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THE RELATION BETWEEN CONCENTRATION OF VISUAL PURPLE AND RETINAL SENSITIVITY TO LIGHT DURING DARK ADAPTATION

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The concentration of visual purple during dark adaptation was first measured by Tansley [1931], who used the eyes of previously light-adapted albino rats, killed after certain standard periods of dark adaptation. A similar experiment was performed by Charpentier [1936], but instead of extracting visual purple and measuring its density, he recorded the size of the electrical response of the rat's eye (the so-called *b*-wave) and found it to increase during dark adaptation along the curve published by Tansley. This general agreement between the two sets of data shows that in a rod eye high concentrations of visual purple are associated with large electrical responses.

Results similar to Charpentier's were obtained with the "mixed" eye of the frog by Wrede [1937] and Riggs [1937]. For the important early period of dark adaptation Wrede's curves showed, however, a delayed onset of the increase in the electrical response and thus did not agree with those of Charpentier. The reason for this may have been that technical difficulties prevented Charpentier from obtaining reliable results with a preparation as lively as the intact white rat until the electrical response had risen well above the base line irregularities. This early phase of the process therefore had to be reinvestigated and it was also thought necessary to obtain parallel observations on the concentration of visual purple (v.p.) under the same conditions as those used for the electrophysiological measurements.

We have now made parallel measurements with the two methods for studying dark adaptation, using both eyes of frogs (over 70), and occasionally eyes of cats. Our results have forced us to a thorough revision of current ideas concerning the relation between sensitivity to light and the rise of v.p. concentration during dark adaptation.

It is perhaps the fault of the duplicity theory that this relation has escaped an experimental analysis for so long. The whole problem has vaguely been regarded as settled in the sense that the rise of sensitivity to light during dark adaptation is merely the sensory equivalent for a rise in the concentration of visual purple. In a recent summary one of us [Granit, 1938] pointed out certain difficulties inherent in this view. Thus, for instance, the electrophysiological measurements of Wrede [1937] and Riggs [1937] could be interpreted along "classical" lines only if curves for V.P. regeneration were assumed which did not agree with those of Tansley [1931].

Recently Granit, Holmberg & Zewi [1938] showed that after moderate light adaptation the electrical response of thoroughly dark-adapted eyes could be greatly reduced although there was no measurable reduction in the concentration of visual purple. This was explained by assuming that most of the visual purple was a store of bleachable, but from the point of view of excitation, inactive material inside the outer limb of the rod cell. Excitation was held to be initiated by the bleaching of a thin surface film, which had to contain only an immeasurably small fraction of the total quantity present. From other points of view Lythgoe [1938] also has suggested that visual purple is active along the surface of the outer limb of the rod.

The essential point, however, in the theory put forward by Granit *et al.* [1938] is the introduction of an intermediate process between V.P. regeneration and the retinal rise of sensitivity. If they are right, the rise of sensitivity should lag behind the rise in the concentration of visual purple during dark adaptation. Our experiments have amply confirmed this expectation of the theory and shed new light on the nature of the process that ultimately determines retinal sensitivity.

TECHNIQUE AND PROCEDURE

Technique. The technique for measuring visual purple concentration has been described in detail by Granit *et al.* [1938]. The same photoelectric colorimeter was used in this work. For the parallel observations on the retinal electrical response a cathode-ray oscillograph and a directly coupled amplifier of the push-pull type were employed. Its proportionality range was checked.

Extraction. Extraction of the retinae from the frogs (*Rana esculenta*) was carried out with 2% digitonin [Tansley, 1931] in the manner described by Granit *et al.* [1938].

With the eyes of cats it was found necessary to modify the technique of extraction, as these retinae are difficult to remove without some measure of preliminary hardening.

