isolated adaptation of the cones. Otherwise as in figure 1. Data for the control period include 3 experiments.

matic" red light of wavelengths longer than 650 mμ. Under these conditions the dark adaptation of the cones may be followed in isolation for about 20 minutes. Data obtained in this way during the control period confirm those obtained with white light in showing cone dark adaptation to involve a fall in threshold of about 1.5 log units (about 32 times) and to be virtually complete within about 10 minutes (fig. 2, open circles).

The general expediency of plotting the visual thresholds in dark adaptation logarithmically has been appreciated for some time (Hecht, 1919-20; Kohlrausch, 1931, p. 1572). Two conditions give this usage special force in the present instance:

1. Each fall of one log unit represents a tenfold decrease in threshold. The normal range of dark adaptation in the present experiments is about 4 log units, or about 10,000 to 1. Following light adaptation the threshold falls to 1/10 of its initial value in about 2 minutes, to 1/32 (the cone plateau) in about 5 minutes, and to 1/100 in about 12.5 minutes. By this time, therefore, 99 per cent of the total absolute fall in threshold is already complete. This consideration has prompted the belief that

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dark adaptation is "practically" over within about 10 minutes. Actually the further drop from 1 to 0.01 per cent of the initial threshold is as significant as the initial 99 per cent decrease, and is negotiated in about the same detail and with the same order of accuracy. This is a "practical" matter: when our subject's threshold failed to fall below 1 per cent, or even 0.1 per cent of its light adapted value, he was definitely night-blind, not only by clinical and laboratory test, but in his daily experience.

2. Dark adaptation data normally vary from day to day. Experimentally induced changes possess significance only with reference to this normal scatter. Figures 1 and 2 show this variation to assume very simple form on a logarithmic scale: it is approximately constant in width at all stages of cone and of rod adaptation. All data for the control period may be enclosed, therefore, between parallel lines. The day-to-day range of variation in each eye is about 0.1 log unit (about 26 per cent) for the cones, and about 0.2 log unit (about 60 per cent) for the rods (fig. 1).

Recently a large number of clinical observations have appeared concerning the behavior of normal and reputedly hemeralopic subjects when examined with the so-called "Biophotometer," using an arbitrary standard technique based upon visual adaptation (Jeans, Blanchard and Zentmire, 1937; Jeghers, 1937a, b). The relation of these to the present measurements can be stated only very roughly, since practically all phases of the procedures differ significantly. The conventional Biophotometer technique employs a 3 to 5 minute light adaptation to a brightness only about 1/21 of that used in the present experiments. This is followed with two measurements of threshold, at 20 seconds and at 10 minutes in darkness. Roughly these correspond with thresholds in the present procedure at about 8 and 18 minutes in darkness.

Rise of hemeralopia. At the close of the control period the subject abruptly eliminated vitamin A supplement and all milk products, eggs and colored vegetables from his diet. To ensure optimal supplies of all dietary factors other than vitamin A, the experimental regime was supplemented daily with 200 I. U. of vitamin B_1 and 150 Sherman units of vitamin B_2 taken in brewer's yeast, 50 mgm. of ascorbic acid, 2000 I. U. of vitamin D ("viosterol"), 8 grains ferrous sulphate, and 2 grams dicalcium phosphate. This basic diet, containing an estimated 50 to 200 units per day of vitamin A, had been found to induce hemeralopia within a short period (Jeghers, 1937b). It was maintained unaltered for 30 days. Its effects upon the course of dark adaptation are shown in figures 1 and 2; in figure 3 are plotted separately the thresholds of the completely dark adapted cones and rods (the cone and rod "plateaus").

At the close of 24 hours on this deficient diet, the first 30 minutes of dark adaptation of the right eye and the entire function in the left eye remained within the normal range. The threshold of the completely dark adapted right eye, however, had already risen significantly (fig. 3).

