THE REGENERATION OF VISUAL PURPLE IN THE LIVING ANIMAL

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I

Vision and Visual Purple

When an animal, which has been in the light, is placed in the dark, its sensitivity to light increases. Measured as the light intensity necessary to elicit a minimal visual response, these changes yield the familiar data of dark adaptation (Aubert, 1865; Piper, 1903; Hecht, 1937).

For many years it has been known that during dark adaptation the vertebrate retina shows changes in concentration of the photosensitive rod pigment visual purple (Kühne, 1878; Gatti, 1897; Fridericia and Holm, 1925; Tansley, 1931; Zewi, 1939). More recently the chemical behavior of this substance has become associated with certain carotenoids (Wald, 1938).

The present research was undertaken to discover the course of the concentration changes shown by visual purple in the intact animal, to relate this information to the data of dark adaptation, and to elucidate the behavior of the carotenoid pigments. As a working hypothesis it is assumed that dark adaptation depends on the accumulation of visual purple in the retina. It is proposed, therefore, to use those conditions during regeneration of visual purple which are known to modify dark adaptation in specific ways and to see whether similar effects are produced on the course of visual purple accumulation.

II

Dark Adaptation and Visual Purple

Due to the work of Hecht (1921) and of Kohlrausch (1922) it is known for the human eye that the two receptor systems, rods and cones, both enter into normal dark adaptation. Following daylight illuminations, human dark adaptation proceeds in two stages. The first is rapid and is practically complete in 3 or 4 minutes; it records mainly cone function. The second is delayed, slow, and takes about 25 minutes for completion; it records rod function.

There are many factors which control the extent, speed, and duration of the two parts of the course of dark adaptation. The most striking are the intensity and duration of the light preceding the beginning of dark adaptation. Following high intensity light adaptation, the threshold falls in two steps. Decreasing the intensity of light adaptation diminishes the extent of the first section and shortens the time at which the transition from cone to rod function

occurs. Following the lowest intensities of light adaptation, cone function does not appear at all, and only the secondary rod adaptation is evident (Johannsen, 1934; Winsor and Clark, 1936; Hecht, Haig and Chase, 1937). In other words, the portion of dark adaptation mediated by the rods is delayed when the eye is light adapted to high intensities, and begins without delay following low intensity light adaptation.

A similar effect on the course of dark adaptation is produced by varying the duration of light adaptation. Müller (1931) found that following a short period of light adaptation only the secondary rod dark adaptation appears. With increasing time of light adaptation, the primary cone adaptation appears and increases in duration until it occupies the first 5 minutes of the dark adaptation process, while the second, rod portion, is delayed for greater periods. These results were later confirmed by Wald and Clark (1937).

Among the many animals whose dark adaptation has been measured, the frog is of particular interest here. It too possesses rods and cones and may be expected to show a two-stage adaptation. This has been found by Riggs (1936–37) who, following the method of Hartline (1930), used as an index the B wave of the retinal potential elicited by a brief illumination of the intact dark adapting eye. This method is comparable with the procedure used on the human eye. In the latter the data represent the light intensity required to produce a constant visual effect at various times in the dark. The procedure of Hartline and Riggs is to determine the intensity of light which produces a constant physiological effect, in this case a given retinal potential, at different times in the dark. The course of adaptation divides into two sections, the first corresponding to cone function, and the second to rod function. The rods do not mediate the function until the cone portion of the data has reached completion approximately 10 minutes after the onset of dark adaptation. The rod function reaches a threshold about 1 hour after the start of dark adaptation.

The results of Granit, Holmberg, and Zewi (1938) and of Granit, Munsterhjelm, and Zewi (1939) on frog dark adaptation seem at first sight completely at variance with the human data and those of Riggs. Instead of a rapid increase in sensitivity as found by Riggs, Granit and his coworkers report delays of as long as 1 hour before a rise in sensitivity begins. Moreover, in spite of the fact that the frog retina contains both rods and cones and that the adapting light is of sufficient intensity to show their separate effects, the data are continuous without any indication of a double function.

This apparent discrepancy befween the work of Granit and of Riggs is probably due to the method used by Granit in which the *size* of the retinal potential evoked by a measuring light of constant intensity is considered the measure of sensitivity. This method is not comparable either to the accepted practice in direct visual measurements or in retinal potential measurements of

visual function (*cf.* Chaffee, Bovie, and Hampson, 1923; Hartline, 1930), in which there is always measured the variation in intensity required to produce a constant physiological or electrical effect. It is quite likely that the reason Granit found only a single and very delayed function in frog dark adaptation is that the *magnitude* of the retinal potential does not correspond to visual sensitivity as measured in the usual ways.

Considering the relationship of visual purple to vision, it would be desirable to know how these changes in the sensitivity of the eye depend upon variations in the concentration of visual purple. For conditions at the stationary state, a fairly successful description of many visual functions has been made upon the basis of simple photochemical theory derived from consideration of the equilibrium concentration of a photosensitive material like visual purple (Hecht, 1938). For kinetic measurements like dark adaptation, however, there exists no adequate description either in terms of theory or in terms of actual measurements of visual purple concentration.

Kühne (1878) showed that both the intact retina and solutions of visual purple, when returned to the dark, would regenerate visual purple which had been bleached by light. Hecht, Chase, Shlaer, and Haig (1936) confirmed the regeneration of visual purple in solution. Gatti (1897), and later Fridericia and Holm (1925), by comparisons of the retina with a series of standardized color charts, investigated the accumulation of visual purple in the dark adapting retina. More quantitative measurements were made by Tansley (1931), who studied spectrophotometrically digitonin extracts of dark adapting rat retinas. She found a gradual increase in visual purple concentration during dark adaptation, and the speed of the process was retarded by lack of vitamin A in the diet. Zewi's (1939) work on the regeneration of visual purple in the intact frog showed among other things that the pigment begins to increase in concentration as soon as the animal is placed in the dark except following short periods of light adaptation or at low temperature.

