SECTION III

The Properties of the Photosensitive Substances and the Mechanism of Excitation

The reddish colour of the retina was noticed by many observers from the middle of the last century onwards, yet Francesco Boll (1876) is rightly regarded as the discoverer of the photosensitive substance, visual purple. The reason why the honour should be his may be seen in the following statement, taken from the first of a series of remarkable papers by Willy Kühne devoted to the study of visual purple:


The late R. J. Lythgoe discovered that the first stage in the breakdown of visual purple under the influence of light is the formation of a substance, ‘transient orange’, from which regeneration may occur under certain circumstances. In this connexion Lythgoe made a statement that may well indicate the trend of future research in this field:

‘It is surprising that the absorption curve and other properties of the (from transient orange) regenerated substance are not quite the same as those of the parent visual purple. One cannot help wondering whether the photosensitive substances responsible for day vision are formed in this way: the bright light may be the essential agent in the preparation of visual purple for the reception of high illuminations.’—R. J. Lythgoe, ‘The Mechanism of Dark Adaptation’, Brit. J. Ophthal., 1940, XXIV, 21-43.
CHAPTER XIII

THE ABSORPTION SPECTRUM OF VISUAL PURPLE AND ITS PHOTOPRODUCTS. VISUAL VIOLET

Historical Commentary

Without the well-planned and careful experiments made by Kühne and his collaborators, reported in a series of fundamental papers from the Heidelberg physiological laboratory from 1877 to 1882 and summarized by himself in 1879, Boll’s (1876, 1877) discovery that the red colour of the retina is destroyed by light and reappears in the dark would hardly have secured, in less than a generation, the central place in visual physiology which it holds to-day. Kühne himself rightly pointed out that his ‘optograms’, bleached patterns produced on the retina by illuminated figures projected on to it, were at that time the best evidence of the significance of the photochemical properties of visual purple in the stimulation of the eye. With his collaborators, Ewald, Sewall, and Ayres, he studied the bleaching and regeneration of this remarkable substance, its spectral transmission and its distribution in the retinas of various animals including man. Visual purple had been demonstrated in the human eye both by Schenk and Zuckerkandl (1877) and by Fuchs and Welponer a year after the publication of Boll’s communication. Both Boll (1876) and Kühne established that visual purple is only present in the rods and not in the cones and is, therefore, lacking in the human fovea. Kühne and Sewall also discovered visual violet in 1879 and they pointed out that the characteristic violet tint of fish visual purple is not found in the eel in which the pigment is the usual colour. We now know (Bayliss, Lythgoe, and Tansley, 1936) that eel visual purple actually has the same absorption curve as that of the frog pigment.

Kühne’s work was repeated, confirmed and extended by Garten (1906) and in 1894, after spectroscopic techniques had been improved, König (1903) made the first absorption curve for human visual purple and found it to agree pretty well with the scotopic luminosity curve. The measurements were complicated by the presence of visual yellow, a substance which Kühne
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had described and which he believed to be the first product of
the bleaching of visual purple. König accepted and confirmed
Kühne’s results and went so far as to suggest that visual yellow
might be the ‘blue’ substance postulated by the Young-Helm-
holtz theory of colour vision. However, König’s pupils,
Köttgen and Abelsdorff (1896) were unable to obtain visual
yellow and nor could Nagel and Piper (1905) or Trendelenburg
(1904) although in other respects all these workers confirmed
König’s findings. We shall see below that, in fact, Kühne was
right and that the presence of visual yellow after visual purple
is bleached depends on the degree of acidity of the solution.

A brief summary of Kühne’s generalizations is still as good
an introduction as any to the problems raised by those who are
engaged in research in this field. Kühne held that visual purple
is decomposed by light to visual yellow and that the latter
undergoes further decomposition to visual white, a colourless
final product with a greenish-white fluorescence. He believed
that visual purple can be regenerated both from the yellow and
from the white stage; regeneration from the former being quicker and even taking place in a bile salts extract, while
contact with the pigment epithelium is necessary if regeneration is to take place from visual white. He did not find that this
second form of regeneration involved an intermediate yellow
stage. He suggested that this regeneration was really a produc-
tion of visual purple de novo and he called it neogenesis, whereas
regeneration from the yellow stage was thought to be a re-
formation of the molecule from its breakdown products and was
called anogenesis.

Garten (1906, 1907), to whom improved methods of measur-
ing absorption spectra were available (Kühne had not been
able to measure them with any degree of accuracy), defended
the idea that visual yellow is formed during bleaching. Kühne
had shown that the regeneration of visual purple is encouraged
by raising the temperature and Garten added the observation
that regeneration from the yellow stage can still take place
after the retina has been frozen and then thawed. He found the
reaction to be very much faster than Kühne had realized.
Garten also showed that the decomposition of visual purple to
visual white is much delayed by cooling.

The diagram shown in Figure 95 illustrates what may be
THE ABSORPTION SPECTRUM  

called the Kühne-Garten concept of visual purple breakdown and regeneration. It is hardly necessary to emphasize that it is hypothetical, inasmuch as the arrows are supposed to represent identifiable chemical transitions. Fairly complete reviews of the work in this field may be found in summaries by Granit (1938), Zewi (1939), Lythgoe (1940), and v. Studnitz (1940). The literature up to 1934 has been summarized by Krause (1934) and the older work discussed in the reviews by Kühne (1879) and Garten (1907).