By the end of the fourth day, the final thresholds of both eyes had risen well above the normal range (figs. 1 and 3). The cone function and the

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initial portions of rod dark adaptation still appeared normal. The threshold of the completely dark adapted eye, therefore, responds first to the vitamin A deficiency.

After six days on the deficient diet, a longer portion of rod dark adaptation had risen above the normal range, but the entire cone function still remained within it.

On the eighth day the cone plateau suddenly rose about 0.25 log unit (about 80 per cent) above the normal average. The rod plateau had by

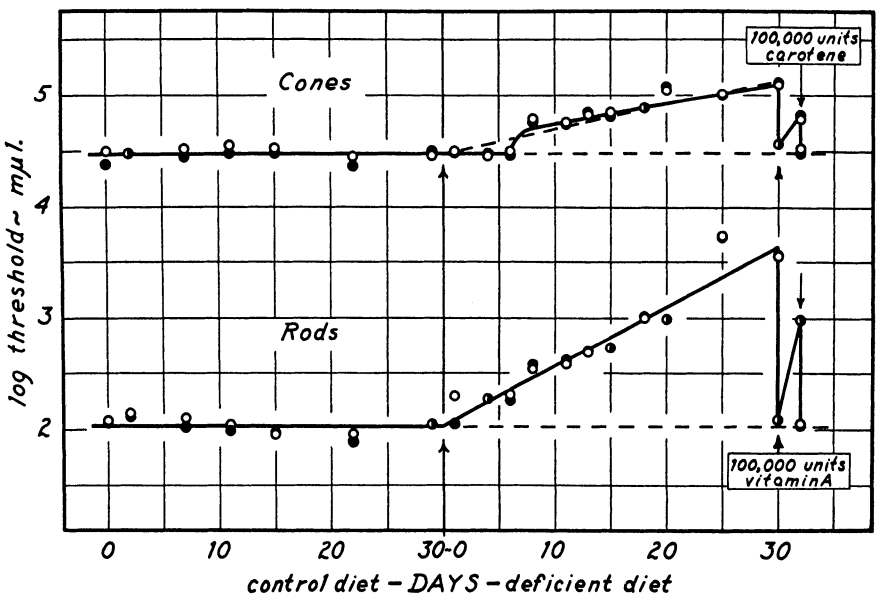

Fig. 3. Thresholds of completely dark adapted cones and rods (cone and rod "plateaus"), during 30 days of optimal vitamin A nutrition (left), and during 30 days on a vitamin A-deficient diet (right). Open and closed circles show thresholds of right and left eyes respectively. On the thirtieth day of the deficient diet vitamin A was administered; the course of cure is shown in figure 4*A*. On the thirty-second day the subject was again hemeralopic and was given carotene; the details of this experiment are shown in figure 5.

this time risen over 0.5 log unit (over 300 per cent) above the normal average. The initial portions of both cone and rod adaptation still remained within the normal range (figs. 1 and 3). It is true of the cones, as of the rods, therefore, that the completely dark adapted condition first shows the effect of the deficient diet. At this stage of the experiment thresholds measured between 4 and 10 minutes or after 15 minutes in darkness give clear evidence of hemeralopia, while those determined at other times are normal.

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By the eleventh day the entire course of dark adaptation beyond the initial descending limb of the cone function had emerged from the normal range. Thereafter all thresholds continued to rise for the remainder of the deficiency period (figs. 1–3). Greater day-to-day variation marked the course of this development than was found during the normal period, probably due principally to fluctuations in the daily intake of vitamin A.

The most severe hemeralopia appeared in the rods on the twenty-fifth day, when their dark adapted threshold had risen about 1.7 log units or to about 50 times the normal average. The greatest change in the cones occurred on the thirtieth day, when their final threshold had risen 0.63 log unit, or to about 4.3 times the normal average.