In addition to the changes in visual purple during dark adaptation, there are also changes in the carotenoids of the retina. Wald (1935, 1936, 1937, 1938) demonstrated the rôle of these carotenoids in the visual cycle. Excised retinas, when freshly bleached, contain a carotenoid which Wald called retinene. If the retinas then remain either in the light or in the dark, the retinene disappears, and in its place vitamin A appears. This change is independent of light and is a thermal process. According to Wald, visual purple is regenerated along two paths. One is directly from the photoproducts acting with other materials which are at hand in the retina. The other is through the conversion of retinene into vitamin A, and the combination of vitamin A with a specific protein to form visual purple.

Much information, however, is still needed to establish the quantitative

interrelations among dark adaptation, visual purple regeneration, and carotenoids. It is hoped that the following measurements will supply some of this information.

III

Apparatus

The apparatus is essentially a device for illuminating the frog so that the eye will become evenly adapted. Two such arrangements were used.

The first consists of a metal sphere S 12 inches in diameter (Fig. 1). At equal intervals on the inside of the sphere, seven automobile headlight lamps of 6–8 volts

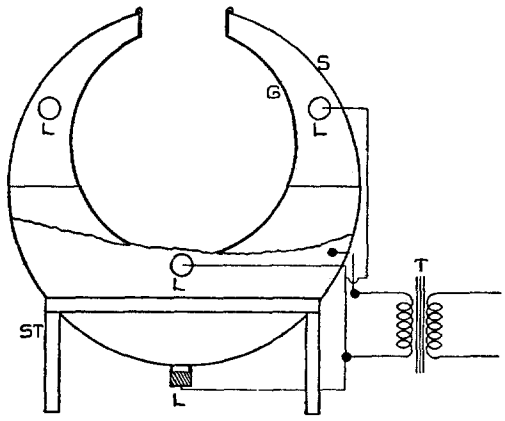

FIG. 1. First apparatus for light adaptation. The metal sphere S has on its inside a series of lights L, which illuminate evenly the opal glass globe. The frogs are in the globe which is kept at constant temperature by circulating water through it.

are mounted, and the whole group is operated at 170 watts through a transformer T. The inside of the sphere is painted flat white to insure a diffuse reflection. A spherical opal glass globe G is suspended inside the sphere and so arranged that it is evenly illuminated.

The temperature inside the globe is regulated by a continuous flow of cooling water entering on one side of the globe and leaving by suction on the opposite side.

For light adaptation the frogs are firmly fixed upon a board and placed inside the globe in the circulating water.

High intensities could not be obtained with this apparatus because the sphere was too small to permit mounting a sufficient number of lamps. Moreover, at high intensities it was impossible to prevent the temperature of the globe from rising.

The second apparatus overcame these difficulties. It consists of a conical, aluminum-painted reflector R, containing a 1000 watt lamp L (Fig. 2). The heat generated by the lamp is carried off through vents. The frog is placed in a white enamel

receptacle A, which is carefully fitted with a cover of opal glass O, over which is a pyrex glass H. The receptacle is then securely inserted underneath the conical reflector in such a way that the opal glass plate, as well as the walls of the receptacle, are evenly illuminated. The animals are cooled during light adaptation by a continuous flow of water which enters at the bottom of the receptacle I and leaves at the top X.

The intensity of the illumination is determined with a Macbeth illuminometer, and is checked during the course of the experiments.

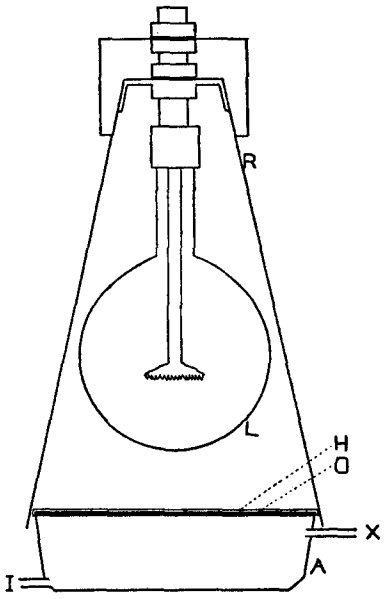

FIG. 2. Second apparatus for light adaptation. Light from a 1000 watt lamp L is reflected by the metal housing R on to the opal glass O through the heat-absorbing glass H. The frogs are in the white enamelled receptacle A and are kept at a fixed temperature by water entering at I and leaving at X.

For dark adaptation, the animals are placed in a water bath kept entirely in the dark and maintained at constant temperature.

IV

Procedure

(*a*) *Manipulations.*—The frogs were all *Rana pipiens*, obtained from Vermont and stored for only a short period in the laboratory before use. They were light adapted two at a time. The cooling water was started and the light turned on for periods of

5, 10, or 20 minutes. After the light was turned off, the animals were immediately put into the constant temperature bath for dark adaptation. The subsequent manipulations were carried out in dim red light from which the frogs and retinas were shielded as much as possible.

After dark adapting for specific periods, the two frogs were removed from the bath and immediately beheaded. The eyes were removed and dropped into water containing cracked ice. The eyes were then immediately sectioned behind the iris, and the retina and pigment layer removed into buffer solution of pH 9.6 which was kept at 0°C. The procedure after this depended on the measurements.

(*b*) *Visual Purple Determination.*—As extractive, 4 cc. of a 4 per cent solution of sodium desoxycholate in Clark and Lubs boric acid-KCl buffer mixture (Clark, 1928) of pH 9.6 were used. This choice of extractive rests on the fact that the regeneration of visual purple in solution (Hecht, Chase, Shlaer, and Haig, 1936) occurs neither in sodium desoxycholate (Chase and Smith, 1939) nor at a pH above 9.0. The extractive containing the four retinas was kept at 0°C. until the second set of retinas was similarly prepared and added to the solution. The suspension was stirred with a glass rod to break up the retinas, and then placed in a bath at 25°C. for 1 hour during which it was frequently agitated.