The opened bulbs, freed from the vitreous body, were put in 3 c.c. of a 4% alum solution for 2 hr. The retinae are then just sufficiently hardened to be removable without loss of substance. No pigment could be seen in them. The retinae were then washed in Tyrode solution and finally placed in 2 c.c. 10% NaCl for 15 hr. After this they were ready to be extracted by 1 c.c. of a 2% solution of digitonin and to this end were put into a test tube. The retinae were crushed against the wall of the tube and shaken. After 2 hr. in digitonin followed centrifuging for 20 min. at a rate of 3800 rev./sec. The V.P. solution was clear and the residue at the bottom of the test tube colourless.

Absorption was measured in the same manner as with solutions from frogs' eyes, but on account of the extensive treatment during extraction one eye of the cat always had to be used as a fully dark-adapted control. For each animal V.P. concentration of the bleached and dark-adapted eye is given in per cent of the value of the control. With frogs, on which most of our observations have been made, we have simply averaged our density measurements for different animals and identical periods of dark adaptation.

In this laboratory Zewi [1939] has collected and averaged a very large number of measurements illustrating V.P. regeneration in frogs. For our experiments we have chosen certain fixed experimental conditions from Zewi's work and hence easily repeatable. His standard curves have been drawn through the points marking our own readings. One eye was used for V.P. measurements, the other eye of the same animal for testing the size of the electrical response to wave-length 0·500μ. (Tutton monochromator and energy control).

Adaptation to light and darkness. A special apparatus was designed for light adaptation of frogs. This consists of an enamelled basin reflecting in all directions about 20,000 m.c. (metre candles) from a lamp with a reflector above it. A thick water filter of the same diameter as the basin prevents this light from warming the water in which the frogs are kept during light adaptation. The frogs were light-adapted in this apparatus at room temperature for 1 hr., except when otherwise stated. The light-adapted frogs have varied surprisingly little with regard to the V.P. content of the retinae just after bleaching. The density values fall to about 0·040. In complete dark adaptation this value rises to about 0·550 (for cats to about 0·300).

Light adaptation completed, the frogs were put into a jug, which stood in a large dark water-bath fitted with an electric thermoregulator and stirred by a motor. The thermoregulator was coupled either to a

large electric heater or to a refrigerator, according to the temperature wanted.

From this water-bath frogs were removed at suitable intervals of dark adaptation, immediately decapitated, and the two eyes excised and opened for measuring both the electrical response and the V.P. concentration. At the same moment that the electrical response to the constant test light was being photographed for one eye, the other eye was dropped into digitonin. After 2 min. a second or third response to stronger test lights of the same wave-length (0·500 μ.) were photographed. Although the latter therefore followed a few minutes later than the response to the lowest intensity they have nevertheless been plotted to the same abscissa in the diagrams. Of these eyes about thirty were immediately afterwards put into formol, fixed in paraffin and stained with Weigert's haematoxylin-eosin. The values for complete dark adaptation have been obtained from unbleached animals kept at the required temperature in the dark for not less than 20 hr. All results are given in per cent of the value for complete dark adaptation and thus electrical and density measurements become directly comparable.

Results of experiments with frogs

Effect of temperature. Fig. 1 shows dark adaptation of twelve frogs at 16·4° in terms of V.P. densities, as well as the size of the electrical response of the retina to two intensities. The initial small responses, at a constant level before the steep rise of the electrical potential sets in, are due to cones. The concentration of visual purple begins to increase immediately, but the retinal response rises only after a delay of about 1 hr. corresponding to a V.P. density of 50–60 %.

In order to find out whether V.P. concentration or time is the decisive factor in the lagging of sensitivity behind regeneration of visual purple, the experiment was repeated with fourteen frogs at a temperature of 8·0°. This very considerably slows down regeneration. The result of this experiment is shown in Fig. 2, and here we have used three intensities of the test light. Six hours must now elapse before the rise of sensitivity starts in the retina, but again this rise corresponds to a V.P. concentration of 50–60 %.