It is clear that cones as well as rods become hemeralopic on the deficient diet. This is shown most unequivocally in the experiments with red test lights (fig. 2). The cones may respond later, and their thresholds rise more slowly than those of the rods. Nevertheless the cone response is of great theoretical and practical importance. It implies that all aspects of vision in dim light, including color vision, are impaired in vitamin A deficiency.

The rise of the rod plateau, plotted logarithmically, is described adequately with a straight line originating on the first day of the restricted diet (fig. 3). Most of the ascent of the cone plateau also is linear in this type of plot, following an abrupt rise on the eighth day. Indeed, a straight line, originating on the first day of the diet, may be drawn so that none of the cone data diverge from it further than is accounted for by the ordinary day-to-day variation. This is shown as a broken line through the cone data of figure 3. It is possible, therefore, that our apparent sudden onset of cone hemeralopia on the eighth day is a fortuitous effect of the scatter of the function, and that actually the hemeralopia of the cones originates on the first day of the deficient diet, as does that of the rods.

It has frequently been asserted clinically that the primary change in dietary hemeralopia is retardation in the velocity of dark adaptation with little or no change in its final level (cf. Dieter, 1931). This is not true in the present experiments. The data of figures 1 and 2 show that dark adaptation of both the cones and rods is completed as rapidly in the hemeralopic as in the normal eye. The primary abnormality is the rise of the cone and rod plateaus, and eventually of the thresholds at all levels of visual adaptation.

The threshold of the completely dark adapted eye not only first responds to the deficient diet; it systematically exhibits by far the greatest change. In the highly hemeralopic eye, as after the eleventh day in the present experiments, the log threshold diverges increasingly from normal as dark adaptation progresses. This observation is of great importance in the design of clinical procedures for the measurement of dietary hemeralopia.

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Cure of hemeralopia. On the thirtieth day of the deficiency the subject's threshold at complete dark adaptation was found to be about 1.5 log units (about 32 times) above normal. He was given about 100,000 units