After extraction, the solution was centrifuged for 60 to 90 minutes. This operation was carried out at 6°C. in order to prevent an undue temperature rise due to the centrifugation, since visual purple is decomposed at high temperature (Kühne, 1878; Lythgoe and Quilliam, 1938). The clear supernatant liquid was carefully removed with a pipette, and transferred to small test tubes which were stored at 6°C.

The concentration of visual purple was determined by measuring the transmission of a 1 cm. layer of the solution at 500 mμ with Shlaer's photoelectric spectrophotometer (Shlaer, 1938) within 20 hours of extraction. Without removing it from the spectrophotometer, the solution was completely bleached by a 10 minute exposure to the light of a 250 watt projection lamp placed 6 inches from the solution and separated from it by a filter of heat-absorbing glass. The rise in temperature was seldom more than 1.5°C. After bleaching, the solution was permitted to return to room temperature and the transmission at 500 mμ again determined. The density of visual purple was then computed from the difference in transmission between the bleached and unbleached solution.

(*c*) *Carotenoids.*—For carotenoid estimation the retinas were separated from the pigment layer. Eight retinas were extracted together three times with petroleum ether, each extract employing 2 cc. of petroleum ether. The successive extracts were combined and evaporated to dryness at reduced pressure. The residue was then taken up in 2 cc. of anhydrous chloroform. To test for the presence of vitamin A and other carotenoids, the Carr-Price (1926) reaction with antimony chloride was used. The chloroform extract was placed in an absorption cell, and 5 cc. of a saturated antimony chloride solution in chloroform were added. The optical density of the resulting blue solution was determined through a thickness of 1 cm. of solution at 612 mμ and 664 mμ, corresponding to the absorption maxima of the $SbCl_3$ compounds of vitamin A and retinene respectively. Since the blue color fades rapidly, successive measurements were made, and from the plot of optical density against time of measurement the density at the moment of mixing was determined by extrapolation.

V

Visual Purple Regeneration

(*a*) *Adaptation at 25° C.*—Two series of experiments were performed at 25° C. In one, light adaptation was to 1700 millilamberts, in the other to 9500 millilamberts, both for 10 minutes, and both followed by dark adaptation at 25° C.

The results are presented in Table I, where each density is the average of the individual measurements shown in Fig. 3. Each individual point represents an extract of eight retinas. The curves are smoothly drawn as close to the averages as possible. They are nearly similar in shape and in position, and in both cases the initial concentration of visual purple is not zero, but a finite value.

The significant difference between the data obtained at the two intensities is that following 1700 millilambert light adaptation, the regeneration of visual purple begins immediately, while at the higher intensity the onset of regeneration is delayed. This delay at 25° C. lasts for about 10 minutes before a rapid rise in the concentration of visual purple begins. After regeneration starts, it proceeds rapidly and reaches a plateau approximately 75 minutes after the frogs have been placed in the dark.

These data may be compared with the behavior of the rods in adaptation. Hecht, Haig, and Chase (1937) found for human vision that recovery of rod sensitivity in the dark begins without any delay following low intensity light adaptation, while at high intensity light adaptations, rod function is increasingly delayed. The data in Fig. 3 show a similar difference between low and high intensity, though the actual intensities of light adaptation differ.

The data for the higher intensity light adaptation agree with the results of Riggs (1936–37) for the intact eye of *Rana pipiens*, which show a delay of about 10 minutes in the appearance of rod dark adaptation. It may thus be that this delay in the assumption by the rods of the threshold function in frogs during dark adaptation is due to the lag in the formation of visual purple.

The data of Zewi upon the regeneration of visual purple differ considerably from those presented here. At 25° C. and at comparable intensities of light adaptation, his data show no suggestion of delay in the recovery of visual purple. His data do not correspond with those of Riggs, which are typical of all dark adaptation data, or with the data of Granit which show very long delays in the recovery of sensitivity.

(*b*) *Adaptation at 15° C.*—The experiments were repeated at 15° C. In this way they duplicate the conditions of Riggs' measurements on the intact eye so that comparisons of the visual purple regeneration curves with the curve describing the recovery of sensitivity in the intact eye can be made.

The data are given in Table II, where each density is the average of the

individual measurements shown in Fig. 4. Each individual point represents an extract of eight retinas. The curves are drawn through the averages as before.

The measurements show that there is little difference between the regeneration of visual purple at the two intensities. The concentration of visual purple increases more rapidly after lower light adaptation, and reaches the maximum slightly earlier. However, it is evident that a decrease in temperature of 10° C. has delayed the process of regeneration to such an extent that the acceleration expected by lower light adaptation has been considerably diminished.

TABLE I

Changes in concentration of visual purple at 25° C. in retinas of frogs after 10 minute light adaptation. Series I, light adaptation at 1700 millilamberts; Series II, 9500 millilamberts.

Time in dark	Photometric density	
	Series I	Series II
min.		
0	0.012	0.015
5	0.014	0.014
10	0.016	0.016
15	0.022	0.027
20	0.028	0.042
25	0.037	0.057
30	0.046	0.070
35	0.051	0.072
40	0.054	0.079
45	0.069	0.089
50	0.075	
60	0.086	0.128
75	0.106	0.135
90	0.108	0.133
120	0.110	0.135

The initial delay in visual purple accumulation appears at both intensities of light adaptation. The limiting factor in this case is apparently the temperature of dark adaptation rather than the intensity of light adaptation.

These data again indicate that following its decomposition by light of sufficient intensity, visual purple does not begin to regenerate immediately. There is an initial period during which little or no visual purple forms, which lasts approximately 10 minutes, and is followed by a rapid rise in concentration.