Short period of light adaptation. Zewi [1939] discovered the curious fact that after 6 min. of light adaptation in our apparatus there was an initial period of delay also in the regeneration of visual purple. This is shown by his standard curve drawn between our own observations, in Fig. 3, for which seventeen eyes were used. In this particular experiment

the concentration of visual purple remains at 20% for 30 min. before it begins to regenerate, but the rise of sensitivity is correspondingly delayed and a fast phase does not set in until the V.P. concentration has reached 50–60%.

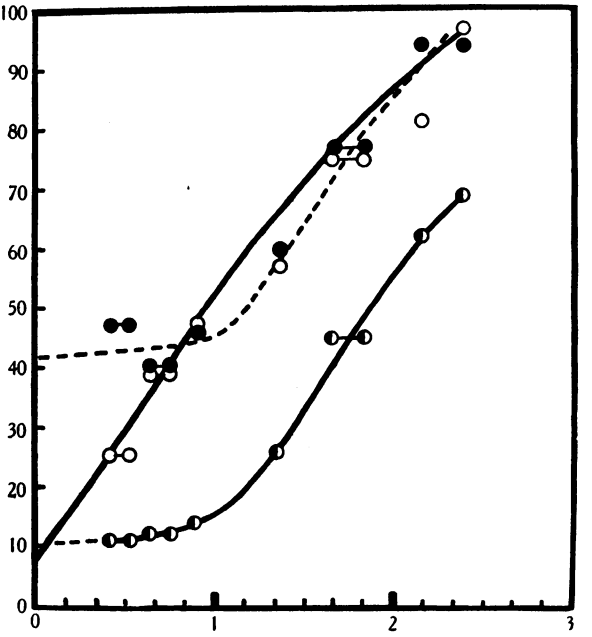

Fig. 1. Ordinates: relative densities of visual purple (○) and relative size of electrical response to a low (◐) and a 20 times higher intensity (●). The latter curve drawn in broken lines. Abscissae: time in the dark in hr. The curve rising immediately from the left corner of the figure is Zewi's [1939] standard curve for visual purple regeneration at 16·4°. Note delay in the rise of the electrical response relative to increase in V.P. concentration. When there are two observations on different eyes not exactly coincident in time, their averages have been joined by lines showing time difference.

Weak light adaptation. In this experiment we did not use our usual apparatus for light-adapting animals, but a small lamp reflecting only about 330 m.c. The frogs were tied to pieces of cardboard in order to ensure constant conditions of illumination. The strength of the adapting light was controlled with resistance and ammeter. Such precautions were deemed unnecessary with the very strong adapting light used above.

Despite this there were greater variations from animal to animal in this experiment, probably because weak light adaptation necessarily leaves room for greater individual variations. In an attempt to compensate for this as many as twenty-seven eyes were used for testing the

retinal sensitivity at wave-length 0·500 μ., and three more retinae for V.P. concentration. In this case (Fig. 4) there is again a fast rising phase after a delay of between 10 and 30 min., varying greatly from eye to eye. An accurate comparison with V.P. concentration is therefore difficult. But it can be safely stated that the appearance of the fast rise of sensitivity presupposes regeneration of visual purple to at least 50–60 %.