Recent research in this field has profited from the general advance in chemical and physical methods which has been made since the beginning of the century. Absorption spectra can now be measured quickly and accurately by photoelectrical methods and an understanding of the significance of the pH of a solution has introduced concepts which were unknown to those who studied visual purple solutions during the last period of active research into this subject. The general chemical technique for the purification of minute amounts of unknown substances has likewise made great progress. Fifty years ago there was hardly a chance of isolating and synthesizing such substances as vitamins. With our present technical equipment the study of retinal photochemistry would seem to be full of promise for the future.

The Absorption Spectra of Visual Purple and the Products of its Decomposition

An analysis of the products formed when visual purple solutions are illuminated must ultimately lead to their description in terms of their absorption spectra and chemical properties. 'Visual yellow' and 'visual white' are terms which merely describe the colours noted at certain stages of decomposition.
Colours intermediate between red and yellow were seen by Kühne and held by him to represent mixtures of visual purple and visual yellow.

In 1929 Nakashima found that bleached visual purple extracts were a deeper yellow when the solution was acid than when it was alkaline, and his suggestion that differences in acidity would explain the discrepancies in the observations of earlier workers as to the existence of visual yellow was endorsed by Krause (1934). The formation of yellow photoproduets was again demonstrated by Hosoya and Bayerl (1933) and by Hosoya (1933), and in 1934 by Hecht and Chase. Later Chase (1936) produced further evidence that the yellow colour only

![Figure 96. Effect of pH on the absorption curve of indicator yellow. Visual purple absorption curve for comparison. The curves at pH 5.2 and 6.1 have been corrected for fading. Abscissa: wave-length in μ. Ordinate: density. (Lythgoe, 1937, J. Physiol., 89)](image-url)

appeared in acid solutions. The results of a thorough study of this problem were published by Lythgoe in 1937. He measured the absorption spectra of both visual purple and its yellow photoproduce in solutions of different pH and obtained the curves shown in Figure 96.

The final stage when visual purple is bleached in solution is, thus, a new substance with the maximum of its absorption curve in the short wave-lengths. In acid solution this maximum is in the blue part of the spectrum, the substance, therefore, appearing yellow in colour, while in alkaline solution the maximum is shifted towards the ultraviolet and the colour disappears. The substance thus has indicator properties and for this reason Lythgoe called it ‘indicator yellow’.
THE ABSORPTION SPECTRUM

Lythgoe's absorption curve for indicator yellow was a great advance on anything previously published on account of the great care which he devoted to the purification of his solutions. His first step was to prepare a suspension of rods (from the frog), after which he extracted the lipoids by treatment with petrol-ether. The proteins were then precipitated by making the rod suspension acid (pH 4.6) and finally the visual purple was extracted by the use of digitonin (Tansley, 1931) which destroys the rod membrane (Kühne had used a bile salts solution). Later others succeeded in obtaining equally pure solutions of visual purple (Krause and Sidwell, 1938; Saito, 1938; Chase and Haig, 1938; Wald, 1938).

Since later on we shall use Lythgoe's values for visual purple absorption for comparison with our own electrophysiological data, they are given in Table 2.

TABLE 2

<table>
<thead>
<tr>
<th>Wave-length (μ)</th>
<th>Visual purple absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.395</td>
<td>0.001</td>
</tr>
<tr>
<td>0.400</td>
<td>0.016</td>
</tr>
<tr>
<td>0.410</td>
<td>0.103</td>
</tr>
<tr>
<td>0.420</td>
<td>0.196</td>
</tr>
<tr>
<td>0.430</td>
<td>0.306</td>
</tr>
<tr>
<td>0.440</td>
<td>0.416</td>
</tr>
<tr>
<td>0.450</td>
<td>0.530</td>
</tr>
<tr>
<td>0.460</td>
<td>0.661</td>
</tr>
<tr>
<td>0.470</td>
<td>0.780</td>
</tr>
<tr>
<td>0.480</td>
<td>0.878</td>
</tr>
<tr>
<td>0.490</td>
<td>0.953</td>
</tr>
<tr>
<td>0.500</td>
<td>0.997</td>
</tr>
<tr>
<td>0.505</td>
<td>0.993</td>
</tr>
<tr>
<td>0.510</td>
<td>0.975</td>
</tr>
<tr>
<td>0.520</td>
<td>0.899</td>
</tr>
<tr>
<td>0.530</td>
<td>0.780</td>
</tr>
<tr>
<td>0.540</td>
<td>0.622</td>
</tr>
<tr>
<td>0.550</td>
<td>0.460</td>
</tr>
<tr>
<td>0.560</td>
<td>0.310</td>
</tr>
<tr>
<td>0.580</td>
<td>0.113</td>
</tr>
<tr>
<td>0.600</td>
<td>0.034</td>
</tr>
<tr>
<td>0.650</td>
<td>0.003</td>
</tr>
</tbody>
</table>
For wave-lengths shorter than 0.430 μ these corrected values are probably too low and the original readings from unbleached solutions more accurate (see discussion of photosensitivity on p. 235, and Figs. 113 and 115).

The values given in Table 2 and used for the ordinates in Figure 96 are densities defined according to the equation:

\[ D = \log \frac{I_o}{I_t} \]

where \( I_o \) is the intensity of the incident light and \( I_t \) that of the transmitted light. We shall see later on that these densities agree very well with the values obtained for the photosensitivity of visual purple to different wave-lengths (cf. also Trendelenburg, 1904).