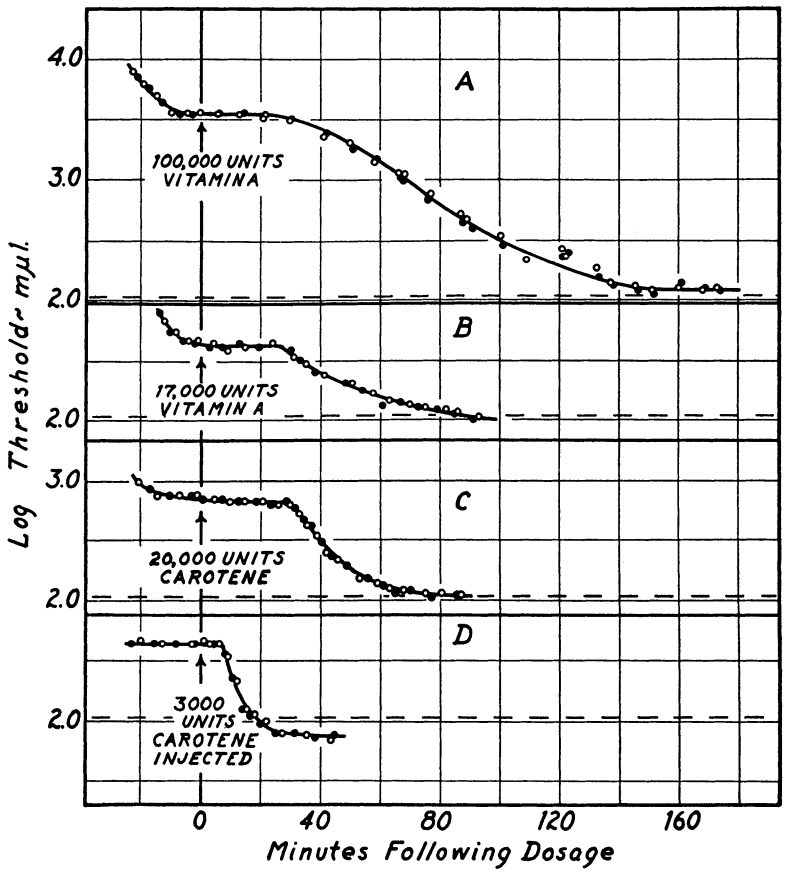

Fig. 4. Cure of hemeralopia with vitamin A and carotene. Thresholds of right and left eyes are shown as open and closed circles respectively. Measurements preceding the dosage show terminal portions of dark adaptation. In each section of the figure a broken line indicates the average threshold of the normal dark adapted eye. The threshold is markedly hemeralopic at the beginning of each experiment; after administration of vitamin A or carotene orally (*A-C*) or by intramuscular injection (*D*) the threshold descends to a final level within the normal day-to-day range.

of vitamin A in the form of 12 gelatine capsules of halibut liver oil, taken orally with water.

For about 24 minutes the dark adapted threshold remained unchanged. Then it began to fall regularly, and at 150 minutes following administra-

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tion of the vitamin it had reached the normal level. The course of this change is shown in figure 4A. When the rod threshold had become normal, the subject was again run through the standard adaptation procedure. Both cone and rod segments of dark adaptation were found to be entirely normal (fig. 3).

Two days later, the deficient diet having been maintained unaltered, the subject was again decidedly hemeralopic (fig. 3 and 5). This second rise of hemeralopia was many times more rapid than its initial development. In these two days the cone plateau rose as high as in 8 to 12 days, and the rod plateau as high as in 18 days of the original deficiency. Presumably this great contrast in the effectiveness of vitamin A-deprivation

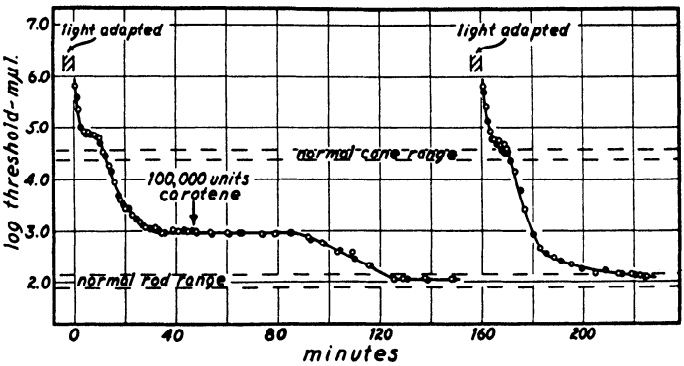

Fig. 5. Complete course of an experimental cure of night-blindness. Data of right and left eyes are shown with open and closed circles respectively. Following a standard 4-minute light adaptation, measurement of dark adaptation shows both cone and rod plateaus to lie well above their normal ranges (broken lines). When dark adaptation is complete, carotene is administered orally. After a "latent period" of 38 minutes, the rod threshold descends to normal. Repetition of the standard light and dark adaptation procedures shows both cone and rod plateaus to have entered the normal range.

is correlated with the presence of maximal vitamin reserves at the close of the control period, of little or no reserves at the end of the long initial deficiency. Since in both situations effects of the deficient diet were appreciable almost at once, it may be concluded that stored vitamin A in this instance retards the progress of hemeralopia, but does not defer its onset.

The entire course of the second "cure" experiment is shown in figure 5. The subject, when completely dark adapted, was given about 100,000 units of carotene in cotton-seed oil, taken orally in gelatine capsules with water. The hemeralopic threshold remained unchanged for 38 minutes. Then it descended rapidly and attained the normal rod level within 80

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minutes following the carotene administration. Repetition of the standdard adaptation procedure showed both cone and rod thresholds to have become normal.

During subsequent weeks the subject was repeatedly allowed to become hemeralopic on the deficient diet, and was brought back to normal temporarily by oral administration of carotene or vitamin A. The effects of these treatments are highly regular (fig. 4*A-C*, fig. 5; table 1). They begin with a "latent period" of 26 to 38 minutes during which the threshold remains constant. The duration of this interval is independent of the nature and concentration of the administered oil. It is probably occupied principally with solution of the gelatine capsules and delivery of the oils to the intestine.