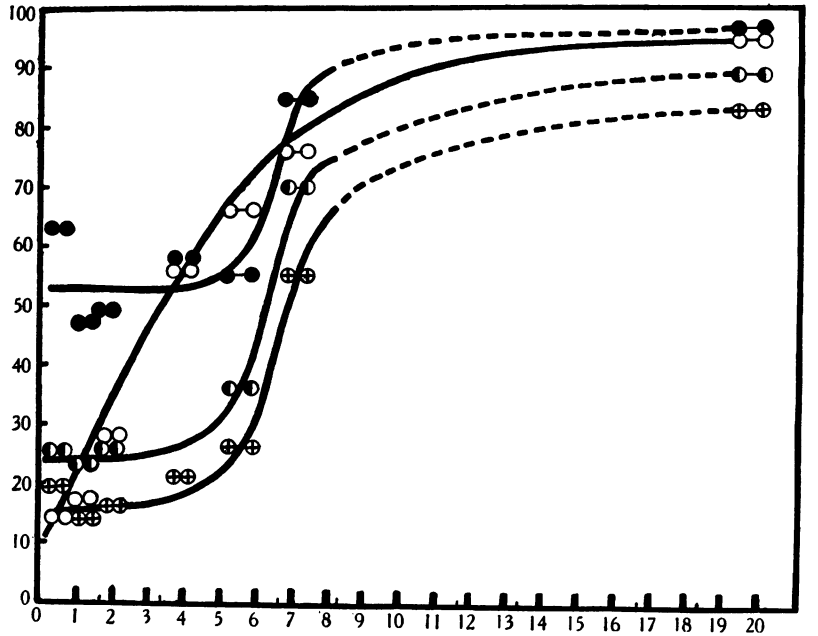

Fig. 2. Ordinates and abscissae as in Fig. 1. Temperature 8·0° and hence time axis shortened relative to Fig. 1. V.P. densities (○) with Zewi's [1939] standard curve for this temperature drawn through the points and rising immediately from left corner of figure. Low (⊕), medium (◐), and high (●) intensity used for eliciting retinal response. Note delay of nearly 6 hr. before rise of sensitivity in the retina sets in.

The role of the pigment. We have seen that the rise of sensitivity lags behind V.P. regeneration and does not appear before there is some 50 % of visual purple. Now this might be due to movement of the retinal pigment. During light adaptation the pigment protrudes between the rods. It will leave them covered for some time afterwards and thereby prevent light from reaching the visual cells.

In order to investigate this supposition we used different levels of intensity in the experiments just described. It was anticipated that the shading by the pigment would be relatively more significant for the weaker

test lights, but the experiments have shown very clearly that the fast rising phase in the electrical response during dark adaptation sets in at the same moment for all intensities tested (see the Figs. 1–4).

Another argument against the view that pigment shields the visual cells has been raised by Therman [1939]. Adrenaline forces the pigment between the rods just as does light adaptation. Owing to the presence of

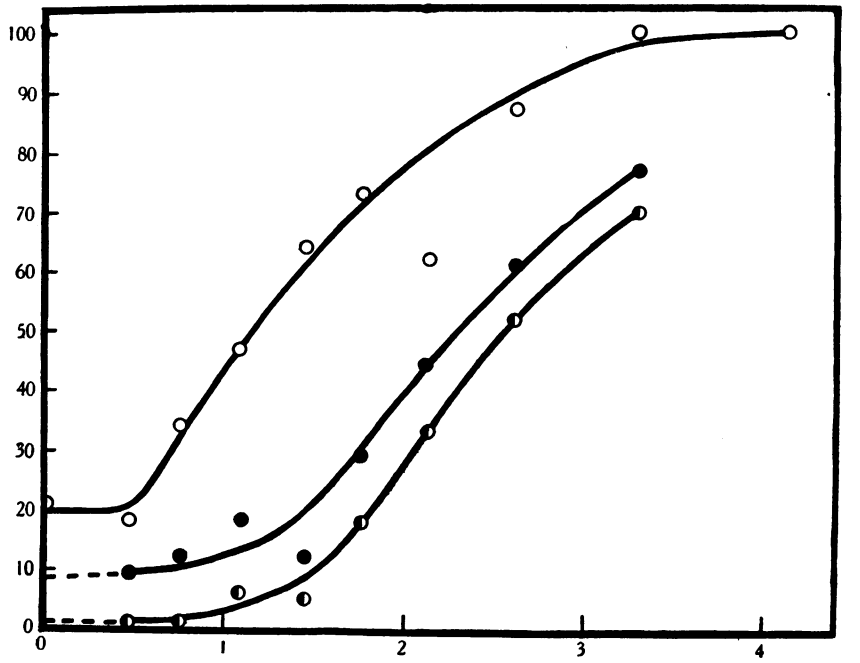

Fig. 3. Ordinates and abscissae as in Fig. 1. Temperature 16·4°. Owing to the short period of light adaptation (see text) V.P. densities (○) begin to increase after a delay of about 30 min. as shown by uppermost curve drawn through the points. The two lower curves show size of retinal electrical response elicited by a low intensity (◐) and one 20 times higher (●).