Indicator yellow is not the first product of the bleaching of visual purple, but it is the first which is stable enough to be easily observed. It is hardly affected by light, but is decomposed slowly by heat. Its low photosensitivity argues against König's suggestion, already mentioned, that 'visual yellow' might be the photochemical substance mediating the sensation of blue.

Lythgoe suspected that the formation of indicator yellow from visual purple under the influence of light involved significant intermediate stages and he therefore tried the effect of temperature on the bleaching process (Lythgoe, 1937). He was rewarded by the discovery of 'transient orange', an orange-coloured photoprodut which is relatively stable in cooled solutions and which has a reproducible absorption curve. At ordinary temperatures this substance is rapidly decomposed and is, therefore, rightly called 'transient'. Kühne's term 'visual yellow' embraces both transient orange and indicator yellow. Lythgoe and Quilliam (1938b) determined the absorption curve of transient orange which is shown in Figure 97 together with those of visual purple and indicator yellow. It will be seen that the curve is similar to that of visual purple, but that the maximum absorption which is at 0.502 μ for the latter substance is shifted to between 0.470 and 0.480 μ for transient orange. The progressive thermal decomposition of transient orange to indicator yellow makes the measurement of its density rather difficult.
Figure 97. The conversion of indicator yellow into transient orange at pH 7.13 and 3° C. The numbers give the time (secs.) at which the reading was taken; the arrows show the direction in which they were taken. The readings on the curve marked T.O. (transient orange) were taken within 1 1/4 min. after bleaching. After the solution had been at 3° C. for 2,500 secs. it was warmed to 20° C. for 20 mins. and cooled again. The curve marked I.Y. (indicator yellow) was then obtained. V.P.: curve for unbleached visual purple. The figure embodies readings on two identical solutions. Abscissa: wave-length in mp. Ordinates: density. (Lythgoe and Quilliam, 1938, J. Physiol., 94)

The discovery of transient orange explained some earlier observations made by Hosoya (1933), Dartnall (1936), and Chase (1936) that partially bleached visual purple solutions continue to lose colour in the dark (‘dark’ reaction or ‘Nachbleichung’). This is due to the thermal conversion of transient orange into indicator yellow as well as to the very much slower thermal decomposition of indicator yellow itself. The formation of transient orange from visual purple is a true photochemical process independent of temperature.

These results are, in the main, the same as those obtained
by Wald (1938) who, however, came to the conclusion that there are other thermal ‘dark’ processes besides the two described above. This conclusion was not accepted by Lythgoe. He and Quilliam pointed out that the losses or gains in density occurring at any given wave-length during the thermal fading of transient orange are proportional to those at any other wave-length so that the transformation of transient orange to indicator yellow can be quantitatively accounted for by following the changes in density at different wave-lengths.

The thermal decomposition of indicator yellow to a colourless product is accelerated in acid (pH 4.0-5.2) solutions. Between pH 7.0 and 10.8 it remains unchanged for several hours at a temperature of 20° C. (Lythgoe, 1937). Visual purple itself is stable in the dark between pH 5.2 and 10, but at pHs either above or below this range it is bleached to indicator yellow. These reactions are summarized in Figure 98.

Krause and Sidwell (1938) measured the ultra-violet absorption of indicator yellow during its thermal breakdown and found that the final product gave a curve (shown in Fig. 99) of the type which is characteristic of proteins. This suggested that the complex visual purple molecule contains a protein nucleus (cf. below, p. 208).

**The Absorption Curve of Visual Violet**

Kühne and Sewall’s (1879) discovery of visual violet in the retinas of certain fish was followed by Köttgen and Abelsdorff’s (1896) measurement of its absorption curve. These workers found that, compared with that of visual purple, the whole
THE ABSORPTION SPECTRUM

curve is shifted about 0.03 \( \mu \) towards the red end of the spectrum.

The retinal pigment of a large number of fish were studied by Bayliss, Lythgoe, and Tansley (1936). They found that the pigments of different species had different absorption curves of which some examples are given in Figure 100. Of these the

![Figure 99. Ultra-violet absorption curves of indicator yellow (y) and 'visual white' (v.w.) showing characteristic absorption due to protein. (Krause and Sidwell, 1938, Amer. J. Physiol. 121)](image)

curve for the gurnard (Trigla hirundo) probably represents a fairly pure visual violet. The tench (Tinca vulgaris) and the carp (Cyprinus carpio) are other species whose retina contains visual violet alone (for measurements on the carp see Saito, 1938) as do the sun-fish (Lepomis) (Grundfest, 1931), and bream (Abramis brama) (Köttgen and Abelsdorff, 1896). Despite Kühne and Sewall’s statement that the fresh water eel (Anguilla
vulgaris) has a visual purple similar to that found in amphibia and mammals, ever since the publication of Köttgen and Abeldorff’s paper fish were generally held to be characterized by the possession of visual violet. Bayliss, Lythgoe, and Tansley showed conclusively that the fresh water eel has the ordinary visual purple and, in this respect, belongs to the same group as the dogfish (Scyllium canicula), the ray (Raja clavata) and some other ocean living species studied by Wald (1936-7). They pointed out that all the fish used by Köttgen and Abeldorff frequent fresh water and that the deviations from visual violet which they found all occurred among ocean living species. ‘It is possible’, they said ‘that the retina of a fish such as the plaice contains a mixture of two types of visual purple having individual maxima of absorption at 505 mµ and 540 mµ which, when combined, give a maximum at 520 mµ. We did, in fact, obtain slight evidence for the simultaneous existence of two forms in the retina of the pollack.’