TABLE 1

Numerical constants associated with the cure of dietary hemeralopia with various dosages and modes of administration of vitamin A and carotene

The "latent periods" in the fourth column are intervals between administration of the active principle and the first appreciable decrease in hemeralopic threshold. The numbers in the fifth column state the maximum fall in log threshold intensity per minute during the period of "cure."

ACTIVE PRINCIPLE	METHOD OF ADMINISTRATION	DOSAGE (U.S.P. XI UNITS)	"LATENT PERIOD"	MAXIMUM SPEED OF "CURE" (LOG THRESHOLD PER MINUTE)
Vitamin A	Oral	100,000	30	0.016
Vitamin A	Oral	17,000	26-30	0.016
Carotene	Oral	100,000	38	0.026
Carotene	Oral	20,000	30	0.033
Carotene (colloidal)	Intramuscular injection	3,000	7	0.068

The descent of the threshold to normal follows. Its velocity also is independent of the dosages we have employed. We find it, however, to be almost twice as rapid following oral carotene as after oral vitamin A administration. The maximum velocities of fall of log threshold in four "cure" experiments (fig. 4*A–C*; fig. 5), shown in table 1, illustrate clearly these characteristics.

Oral administration in gelatine capsules is obviously a very slow method for delivering the vitamin to the retina. Even the short periods for cure measured in the experiments described above must have been largely occupied with processes of digestion, absorption and transport. That this is true is demonstrated in an experiment in which 3000 units of colloidal aqueous carotene were injected intramuscularly into the gluteal region of the hemeralopic subject. The result is shown in figure 4*D*. Seven minutes following the injection the threshold broke sharply in descent. Within 20

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minutes it had reached the normal rod level, and within about 35 minutes had become stable at as low a level as was ever attained by the normal eye during the control period.

This experiment shows that at least $\frac{3}{4}$ of the "latent period" following oral administration of the A factor and at least half of the period of "cure" are occupied with processes which deliver the vitamin to the tissues. Our observation that orally administered carotene acts more rapidly than vitamin A therefore is probably associated with greater efficiency in the absorption and transport of carotene, not necessarily at all with the efficiency of its utilization by the retina.

DISCUSSION. Aykroyd's report in 1930 that marked dietary hemeralopia may develop within less than a month of vitamin A-deprivation and may be cured with one or two doses of cod-liver oil within 24 to 48 hours occasioned considerable interest. By 1932 the reported period for cure had been reduced to 12 to 24 hours.[5] With the introduction of sensitive procedures for estimating night-blindness, these times have since dwindled greatly. Jeghers (1937) observed evidences of hemeralopia on the sixth day of an experimental vitamin A deficiency, and some improvement of the hemeralopic threshold within 2 hours after oral administration of vitamin A. Edmund and Clemmesen (1936) have reported that following intramuscular injection of a vitamin A preparation the visual discrimination of three hemeralopic clinic patients became normal within from 7 to 10 minutes; the procedure employed, however, may be quite unreliable when applied as in this instance to untrained observers (cf. Groth-Petersen, 1938).

Our experiments show that an appreciable rise of visual threshold may appear within 24 hours on a vitamin A-deficient diet, in a normal subject previously optimally supplied with the vitamin. Pronounced hemeralopia may be improved within about 30 and cured within about 80 minutes after oral administration of vitamin A or carotene; intramuscular injection reduces these periods to about 7 minutes for improvement of threshold, and 20 minutes for complete cure. Even these very short intervals are probably limited principally by the method of administration of the vitamin.