a Purkinje shift in the frog's eye one might therefore expect the retinal response to short wave-lengths (rods) to become relatively smaller than the response to long wave-lengths (cones) for the adrenalinized eye, as is the case with the light-adapted eye. Actually normal and adrenalinized frogs were found to have the same spectral distribution of light sensitivity, though in the latter the pigment was fully expanded.

In our experiments a large number of sections were made from eyes representing different stages of dark adaptation. We also noted whether

the retinae could be detached easily from the bulb and how much pigment could be seen in them. It was our general impression that the largest electrical responses were obtained from animals from which the twin retina used for density measurements had been easy to remove. When this was so the retina was also relatively free from pigment. Exceptions were, however, not uncommon.

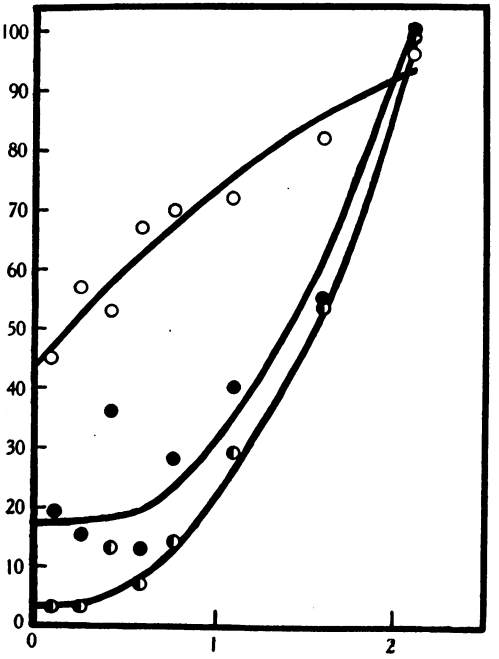

Fig. 4. Ordinates and abscissae as in Fig. 1. Uppermost curve illustrates rise in concentration of visual purple, the two lower curves increase in size of the electrical response. Observations marked as in Fig. 3. Note that, despite the presence of over 40% visual purple, the retinal response of the rods is almost absent and begins to rise after a period of delay just as in previous figures.

Examination of a large number of stained sections did not lead to the expected close correlation between dark adaptation and withdrawal of the pigment. In general less pigment was found in the retinae of the animals which had been dark-adapted for a very long time, but even animals which had not been light-adapted were found to have some pigment between their rods. The most definite way of dividing stages of dark adaptation was to separate those retinae in which the inner limb was covered from those in which the inner limb was free from pigment. We do not know whether the reason for this equivocal result was faulty technique,

the brief flash of the test light, the season (autumn), or merely the fact that a very large mass of material, not restricted to the two end states of photopic and scotopic vision, was examined.

More light can be thrown on the problem, however, by checking our results on the mammalian eye for which so far it has not been possible to demonstrate pigment movement.

Results of experiments with cats

Intensity and duration of light adaptation. Cats were decerebrated in the usual manner and some hours were allowed for the ether to evaporate. The carotids were left untied and only temporarily occluded during decerebration.