Work along the same lines was carried out by Wald (1935-9) who has come to the conclusion that all the known facts can be explained by assuming that there are two forms of visual purple, true visual purple (rhodopsin) and visual violet (porphyropsin). The presence of a mixture of these in varying proportions would account for absorption curves with intermediate maxima.
such as those shown in Figure 100. Bayliss, Lythgoe, and Tansley’s observation that some ocean living species possess visual purple and fresh water fish visual violet has been extended by Wald who (1941) concludes that the violet system is found in true fresh water fish while fish living in salt water have the purple system and those capable of existence in a wide range of salinity (salmon, etc.) possess both. He has now correlated these findings with the phylogenetic development of the different species concerned.

Wald’s (1939) work on solutions of visual violet has shown that the breakdown of this substance follows the lines of the breakdown of visual purple, a russet coloured product corresponding to the yellow substance of the purple system. It is interesting to note that he finds that, compared to the visual purple product, the spectral absorption curve of the visual violet breakdown product is shifted towards the red by about the same amount as the visual violet curve is shifted from the visual purple one. Wald (1939b) and Saito (1938) found the maximum absorption of visual violet to lie between 0.520 and 0.525 μ, while the values given by Bayliss, Lythgoe, and Tansley suggest a figure of about 0.530 μ. Since the photosensitivity of visual violet has not yet been measured directly it is perhaps best to await this final confirmation of the exact position of its absorption maximum (cf. Section IV, on fish spectra).

Visual Purple in the Retina

The old observation that visual purple is only to be found in the rods led Tansley (1933b) to examine its formation in the undeveloped rat retina. Boll (1876) had discovered that visual purple occurs in the outer limbs of the rods and nowhere else in the retina and Tansley found that visual purple does not appear until the outer limbs have developed and that if for any reason these fail to develop no visual purple is produced. She also showed (1933a) that if the immature eye is trephined the retina may develop ‘rosettes’ in which the rods are not in contact with the pigment epithelium. Such rods develop both outer limbs and visual purple in spite of their separation from the pigment epithelium, contact with which has, since the work of Ewald and Kühne, been believed to be necessary for visual purple regeneration. Tansley’s result suggests that such contact
CHAPTER XIV

CHEMICAL ASPECTS OF THE PROBLEM. THE SIGNIFICANCE OF VITAMIN A

Early Work on Vitamin A and Visual Purple

The process of dark adaptation, first elucidated from the sensory point of view by Aubert (1865), appeared in a new light after the publication of Boll's and Kühne's observations on the regeneration of visual purple. And when König (1903) showed, in 1894, that the sensitivity of the dark-adapted eye to different wave-lengths agrees approximately with the spectral absorption curve of visual purple and this finding was confirmed by Trendelenburg (1904), the evidence for the connexion between dark adaptation and the regeneration of visual purple must have seemed almost conclusive. Despite minor discrepancies (see below, p. 234) such a connexion has long been regarded as firmly established. We need not now enter into a discussion of the further investigation of the problem by sensory methods.

These facts make up the background of the general problem as to how states of poor dark adaptation (night blindness, hemeralopia) are connected with the amount of visual purple available in the eye. Dietary night blindness, due to vitamin A deficiency, was produced experimentally in rats both by Holm and by Sugita in 1925, while Fridericia and Holm, in the same year, showed that the vitamin deficient state was accompanied by a delayed regeneration of visual purple. They used a colorimetric method for estimating the amount of visual purple in the retina. This important result, soon confirmed by Tansley (1931, see also 1933b) with a more accurate photographic method, inaugurated a period of biochemical investigation inspired by the accumulating knowledge of the chemistry of vitamin A and the carotenoids in general. Tansley's regeneration curves for both normal and vitamin deficient rats are shown in Figure 102. A similar pair of curves were later obtained by Charpentier (1936) using the size of the b-wave as an index of dark adaptation. Fridericia and Holm's work has also been confirmed, with colorimetric methods, by Amenomiya
(1931) and Kuwana (1934). Tansley (1936) further showed that the first appearance of visual purple in young rats borne by vitamin A deficient mothers is retarded.

A rapidly increasing volume of work, of which a large amount is based on inadequate methods, has since been devoted to a study of dark adaptation as a measure of vitamin A deficiency. The shortcomings of many of these investigations have been pointed out by several workers in laboratories devoted to the study of the physiology of the eye (e.g. by Hecht and Mandelbaum, 1940; by Tansley, 1939; by Nylund in several papers published in Swedish and summarized by him in 1941).