The curves are sigmoid in shape, the only differences between them being the time elapsing before the rapid upward portion starts. It is also to be noted that there is a slight difference in the total period elapsing between the cessation of light adaptation and the time at which the final level of visual purple is reached.

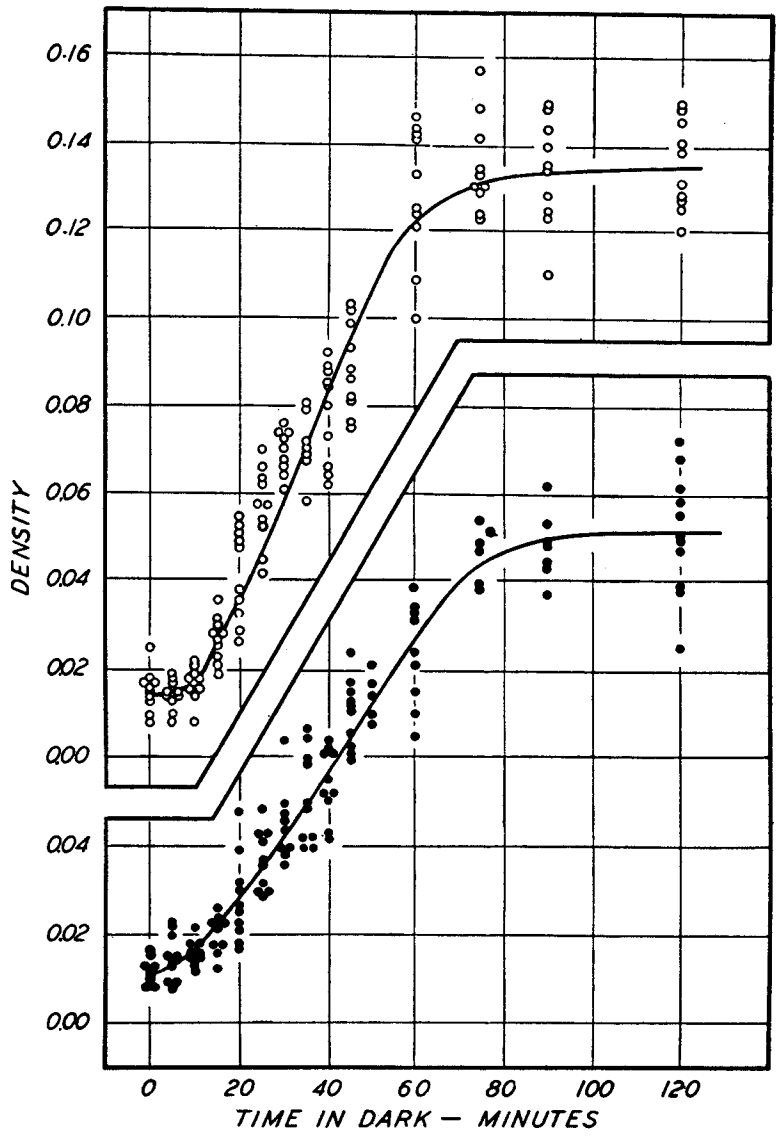

FIG. 3. Regeneration of visual purple in the intact eye of the frog at 25°C. The concentration is measured as density at 500 mμ. Open circles, visual purple concentration following light adaptation to 9500 millilamberts; closed circles, following light adaptation to 1700 millilamberts. Each point records the measurement with 8 retinas. The curve is drawn through the averages.

The data in Fig. 4 agree with those of Riggs for the dark adaptation of *Rana pipiens*. The duration of the initial delay in visual purple accumulation is the same as the period elapsing before rod function begins. Moreover, the time required for complete regeneration is approximately the same as that found by Riggs for the attainment of a constant threshold in the frog.

These data also correspond to human dark adaptation, because the visual purple regeneration is delayed just as is the decrease in the rod threshold. However, the rod threshold reaches its minimum in about 25 to 30 minutes, while the visual purple maximum is reached at about 90 to 100 minutes. This difference in time is probably due to the difference in temperature between the

TABLE II

Changes in concentration of visual purple at 15° C. in retinas of frogs after 10 minute light adaptation. Series I, light adaptation at 1700 millilamberts; Series II, 9500 millilamberts.

Time in dark	Photometric density	
	Series I	Series II
min.		
0	0.025	0.015
5	0.026	0.018
10	0.025	0.017
15	0.031	0.023
20	0.033	0.021
30	0.039	0.034
45	0.060	0.042
60	0.073	0.059
90	0.104	0.102
120	0.113	0.120

human and the frog measurements. The temperature coefficient of regeneration is about 1.8. Computed for 37.5° C., regeneration has approximately the same time characteristics as the human dark adaptation data.

Comparison with Zewi's measurements shows little agreement. Zewi reports no delay in visual purple regeneration following conditions of light adaptation similar to those here used. Thus his data correspond neither to frog dark adaptation (Riggs, 1936–37, and Granit, Munsterhjelm, and Zewi, 1939) nor to human dark adaptation. The occasional delays found by Zewi occur at low temperature, as is to be expected, but only following short periods of light adaptation. Computing these data to 37.5° C. results in a negligible delay.