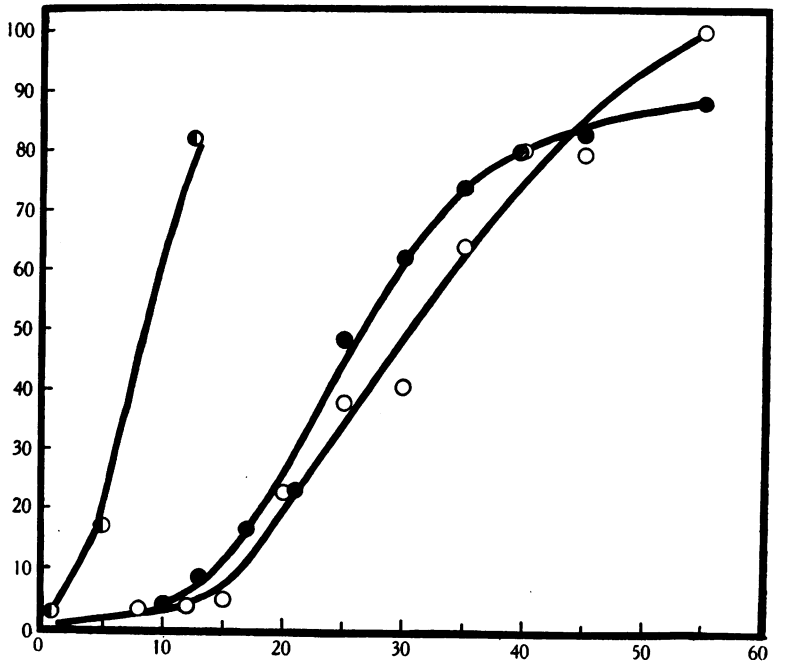

Fig. 5. Ordinates: relative size of electrical response during dark adaptation of cat's eye. Abscissae: *minutes* in the dark after light adaptation, as described in text, for 30 (○), 10 (●), and 1 min. (◐). The slightly lower value for the last point on the curve obtained after 10 min. light adaptation may have been due to a change in the preparation.

The cat's eye contains relatively few cones and its pupil, which in daylight is a mere slit, was wide open in our decerebrate preparations. This probably accounts for the very small electrical responses obtained

after adaptation to 20,000 m.c. This light was far too strong. Nevertheless a definite and long period of delay preceded the small rise of sensitivity which ultimately took place.

In Fig. 5 results are shown illustrating the increase in the electrical response to the test light (0·500 μ.) after adaptation to 3900 m.c. for 30, 10, and 1 min. All readings were made on one animal, beginning with adaptation for 30 min. An arbitrary value of 100 was taken for the electrical response after about 90 min. of dark adaptation in the box before the experiments on light adaptation were begun. During the whole experiment the animal's head was kept in a fixed position relative to test light and adapting source. For light adaptation a small aeroplane lamp, run at a constant amperage, was turned into the opening of the tube entering the dark box. The latter ended in a ground glass disk just in front of the cat's eye. On this disk both test light and adapting light were projected.

It is very obvious from the curves of Fig. 5 that in the cat's eye there is also a period of delay before the rise of sensitivity sets in. This period shortens with duration of light adaptation. After the longer times of light adaptation no response whatsoever or a small negative deflexion is obtained for the first 6–8 min. in the dark. The same observation was made by Charpentier [1936], though its significance as a period of delay was not then fully appreciated. In the frog's eye there is a sufficient number of cones to ensure a positive electrical response in the completely light-adapted eye.

Parallel observations on V.P. *regeneration.* Tansley's [1931] experiments showed that with albino rats there is no delay in V.P. regeneration equivalent to the phenomena described above for the electrical response of the cat's eye. We felt it was important to make a few experiments on the cat since with this animal light adaptation led to complete abolition of the electrical response to the test light chosen. We wanted to know how much visual purple was then left. The outcome of this experiment is shown in Fig. 6. Light adaptation for 20 min. to 3400 m.c. preceded regeneration. The curve for the electrical response is an average for two cats, one representing a relatively long, the other one a very short period of initial delay. The readings for V.P. regeneration refer to nine animals. One eye was used as dark-adapted control. There is one exceptionally low value, but as no noticeable technical error was committed the result may have been due to a diseased eye.

The curves conform to the type found with frogs. The initial absence of rod reactions runs parallel with a V.P. concentration as high as about

40% and the rise of sensitivity sets in when the concentration of visual purple has risen to about 50% (cf. Fig. 4). This is further confirmation of our conclusion that the phenomena discovered with the frog's eye are general and independent of the retino-motor processes.