![Figure 102. The effect of vitamin A deficiency on visual purple regeneration in the rat. □ normal; ○ vitamin A deficient. The curve was calculated from the equation for a bimolecular reaction. (Tansley, 1931, J. Physiol., 71)](image)

Feeding experiments carried out by Holm (1929), Yudkin (1931), Yudkin, Kriss, and Smith (1931), and by Wald (1934-5) soon proved that the retinal tissues are a rich source of vitamin A or its provitamin, carotene. At the same time visual purple itself was found not to contain the vitamin (Haurowitz, 1933; v. Euler and Adler, 1933, 1934; v. Euler and Hellström, 1933). Both carotene and vitamin A have been shown, by chemical methods, to be deposited in the retina (cf. Brunner et al., 1935; v. Euler et al., 1933–34, and others), although apparently in quantities which vary greatly according to the season and
the diet of the animal used (Haurowitz, 1933; Krause, 1937; Krause and Sidwell, 1938). The pigment epithelium seems to be the chief source of these substances in the eye; for instance, Wald (1934, 1935) gives the following figures for the vitamin A content of various ocular tissues; mammalian retina 0.22, frog retina 0.40, frog pigment epithelium 2.012 mg. per gm. dry weight. Lönnberg published the results of his examination of the eyes of a very large number of fish in a series of papers between 1935 and 1940. He found that vitamin A, Wald's retinene (see below) and two other carotenoids, xanthophyll and taraxanthin, might all occur. The last substance was rarely encountered and the occurrence of the others was variable, although xanthophyll was present in most of the eyes and was the only carotenoid found in some (Lönnberg, 1938). Wald (1935, 1936, 1936-7) also reported the presence of xanthophyll in some of the eyes examined by him. (Boll's collaborator, Capranica, 1877, appears to have been the first to demonstrate this pigment in the eye.) Wald and Zussman (1938) believe that the pigments of the oil globules of the chicken retina are related to the carotenoids xanthophyll and astacene.

That lack of vitamin A has a definitely deleterious effect on the rods has been convincingly demonstrated by Tansley (1936) and Johnson (1939, 1943). This is probably one of the factors responsible for hemeralopia in conditions of vitamin A deficiency.

_The Chemistry of Visual Purple and its Decomposition Products_

Ever since the work of Ewald and Kühne (1877-9) it has seemed likely that visual purple is a protein, and this view has been supported by much of the recent work. Ewald and Kühne found that visual purple is destroyed by heat and they compared this reaction to the coagulation of albumen. Lythgoe and Quilliam (1938a) found the behaviour of visual purple solutions, as regards the kinetics of their thermal decomposition, and their dependence on the concentration of salts (Ewald and Kühne), to be very similar to that of protein solutions, the heat of activation being 44°O calories per gm.-molecule. Krause and Sidwell (1938) and, still more convincingly, Goodeve, Lythgoe, and Schneider (1942) have shown that visual purple has the ultra-violet absorption curve characteristic of proteins.
Further, the general result of all the recent work on visual purple is to show that procedures, such as heating, treatment with acids, alkalis, and other protein precipitants, which cause the denaturation of proteins also destroy visual purple and produce indicator yellow (Wald, 1935; Lythgoe, 1937). Visual purple has cataphoretic properties with an isoelectric point at pH 4·47 (Broda and Victor, 1940). The molecular weight, calculated from the diffusion coefficient (Hecht, Chase, and Shlaer, 1937), is very high and the figure, about 270,000, obtained by using both the sedimentation constant (Svedberg’s principle) and the diffusion coefficient (Hecht and Pickels, 1938) is probably a better approximation to the true value.

After bleaching, when indicator yellow is produced, the cataphoretic mobility was found by Broda and Victor (1940) to have altered so that the isoelectric point was shifted to pH 4·57. These authors point out that, owing to its photosensitivity, visual purple is unique in this respect since no other protein is so affected by moderate illumination. The location of its isoelectric point so far on the acid side places this substance among the so-called acid proteins. Broda (1941) believes that it may be a globulin and that, in the retina, it is associated with a phospholipin (see also Krause, 1934).

Wald (1935) showed that a yellow substance can be extracted with petrol-ether from retinas made yellow either by illumination or by other methods and that this substance has a characteristic absorption band at 0·664 μ after treatment with antimony trichloride. The production of a blue colour with antimony trichloride is characteristic of carotenoids and his results led Wald to conclude that the prosthetic group of visual purple is a carotenoid which he called retinene. The thermal conversion of retinene to a colourless substance is accompanied by the disappearance of its absorption band. Fully bleached retinas, on treatment with petrol-ether, yield a substance which, on the addition of antimony trichloride produces the absorption band of vitamin A, a substance which could never be extracted from the dark adapted retina. Wald believes that disruption of the visual purple molecule leads to the appearance of retinene which is then transformed into vitamin A. He claims that the transformation is a quantitative one and that vitamin A is the final product of the breakdown of visual purple. During re-
generation visual purple is reformed de novo so that vitamin A is a precursor of visual purple as well as a final product of its breakdown. In this sense the reactions of visual purple form a cycle from which some vitamin A is continually being lost and must be replaced from the circulation. In this way Wald explains the dependence of dark adaptation on the presence of sufficient vitamin A and the occurrence of hemeralopia when it is lacking.