(*c*) *Light Adaptation.*—The third series of measurements was made at one light intensity but with varying periods of light adaptation. They were under-

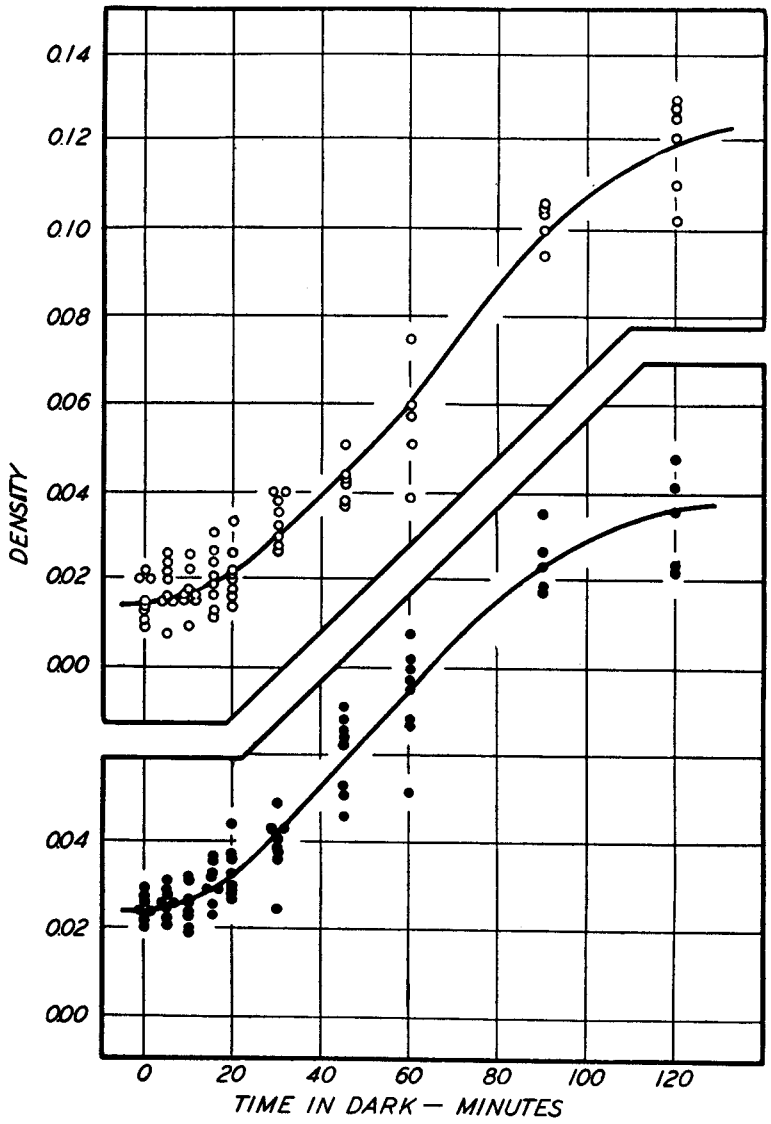

FIG. 4. Regeneration of visual purple in the intact eye of the frog at 15°C. The concentration is measured as density at 500 mμ. Open circles, visual purple concentration following light adaptation to 9500 millilamberts; closed circles, following light adaptation to 1700 millilamberts. Each point is from an extract of 8 retinas. The curves are drawn through the averages.

taken because of Zewi's failure to find delays in the regeneration of visual purple except following short periods of light adaptation.

TABLE III

Changes in concentration of visual purple at 15° C. in retinas of frogs after light adaptation to 9500 millilamberts. Series I, 5 minute light adaptation; Series II, 20 minute light adaptation; Series III, 10 minute light adaptation.

Time in dark	Photometric density		
	Series I	Series II	Series III
min.			
0	0.029	0.011	0.015
5	0.033	0.011	0.018
10	0.038	0.013	0.017
15	0.041	0.025	0.023
20	0.043	0.029	0.021
30	0.057	0.051	0.034

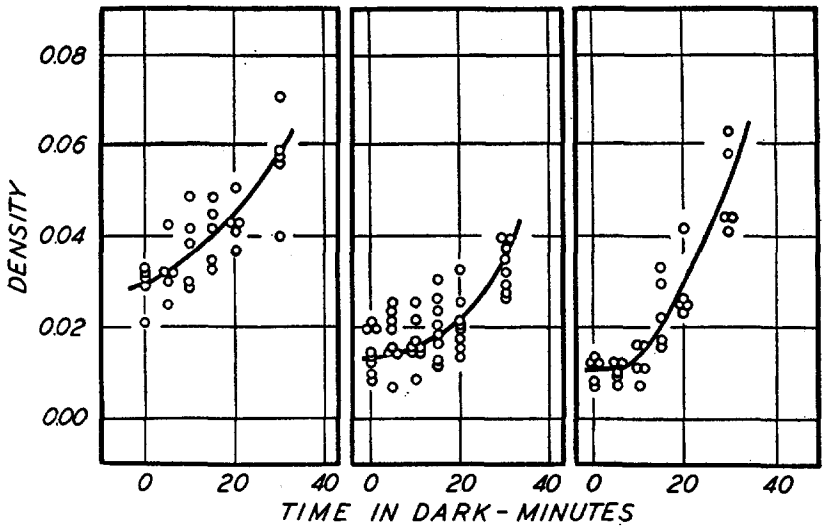

FIG. 5. Regeneration of visual purple in the intact eye of the frog at 15°C. following light adaptation to 9500 millilamberts. Left, after 5 minutes of light adaptation; center, following 10 minutes light adaptation; right, following 20 minutes light adaptation.

The procedure was as before. Animals were light adapted at 9500 millilamberts, one group for 5 minutes and another for 20 minutes. Dark adaptation occurred at 15° C., and was followed for the first 30 minutes only.

The data are in Table III where each value represents the average of 5 measurements with 40 retinas. The individual points are in Fig. 5. Included

in the table and figure are the data from the previous experiment for light adaptation at 9500 millilamberts for 10 minutes at 15° C. obtained under the same conditions of light and dark adaptation but at a different season.

The curves in Fig. 5 show that the delay depends on the duration of light adaptation. Regeneration after 5 minute light adaptation begins immediately, whereas after 10 or 20 minute light adaptation, regeneration is delayed. Both delays are approximately the same, the 20 minute data showing a slightly longer one than the 10 minute data.