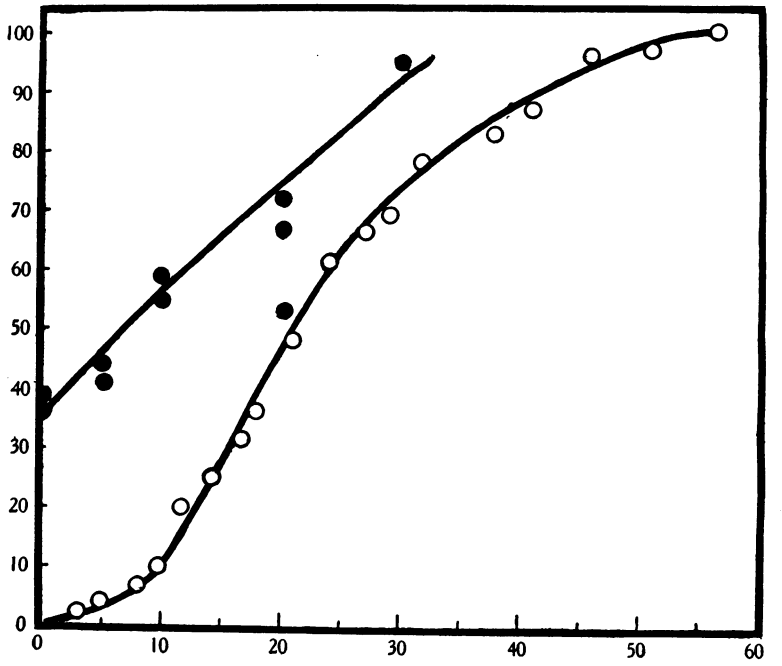

Fig. 6. Ordinates: relative densities of visual purple (●) and relative size of the electrica response (○) during dark adaptation. The observations illustrating V.P. densities represent single eyes (nine cats) with fully dark-adapted control eye, as explained in text. Abscissae: minutes in the dark. Note absence of retinal electrical response despite presence of about 40% of visual purple just after light adaptation (zero abscissa).

Discussion

The intermediate process determining sensitivity. Let us return to the hypothesis put forward by Granit *et al.* [1938]. According to their view (i) most visual purple is a store, inactive physiologically in the sense that neither its presence nor its breakdown *directly* determines the retinal sensitivity to the light absorbed by it. This assumption is amply confirmed by the experiments just reviewed. Granit *et al.* also found it necessary to postulate (ii) an intermediate process bringing about sensitization to light. As a working hypothesis they suggested a process of

diffusion leading to the formation of a surface film of visual purple along the outer limb of the rod cell.

The results of our work strengthens the evidence for an intermediate process between V.P. regeneration and the sensitization of the rod cell. Most striking is perhaps the demonstration that with the cat's eye a degree of light adaptation which leaves 40% visual purple nevertheless abolishes the electrical response. The intermediate process is also assumed to cause the lag observed in the rise of sensitivity relative to V.P. concentration. This period of delay has some interesting properties which serve to characterize the intermediate process:

(i) It has a high temperature coefficient and (ii) a long duration. In order to simplify matters we will assume that these two properties merely depend on

(iii) The seemingly crucial factor, namely, that the intermediate process is not initiated until the V.P. concentration reaches 50%. The high temperature coefficient would then follow automatically from the temperature coefficient for V.P. regeneration whilst the long duration would be dependent on the time required for the latter process to reach the necessary 50%.

(iv) The range of possible modifications in retinal sensitivity is confined to the upper part of the curve for V.P. concentration. In view of this it is interesting to recall once more that the fully sensitized eye, containing its full amount of visual purple, is capable of giving its full performance without detectable loss of material.

Without being dogmatic it will nevertheless be profitable to speculate on the possible ways of explaining the intermediate process.