These hypotheses have not remained unchallenged. In 1938 Krause and Sidwell showed that no vitamin A is formed when visual purple decomposes into the colourless stage in solution, and this particular result was confirmed by Wald (1938). These workers, as well as With (1938) also refused to accept the identification of retinene as a carotenoid on the sole basis of the antimony trichloride reaction but later work of Wald's (1939) as well as that of Morton and Goodwin (1944) with which we shall deal in a moment, appears to have substantiated his opinion. However, the fact that vitamin A cannot be demonstrated in bleached visual purple solutions (see also Haurowitz, 1933) does throw doubt on Wald's simple scheme of visual purple breakdown. Further, Krause and Sidwell (1938) could not find any increase in the vitamin A content of the whole retina after bleaching, on the contrary the total quantity of the vitamin (about 0.005 mg.) diminished when the retina was exposed to sunlight. They used the bovine retina for this investigation and claim that most of the vitamin A is located in the layers behind the receptors. The difficulty of separating the pigment epithelium from the retina has been well known since Boll and Ewald and Kühne first encountered it and we have ourselves often come up against this difficulty in our experiments on visual purple regeneration (Granit, Munsterhjelm, and Zewi, 1939). Now, if as much as 14.0 μg. of vitamin A can be found in the pigment epithelium of the frog (Wald, 1935), this is clearly a source of error which it must be difficult to eliminate. It is true that when a dark adapted retina is removed from the eye and then bleached it will be contaminated with less tissue from the pigment epithelium, but even so it is extremely hard to be certain that this particular cause of inaccuracy has been completely avoided, especially when the frog is used (see Ewald and Kühne, 1877-8; and Hosoya and
Sasaki, 1938). One therefore hesitates to accept Wald's conclusion that retinene is quantitatively transformed into vitamin A during light adaptation, the more so since the actual quantities concerned are so small, of the order of 1.0 μg. or less. Jancsó and Jancsó (1936), as well as Greenberg and Popper (1941), attacked this problem by studying the changes in fluorescence of the ocular tissues in different stages of adaptation. Jancsó and Jancsó noticed that, while the epithelial cells of the dark-adapted albino rat showed no fluorescence, this was very marked after light adaptation (cf. Kühne's description of the fluorescence of 'visual white'). They ascribed this reaction to the appearance of vitamin A and claimed that it confirmed Wald's cycle theory, but did not realize that this theory requires that the increase in vitamin A should take place in the receptors! Greenberg and Popper recorded 'minute traces' of vitamin A in the receptors of light adapted rats while in dark adapted animals it was 'only seen in traces' in rods and epithelium. The fluorescence work, therefore, seems to confirm Wald's view that vitamin A is formed during illumination but suggests that the process takes place in the epithelial cells. However, Wald himself (1935) did not find any significant difference in the vitamin content of the pigment epithelium of dark and light adapted frogs.

Even though the actual part played by vitamin A in the synthesis and breakdown of visual purple must still be regarded as somewhat obscure, the fact that a substance with carotenoid properties can be obtained from indicator yellow is good evidence of the importance of some carotenoid, and thus probably of vitamin A, in the formation of the prosthetic group of the visual purple molecule. The question as to whether the prosthetic group can actually be identified with a carotenoid will be discussed later.

Another carotene derivative, xanthophyll, has also been found in the eye (Lönnberg, 1935-40; Wald, 1934-5). Lönnberg (1938) has shown that, in some fish (e.g. Pleuronectes limanda), vitamin A is replaced by xanthophyll and, in much smaller amounts, by taraxanthin, suggesting that in some species these two carotenoids may perform the task carried out by vitamin A in others. A general criticism which has been made of Wald's scheme is that there is no place in it for xanthophyll (Lythgoe, 1940).
Neither can the degeneration of the rods produced by vitamin A deficiency (Tansley, 1936; Johnson, 1939) be altogether neglected. Johnson found that Kolmer's droplets are lacking in the visual cells of vitamin A deficient animals and suggests that these may play some part in vitamin A metabolism.  

The discovery that vitamin A₂, found in certain fish by Lederer and Rosanova in 1937, takes the place of vitamin A (A₁) in the eyes of those fish which possess visual violet (Wald, 1937, 1939a, b) provided a potent argument in favour of the carotenoid nature of retinene. In the visual violet system another carotenoid, the russet coloured retinene ₂, corresponds to the yellow retinene (retinene₁) of the visual purple system. The spectral absorption curves of the various components of the two systems are given in Figure 103 (from Wald, 1939b). Not only is the general similarity of the two systems very striking, but so is the fact that the displacement of each curve from the position of its opposite number in the other system is always approximately the same.

It appears, therefore, that Wald is right in assuming that the chromophoric groups of the complex chromoprotein, visual purple, actually is a carotenoid, particularly since Morton and Goodwin (1944) have recently identified retinene as vitamin A aldehyde on the basis of the position of its spectroscopic absorption bands both in chloroform and after treatment with antimony trichloride. The light absorbing electrons which are responsible for the colour of the compound are localized at the bond between the protein and the chromophoric group.

1 Johnson, 1943, (Arch. Ophthal., 29, 793-810), in further investigations on the effect of vitamin A deficiency on the rat retina, has found that the degenerated rods may, if the damage is not too severe, recover on a diet supplemented by sufficient vitamin A. Anderson and Hart, 1943 (Amer. J. Vet. Res., 4, 307-17), have also demonstrated rod degeneration in the retina of the vitamin A deficient horse, but Mann, Pirie and Tansley (in course of publication) have been unable to show this effect of deficiency in the rabbit.

2 Johnson and Detwiler, 1942 (J. Exp. Zool., 89, 233-49), now believe that Kolmer's droplets are actually composed of retinene. It should, however, be mentioned that Walls, 1934 (Arch. Ophthal., 12, 914-30); 1939, (Anat. Rec., 72, 373-85), as a result of very careful histological studies has come to the conclusion that these droplets are artefacts bearing no relation to the visual cycle.
Visual purple is thus the conjugated product of a chromophoric group and a carrier, the latter being the bulky protein, combined with phospholipins, to which we have already referred. The effect of light is to alter the bond between the chromophoric group and the protein in such a way as to render the former more easily removable by organic solvents (Wald, 1935–9; Lythgoe, 1940). A number of agents besides light may have the same effect (see above).