These experiments do not corroborate Zewi's measurements in which he finds that only short periods of light adaptation are followed by a delay in visual purple regeneration. The data agree with the work on human dark adaptation by Müller, by Johannsen, and by Wald and Clark, all of whom found that short light adaptation periods produce no delay in rod adaptation, while longer periods of light adaptation are followed by a delay before the increase in rod sensitivity begins. Only the data of Granit on frog retinal potentials show a delay in the recovery of sensitivity under all conditions of light adaptation.

VI

Carotenoids

The changes in the vitamin A and retinene concentrations were followed during dark adaptation. Estimation of the carotenoid content of the entire optic cup showed no regular changes in vitamin A or retinene. Consequently, only those retinas which could be entirely freed of pigment were used. The data are presented in Table IV and Fig. 6. Each point represents the average of five measurements with 40 retinas.

The amount of vitamin A and retinene present in retinas dark adapted for 24 hours is also given in the table and indicated in the figure. We see that the amount of free retinene changes very little during dark adaptation at 25° C. following 1700 millilambert light adaptation. The vitamin A content, however, is high at the conclusion of light adaptation and rapidly decreases until it shows little change after 60 to 90 minutes in the dark. The value obtained with completely dark adapted retinas indicates that vitamin A has not yet fallen to its final level during the period of the experiment.

Wald (1935, 1936) found that when isolated retinas are exposed to light, the visual purple bleaches and retinene appears. Upon continued illumination, the retinene disappears and vitamin A appears in its place. This process takes about 1 hour. Our data show that in the eye of the intact animal a somewhat different series of events takes place. Even after 10 minutes of light adaptation, there is little retinene and much vitamin A in the retina.

The difference between isolated retina and intact animal does not lie merely in the possibility of the circulation removing the bleaching products of visual purple. If the failure of retinene to accumulate in the intact eye were due to

TABLE IV

Changes in concentration of carotenoids at 25° C. in retinas of frogs after light adaptation to 1700 millilamberts for 10 minutes. Series I, retinene, 664 mμ; Series II, vitamin A, 612 mμ.

Time in dark	Photometric density	
	Series I	Series II
min.		
0	0.089	0.215
10	0.062	0.145
15	0.068	0.150
20	0.062	0.132
30	0.072	0.109
45	0.062	0.106
60	0.075	0.120
90	0.068	0.110
D. A.	0.059	0.086

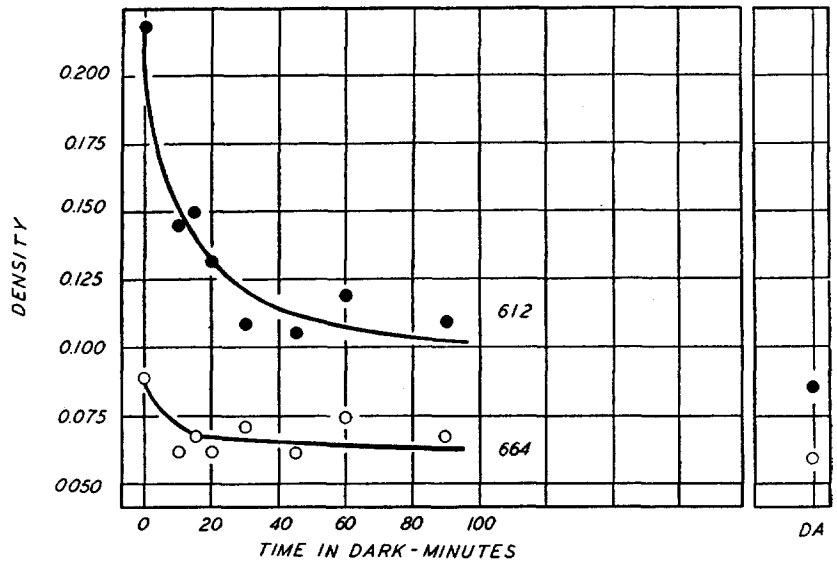

FIG. 6. Changes in retinal carotenoids of the intact eye at 25°C. following light adaptation to 1700 millilamberts. Open circles represent retinene, closed circles vitamin A.

its removal by the blood system, it is to be expected that vitamin A would also be removed. It is therefore possible that the circulation in the eye permits a rapid conversion of retinene to vitamin A, or that vitamin A is formed directly from visual purple.

VII

Kinetics of Visual Purple Regeneration

The course of visual purple regeneration as shown in Figs. 3 and 4 is sigmoid. A comparison of these data with those of dark adaptation can be made by plotting the dark adaptation data as the reciprocal of the threshold intensity against time, much as Piper (1903) did originally. This rests on the obvious

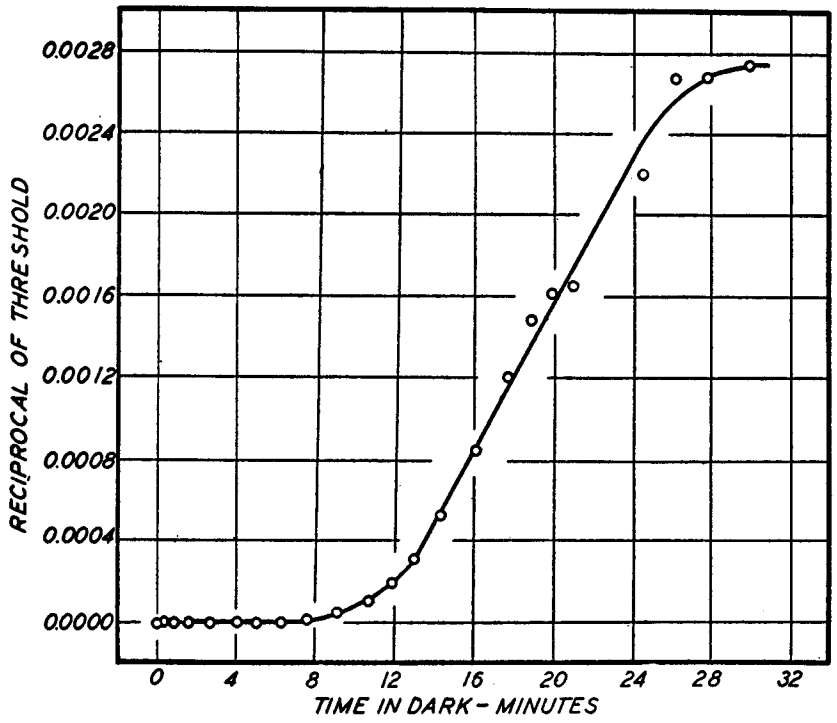

FIG. 7. Dark adaptation of human eye; data of Hecht, Haig, and Chase (1937).