(1) The delay is caused by the necessity for a removal of photo-products of V.P. breakdown caused by light adaptation. This view does not at present seem probable. The main objections to it are: the absence of an effect of intensity of the test light on the initial delay, as well as the fact that the sensitivity of the eye may be greatly reduced without a measurable reduction in its content of visual purple [Granit *et al.* 1938].

(2*a*) A simple diffusion of V.P. molecules to the surface may cause the lag attributed to the intermediate process, but it is difficult to understand why with a simple diffusion the electrical response should require a minimal concentration of about 50% of visual purple.

(2*b*) It really seems most probable at the present moment that the visual purple enters into some molecular combination, or that it has to be distributed in an orderly manner along a membrane or, to put it very generally, that it must cease to be a bleachable absorbing substance

which is physiologically inert. The high concentration necessary for sensitivity suggests the participation of the V.P. molecule in the building up of the excitable locus in the cell.

Dark adaptation of the human eye. We may safely infer from our experiments that the current interpretation of sensory dark adaptation as the perceptual counterpart to the regeneration of visual purple is fundamentally erroneous. By measuring the threshold to light we are measuring sensitivity and not V.P. concentration. This probably means that we are tapping off points along the rising level of the intermediate process, and that therefore the properties of visual purple regeneration are very indirectly reflected in the time course of sensory dark adaptation.

Dark adaptation has always been an easy test case to those interested in interpreting sensory data in terms of photochemical concepts. Best known is perhaps Hecht's [1920] demonstration that the human data for the rods can be fitted to the curve for a bimolecular reaction, the explanation being that this is the underlying mechanism of V.P. regeneration. Our results which necessitate the introduction of an intermediate process do not support it, nor do Zewi's [1939] many observations on V.P. regeneration fit the equation for the bimolecular reaction.

The intermediate process is capable of explaining in the human eye the period of delay before the rise of sensitivity in the rods, which was first clearly seen by Kohlrausch [1922, 1931]. Variations in the recovery curves during dark adaptation resulting from the use of various intensities and times for light adapting the observers, may also be due to the intermediate process, and not to regeneration from visual yellow as against regeneration from visual white [Wald & Clark, 1937]. The intermediate process may even be sensitive to lack of vitamin A. Without fixing our standpoint with regard to such problems we feel it worth mentioning them in order to stimulate criticism of the all-too-readily-accepted dogma that every phenomenon of dark adaptation is some simple function of visual purple regeneration.

SUMMARY

Two methods have been used in parallel: (i) measurements of the size of the electrical retinal response with a cathode-ray oscillograph and a directly coupled amplifier, (ii) measurements of the total quantity of visual purple of single eyes (digitonin extracts) with a photocell and a Christiansen filter adjusted for wave-length 0·498 μ. The preparation was the dark-adapted excised eye of the Hungarian frog and the decerebrate cat.

The aim of the work was to find out how the rise in sensitivity during dark adaptation, as measured by the increase of the electrical response, is related to the rise in the concentration of visual purple. Experimental variables have been: intensity and duration of previous light adaptation and temperature of the frogs. Curves are given showing the two functions analysed for these conditions.

The general result was that the rise in sensitivity lags behind the increase in the concentration of visual purple. For all the experimental modifications used the fast rise of sensitivity was found to set in when the concentration of visual purple had reached 50–60% of the maximum value of the completely dark-adapted eye.

With the cat's eye it was found that, when the concentration of visual purple had been reduced by light adaptation to about 40%, this led to complete abolition of the electrical response. Sensitivity therefore is near zero with as much as 40% of visual purple left.

These facts support the conclusion that the rise in sensitivity, as measured electrically during dark adaptation, is not a simple function of the curve depicting visual purple regeneration in terms of density values (which are proportional to the concentration of this substance).

The return of sensitivity during dark adaptation is due to some other secondary or intermediate process, which in order to start requires a minimal concentration of 50% of the maximal value for visual purple. In the discussion it is tentatively suggested that the intermediate process consists in the building up of the excitable locus in the rod cell with the aid of visual purple molecules.

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