What now is the relationship between indicator yellow and retinene? Apparently they are different stages in the process of separation of the two components of the conjugated protein. Wald (1938) states that ‘retinene may remain in part chemically bound to protein in the final bleached product’, so that it may not, in fact, be the isolated prosthetic group as his earlier papers suggest. Lythgoe (1940) says that ‘there are some grounds for thinking that indicator yellow is itself a conjugated protein. We are justified in saying no more than that light weakens the chemical link which is responsible for the characteristic absorption curve of visual purple. It is possible that the essential chemical change is one of hydrolysis’ (see also Lythgoe and Quilliam, 1938 a; Dartnall, Goodeve and Lythgoe, 1938).
Some interesting information about the structure of the visual purple molecule was brought forward by Broda, Goodeve, and Lythgoe (1940) when they estimated the 'carrier weight' and the number of chromophoric groups combined with each molecule of the compound. The 'carrier weight' of a chromoprotein is the weight of that part of the molecule which contains one chromophoric group; it was found to be about 26,500 for visual purple. This value is of the same order as those found by Svedberg (1938) for the carrier weights of a number of other chromoproteins as well as the value for Svedberg's fundamental protein unit (17,600). If the carrier weight of visual purple is approximately 26,500 (Broda, Goodeve, and Lythgoe, 1940) and its molecular weight about 270,000 (Hecht and Pickels, 1938), it follows that each molecule must 'carry' about ten chromophoric groups connected to its protein nucleus. The latter, as already stated, is also linked to phosolipins.

Addendum

Since the above account of visual purple was written Krause (1942) has presented, in a preliminary communication, a radically different conception of its chemistry. The evidence on which Krause's statements are based has not yet become available to the author.
CHAPTER XV

THE REGENERATION OF VISUAL PURPLE

*Kühne’s Rhodophylin Hypothesis*

It was Boll in 1876 who first discovered that, after bleaching, visual purple is regenerated in the dark, and Kühne made a very thorough study of this process (see, in particular, Ewald and Kühne, and Ayres and Kühne, 1878-82). The essential facts and concepts at which Kühne arrived as a result of this study, which were later largely confirmed by Andogsky, 1897, and Garten, 1906, 1907, are the most valuable parts of his contribution to our knowledge of visual purple which, as a whole, is notable for the unerring instinct with which the fundamental problems were understood and solved.

The central and most important of Kühne’s ideas was his ‘conviction’ (his own word) that the pigment epithelium contains everything necessary for regeneration so that even extracts of epithelial tissue lacking the sensory cells should be able to regenerate visual purple in the dark. Kühne, no doubt, went too far but his statements contain a very essential element of truth. He suggested that a hypothetical substance, *rhodophylin*, is delivered to the rods by the epithelial cells and that this substance can be washed out of the rods with 0.5 per cent saline. A retina, carefully isolated before bleaching, can regenerate visual purple if rhodophylin is not removed by washing during the exposure to light. The process of regeneration, then, does not require a living retina so long as rhodophylin has first been produced by a living epithelium. Once formed rhodophylin is transported to the receptors and the rest of the process can take place in the rods or even on the laboratory bench. The final vindication of this theory was obtained when Ewald and Kühne demonstrated that visual purple, bleached to the yellow stage in solution in the presence of epithelial tissue, is regenerated in proportion to the amount of such tissue present. This process of bleaching and regeneration of visual purple in solution could be repeated several times although the photosensitive pigment gradually diminished and finally failed to reappear.
In the living animal (most of this work was done on the frog) the photoproducts are apparently returned to the epithelium since, if the retina is removed after bleaching to the white stage, no regeneration takes place. If, on the other hand, the retina is removed before bleaching it retains its photoproducts and normally contains enough rhodophylin in adherent remnants of epithelial tissue to regenerate its visual purple.

The differences between visual purple regeneration from its yellow photoproducts and from the final, colourless, stage have already been referred to in Chapter XIII. Kühne's explanation of these was that in anagenesis (regeneration from the intermediary breakdown products) rhodophylin combines with the photoproducts to produce rapid regeneration whereas in neogenesis (regeneration after the final stage is reached) the primary process has to take place in the pigment epithelium. Rhodophylin is not the brownish pigment of the pigment epithelium. In the eye regeneration is increased by raising the temperature, but bleaching is independent of temperature so long as this is not high enough to destroy the visual purple molecule (see above). Pilocarpine also increases regeneration.
and, in the mammal, an adequate oxygen supply is important (Ayres and Kühne).

Regeneration in Solution

The fact that visual purple can be regenerated in solution has been confirmed by Hecht, Chase et al. (1936), by Lythgoe (1937), Wald (1938), and Hosoya and Sasaki (1938). In addition the last workers confirmed that some substance from the epithelial cells which is not the pigment is necessary for the reaction.