supposition that the concentration of sensitive material is inversely proportional to the intensity required to produce a constant photochemical effect. Fig. 7 shows such a curve for human dark adaptation from the data of Hecht, Haig, and Chase, while Fig. 8 shows a similar treatment of frog dark adaptation from the data of Riggs. Because of the ordinate scale, the separation of rod and cone function is not apparent. But neglecting the first few minutes of cone adaptation, one sees that the data show a slow rise in sensitivity (= concentration), followed by a rapid rise in a typical sigmoid manner. These curves are roughly similar to the regeneration curves of Figs. 3 and 4, and taken in conjunction with the similarity of effects produced in both cases by

temperature, and time and intensity of light adaptation, show that the process of dark adaptation depends upon the accumulation of visual purple in the retina.

The curves in Figs. 3 and 4 do not start from zero concentration, and the initial concentration following light adaptation to 9500 millilamberts is lower than that following adaptation to 1700 millilamberts. This difference between the two series, and to some extent the presence of an initial concentration following light adaptation is understandable in terms of the stationary state (Hecht, 1937).

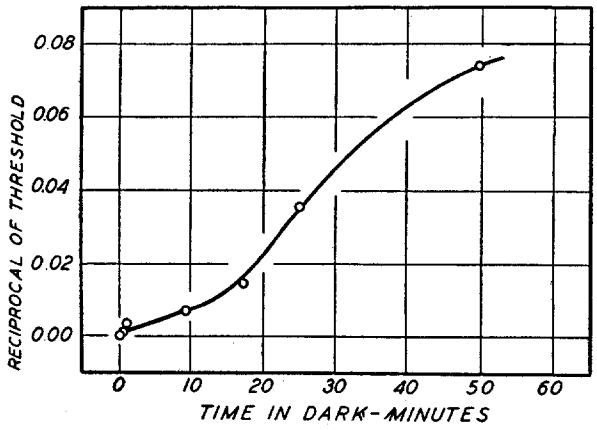

FIG. 8. Dark adaptation of the frog eye measured by electrical method; data of Riggs (1936–1937).

When the photochemical system in the eye has reached a steady state, the concentrations are described by the equation

$$KI = x^n/(a - x)^m$$

where K is the equilibrium constant, I the light intensity, $a - x$ the concentration of unbleached pigment, x the concentration of bleached products, and m and n the order of the reactions. It follows that the fraction $x^n/(a - x)^m$ becomes larger as the intensity increases. Thus the higher the intensity, the smaller the amount of undecomposed photosensitive material remaining.

This expression also indicates that even at high intensities we can always expect to find a finite amount of photosensitive material in the retina. However, this amount is fairly small, and another factor is involved in producing residual visual purple in the light adapted retina. This is the fact that even with illumination from all sides, the entire surface of the retina is not exposed to light. This means that a small amount of visual purple, especially that in the region of the ora serrata, will not be bleached. Thus the apparent

initial concentration of visual purple is increased over that expected from stationary state considerations by a value which depends upon the area of the retina which is unbleached. Since the extent of the unbleached area remains sensibly constant by the use of identical procedures on all animals used, the concentration of visual purple is increased by a constant amount.

Attempts to fit theoretical equations to the data of visual purple regeneration have in the past been only partly successful. Tansley found that monomolecular and bimolecular equations described her data, while Zewi was unable to obtain a satisfactory theoretical description of his data. In both cases the data are too sparse for a critical test of the theoretical curves.

When visual purple is exposed to light, one or more photoproducts are produced. In the dark the decomposition products may recombine, probably with additional materials, to form visual purple again (*cf.* Hecht, 1937).

There are several possibilities which may be considered as the basis for a description of the kinetics of visual purple regeneration. The first is that it is a direct and simple transformation of one molecular species into another. The regeneration should then correspond to a simple monomolecular or bimolecular kinetics. The data in Figs. 3 and 4 exclude this possibility because of the sigmoid shape of the course of regeneration.

Two common chemical systems yield sigmoid curves for their kinetics. One is a catenary series of reactions in which an intermediate compound is formed between the beginning product and the end product. Such chemical reactions yield curves which are only slightly retarded at the beginning. Comparison of the relevant equations with the present data shows them to be inadequate in this respect. The regeneration of visual purple as recorded here has much too great an initial lag.

The other system producing a sigmoid kinetics is one involving autocatalysis. If we assume that the formation of visual purple is a direct chemical transformation which is catalyzed by visual purple itself, we can describe the present data adequately by the usual equation for such an autocatalyzed system. The general equation describing the process is

$$dx/dt = k_2 x^n (a - x)^m$$

where k_2 is the velocity constant, $(a - x)$ the concentration of visual purple at moment t, a the final concentration of visual purple, x the concentration of photoproduct, also at moment t, and m and n the order in which $(a - x)$ and x enter the reaction. Taking m and n as 1, and integrating, the equation yields

$$k_2(t - t_{1/2}) = \frac{1}{a} \log \frac{a - x}{x}$$

where $t_{\frac{1}{2}}$ is the time when the reaction is half complete. Other values of m and n may be tried, but for the present the value of 1 is sufficient.