It is necessary to distinguish clearly the acute "pure" vitamin A deficiency which we have studied from conditions more likely to be encountered outside the laboratory. Chronic vitamin A deficiency may involve widespread epithelial metaplasia (Wolbach and Howe, 1925) and nerve degeneration (Mellanby, 1934); and usually human malnutrition includes deficiencies in a wide variety of dietary essentials in addition to vitamin A. Hemeralopia in all these cases may be aggravated by anatom-

[5] Cited as a personal communication from Mori in the Medical Research Council Report on Vitamins (1932).

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ical lesions which may respond to treatment comparatively slowly. To the extent, however, that a nutritional deficiency involves vitamin A, it includes a loss of visual pigments from the retina; and one initial effect of vitamin A therapy is the replacement of these pigments. These are the specific processes which we have studied. There is no present reason to believe that as components of more complicated situations their characteristics are altered. For these processes our observations, from their consistency with existing data, appear to furnish a reliable description.

Cones and rods. In the belief that hemeralopia is a specific disorder of the rods, Parinaud (1881) advanced it as a principal support of the "Duplicity" theory. Hess (1909) removed the acquired—as opposed to congenital—hemeralopias from this position, with the observation that in subjects hemeralopic from various causes, particularly in one case of chronic liver disease, the rod-free foveal area was pathological "in the same sense" as the rod apparatus. Hess' measurements were very crude by present standards. His conclusions, however, have recently been confirmed by precise measurements of dark adaptation in 13 persons afflicted with chronic liver diseases (Haig, Hecht and Patek, 1938). These determinations reveal an exact parallelism in the behavior of cone and rod thresholds in hemeralopia and in its response to vitamin A therapy.

The present experiments extend these observations to the field of "pure" vitamin A deficiency and its cure with carotene or vitamin A. In every instance complete parallelism in the behavior of cones and rods has been observed. The physiological significance of this situation is not yet certain. Numerous sharp differences in the physiological responses of cones and rods (Hecht, 1937), and the recent discovery of one specific photosensitive substance in cones (Wald, 1937) make it reasonably certain that cones contain photolabile pigments chemically distinct from rhodopsin. It is difficult to avoid the conclusion that vitamin A is the precursor not only of rhodopsin, but of some or all of the visual pigments in the human cones.

Vitamin A and rhodopsin. The dependence of adaptation in the rods upon vitamin A appears to be no more complicated than is implied in the equations of the retinal cycle: rhodopsin $\overset{\text{light}}{\rightleftharpoons}$ retinene-protein $\rightarrow$ vitamin A-protein $\rightarrow$ rhodopsin (Wald, 1935-36). Decrease in the concentration of available vitamin A results in corresponding decreases in retinene and rhodopsin, and consequent rise in the visual threshold. On readmission of vitamin A to the retina, rhodopsin apparently is at once synthesized to its normal concentration, and hemeralopia ceases. Dietary hemeralopia is essentially incomplete dark adaptation, its correction the completion of dark adaptation. It is probable that the limiting velocity with which hemeralopia might be cured, when vitamin A had been administered under

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optimal conditions, should correspond with the concluding portions of a normal dark adaptation.

It is of some interest to estimate the absolute chemical significance of the changes which we have recorded. There is good reason to believe that the reciprocal of the rod threshold, as measured in the present experiments, is directly proportional to the concentration of rhodopsin.[6] If this is true, a rise in threshold of the dark adapted rods of 1 log unit (10 times) above the normal level implies a fall in rhodopsin concentration to $\frac{1}{10}$ its normal value. In his most extreme hemeralopia, therefore, our subject's dark adapted retina probably contained only about $\frac{1}{50}$ of its normal complement of rhodopsin.