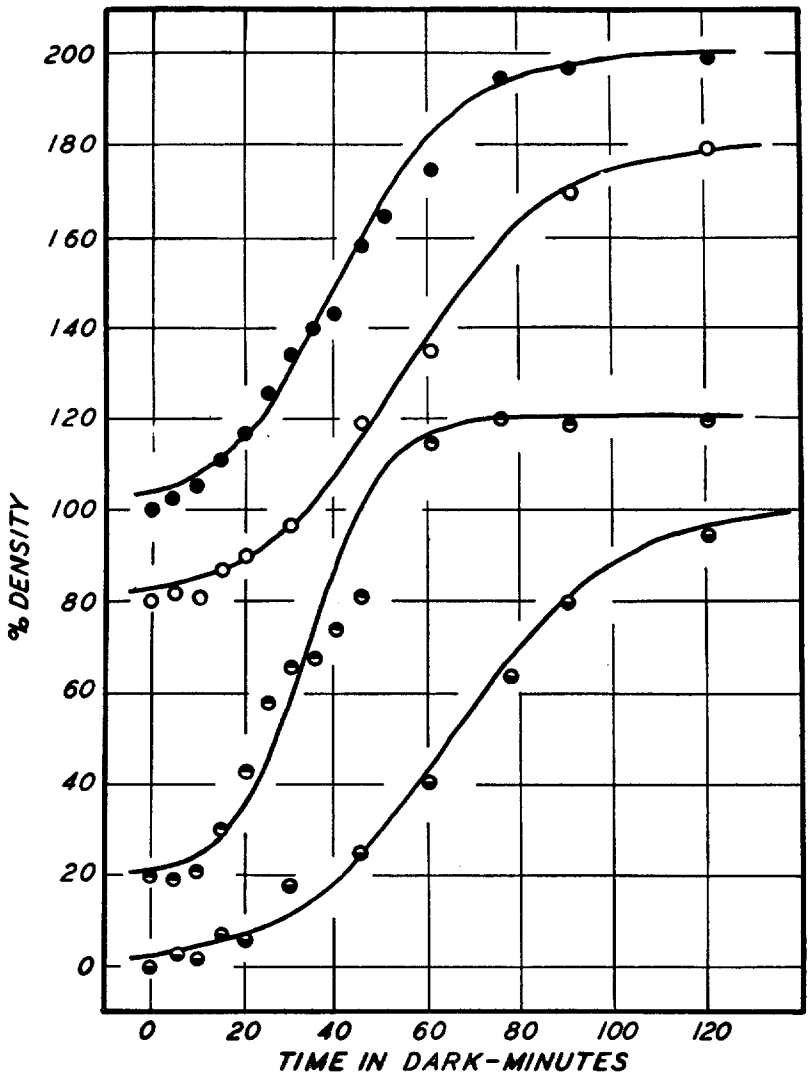

FIG. 9. Computation of visual purple regeneration in terms of the equation for an autocatalyzed chemical reaction. The data are those of Figs. 3 and 4. The three upper curves and data are displaced 20, 80, and 100 per cent units upward.

Fig. 9 shows the application of the integrated equation to the measurements. The data are plotted on the basis of a change in visual purple concentration from 0 to 100 per cent. Since the concentration of visual purple is never zero after the completion of light adaptation, this residual visual purple con-

centration has to be eliminated as a factor in computing the data. The density at zero time of dark adaptation is therefore subtracted from every other density determination of the series. The per cent of the maximum density is then computed for each point, thus giving a series which varies from 0 to 100 per cent.

In Fig. 9 the curve of the autocatalyzed monomolecular equation is drawn with specific values of the velocity constant k_2 for each series of data. It is apparent that the measurements are adequately described by the equation. The slopes are different for the different series. In particular, it is to be noted that k_2 is greater at 25°C. than at 15°C. in accordance with general knowledge.

The precise chemical meaning of the autocatalyzed reaction may really be that visual purple, or perhaps another material formed in equivalent amount as visual purple regenerates, catalyzes the formation of visual purple from the material present in the light adapted retina. At present it is too soon to say. That the equation is first order merely may mean that the other precursors are present in excess, or it may mean that no great chemical change is involved. This is in keeping with the work of Hecht and Pickels (1938) which showed that the bleaching of visual purple in solution corresponds to only a slight change, if any, in the size of the molecule.

SUMMARY

1. The accumulation of visual purple in the retina after bleaching by light has been studied in the intact eye of the frog. The data show that duration and intensity of light adaptation, which influence the course of human dark adaptation as measured in terms of visual threshold, have a similar influence on the course of visual purple regeneration.

2. At 25°C. frogs which have been light adapted to 1700 millilamberts and then placed in the dark, show an increase in visual purple concentration which begins immediately and continues for 70 minutes until a maximum concentration is attained. The increase, although beginning at once, is slow at first, then proceeds rapidly, and finally slows up towards the end. Frogs which have been adapted to 9500 millilamberts show essentially the same phenomenon except that the initial slow period is strongly delayed so that almost no visual purple is formed in the first 10 minutes.

3. At 15°C. the initial delay in visual purple regeneration occurs following light adaptation to both 1700 and 9500 millilamberts. The delay is about 10 minutes and is slightly longer following the higher light adaptation.

4. The entire course of visual purple accumulation in the dark takes longer at the lower temperature than at the higher. The temperature coefficient for 10°C. is about 1.8.

5. In contrast to the behavior of the isolated retina which has small amounts

of vitamin A and large amounts of retinene immediately after exposure to light, the intact eye has large amounts of vitamin A and little retinene after exposure to light for 10 minutes. In the intact eye during dark adaptation, the amount of vitamin A decreases markedly while retinene decreases only slightly in amount. If retinene is formed in the intact eye, the change from retinene to vitamin A must therefore occur rapidly in contrast to the slow change in the isolated retina.

6. The course of visual purple regeneration may be described by the equation for a first order autocatalyzed reaction. This supposes that the regeneration of visual purple is catalyzed by visual purple itself and accounts for the sigmoid shape of the data.

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