Carotene and vitamin A. Carotene introduced into the blood circulation is very rapidly and almost quantitatively absorbed by the liver (Drummond, Gilding and Macwalter, 1934). It is commonly supposed that carotene must be converted in the liver to vitamin A before being used by the tissues. This is generally assumed to be a disadvantage in the use of carotene as opposed to direct vitamin A administration. Our experiments show, however, that in a normal subject such disadvantage, if it exists at all, is over-compensated by the greater efficiency with which orally administered carotene is absorbed and transported. This observation probably possesses no practical importance whatever for the normal subject. However, in a number of cases of hemeralopia encountered clinically, oral administration of vitamin A has proved ineffective (cf. Edmund and Clemmesen, 1936). Apparently pathological conditions exist in which the absorption and transport of vitamin A become limiting factors in its utilization. It is possible that in such cases carotene possesses a significant therapeutic advantage.

The period between intramuscular injection of carotene and its effect on the visual threshold is so short as to suggest that the eye may use carotene directly. This possibility is subject to certain limitations. Though vitamin A has been found in all retinas so far examined which contain rhodopsin, none of them normally contains carotene (Wald, 1934–34; 1935–36). Also it is improbable chemically that carotene can substitute directly for vitamin A in the rhodopsin cycle. It must almost necessarily be assumed that if the eye can use carotene, some eye tissue in addition to the liver can convert it to vitamin A. This function might possibly be fulfilled by the pigmented epithelium, which is known to participate in rhodopsin synthesis, and often contains large quantities of the colored carotenoids (Wald, 1935–36).

[6] It is assumed that at the threshold a test flash of constant short duration, dt, decomposes a constant quantity of rhodopsin, dr. The rate of photolysis of rhodopsin is governed by the equation, $dr/dt = k\ I\ r$ in which k is a constant and I the intensity (Hecht, 1923–24). The assumption states that $dr = constant = k\ I\ r\ dt$, in which both k and dt are constants. Therefore $r = constant/I$.

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Clinical procedure. Apparently the most appropriate visual measure of vitamin A deficiency is the threshold of the completely dark adapted eye. Only this threshold may conveniently be determined repeatedly without change, and the accuracy of the measurement consequently increased enormously by repetition and averaging. This threshold responds first and most to vitamin A deficiency, and appears to be the most sensitive available measure of hemeralopia.

We suggest, therefore, that in future clinical procedures for estimating dietary night-blindness, preliminary light adaptation be omitted. Subjects may be dark adapted in a darkened room or by some other device for withholding light from the eyes. Following 30 to 45 minutes in darkness, the visual threshold may be determined repeatedly. The scatter of the measurements provides a clear indication of the reliability of the subject's reports. Their average, a single number, should constitute the most exact index now available of the hemeralopic state, and therewith of the vitamin A nutritional level.

SUMMARY

1. The dark adaptation of a human subject was measured at regular intervals during a long control period; and during a subsequent period on a diet containing only 50 to 200 U. S. P. units daily of vitamin A, but otherwise complete.

2. A first effect of the deficient diet was noted within 24 hours. Within 25 days the threshold of the dark adapted rods had risen about 50 times, that of the dark adapted cones about 4 times. The threshold of the dark adapted rods responds first and most strongly to the vitamin A deficiency.

3. Following temporary cure of the initial night-blindness with a single dose of vitamin A, hemeralopia re-appeared with greatly increased rapidity, presumably due to depletion of vitamin A reserves during the initial deficiency.

4. The development of hemeralopia was repeatedly checked temporarily by oral administration of vitamin A or carotene. Ingestion of either of these is followed by a "latent period" of about 30 minutes during which the hemeralopic threshold remains unchanged. Then the threshold descends to normal, about twice as rapidly after carotene as after vitamin A administration. This difference in effectiveness appears to be due to greater efficiency in the absorption and transport of carotene.

5. Following intramuscular injection of colloidal carotene the hemeralopic threshold improved within 7 minutes and fell to normal within 20 minutes.

6. In all these changes the behavior of cones parallels that of rods. Vitamin A appears to be the precursor of cone visual pigments as well as of the rhodopsin of the rods.

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7. It is suggested that clinical measurements of hemeralopia omit preliminary light adaptation of the subject and accept the threshold of the completely dark adapted eye as the most satisfactory hemeralopic index.

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