The most complete recent study of regeneration in solution is that of Chase and Smith (1939). Some of their curves, which can be fitted to the equation for a monomolecular reaction, are given in Figure 104. They found that regeneration is most easily obtained at about pH 6.7 and that if the pH falls below 6.0 or rises above 7.5 the amount of visual purple regenerated diminishes rapidly. The spectral absorption curve of the regenerated substance is identical with that of the original. The three curves in Figure 104 also show that something is being used up during the regeneration process since each successive reaction produces less visual purple than the one before, and Chase and Smith suggest that this may be Kühne’s epithelial substance rhodophylin.

In 1911 Bauer showed that light itself stimulates the regeneration of visual purple. He found that more visual purple was produced in a strongly bleached eye kept in a moderate light than in a moderately illuminated eye kept afterwards in the dark. These results have since been confirmed by Zewi using a quantitative method. Chase (1937) and Chase and Smith also noticed that illumination has a favourable effect on regeneration in solution and they found this to be dependent on the wave-length used; solutions illuminated with short wave-lengths produced more visual purple than those subjected to long wave-lengths. This result suggests that some yellow (blue-absorbing) substance, either formed as a result of illumination or present in the solution from the beginning, accelerates the process of regeneration.

What is the nature of this substance?

Chase and Smith put forward three possibilities: it may be one of the yellow decomposition products of visual purple.
(apparently then transient orange or indicator yellow) or one of the flavins with a maximum absorption at about 0.445 μ which have been found in the retina by v. Euler and Adler (1933) and others (see Granit, 1938), or it might be the substance discovered by Granit and Wrede (1937) in the blue-sensitive receptors of the frog (see also Section IV). Of these three possibilities it seems unlikely that the substance in question is lactoflavin. Zewi (1939) has shown that lactoflavin has no effect on

![Figure 105. Relative 'bleaching capacities' of different wavelengths as determined by their effect on the size of the b-wave (as only 5 observations were available at 0.400 μ the general direction of the curve only is indicated in this region). Ordinates: bleaching capacity calculated as a percentage of the maximum at 0.500 μ (Granit, Therman and Wrede, 1938, Skand Arch. Physiol., 80). Dotted line: visual purple curve as determined by the size of b-wave elicited by wave-lengths from an equal energy spectrum. (Granit, 1937, Nature, 140)](image)

the amount of visual purple regenerated when it is either dropped on to the retina or injected into the animal (frog), nor does its concentration decrease after strong illumination of an excised eye (Zewi, 1937). Yellow photo-products or the blue-sensitive receptor substance remain to be considered.

Light was thrown on this subject by some electrophysiological experiments on the effect of the wave-length of the bleaching light on the size of the b-wave of the electroretinogram (Granit, Therman, and Wrede, 1938). The retinas of dark adapted excised frog eyes were bleached by different parts of an equal
energy spectrum and the rate of recovery of the greatly reduced b-wave measured. It was found that the b-wave was reduced far less after five minutes’ bleaching with short than with long wave-lengths. When bleaching and recovery were repeated several times in the same eye it was sometimes found that the short wave-lengths succeeded in eliciting an actual increase in the b-wave produced by a test light.

Granit, Therman and Wrede next investigated the spectral position of the wave-lengths responsible for enhancing the excitability of the retina. For this purpose it is necessary to correct the actual results for the differences in sensitivity (here represented by the size of the b-wave) with wave-length due to visual purple, since one can assume that the depression of sensitivity caused by bleaching will, in the first instance, be proportional to the visual purple absorption curve. A curve showing the effect of wave-length on retinal sensitivity corrected in this manner is given in Figure 105 together with the absorption curve of visual purple (dotted line) calculated from electro-physiological data. If this figure is inverted one has the probable absorption curve of the substance which favours recovery of the b-wave in the frog eye during dark adaptation. Clearly this result is quite in harmony with the view that the substance might be transient orange.

Regeneration in the Living Animal

It is not necessary to review here the older work on regeneration in the living animal in which qualitative methods were used (see above) as the problem has since been attacked with quantitative methods by Tansley (1931), Zewi (1939), and Granit, Munsterhjelm, and Zewi (1939). The work in this laboratory was done mainly on frogs with one set of experiments on cats; Tansley used rats. Zewi’s work on the frog is the most extensive analysis hitherto published, but it should be remembered that this retina differs from the mammalian one in its dependence on the choroidal rather than the retinal circulation for its nourishment and that this may well be responsible for differences, especially with respect to time constants, between the reactions of these two types of eye, the more so as one of them belongs to a cold-blooded animal.

Tansley’s curve for regeneration in the rat is given above in
Figure 102. She tried to fit the equation for a bimolecular reaction, used by Hecht (1920), to describe the data obtained by him with the human and certain invertebrate eyes (Hecht, 1927) to this curve. According to this equation:

\[ k = \frac{1}{t} \cdot \frac{x}{a(a-x)} \]

where \( k \) is the velocity constant of regeneration, \( t \) the time in

![Figure 106](image)

**Figure 106.** The effect of temperature on the regeneration of visual purple in the frog. Temperature: \( +7.2^\circ\text{C.} \) (55 eyes); \( -12.2^\circ\text{C.} \) (63 eyes); \( -17.2^\circ\text{C.} \) (44 eyes); \( -22.1^\circ\text{C.} \) (41 eyes). Light adaptation: 1 hr. to 20,000 m.c. in a special reflector. **Abscissae:** time in dark (hours). **Ordinates:** density of visual purple. (Zewi, 1939, Acta Soc. Sci. Fenn., N.S.B., 2)

the dark (after bleaching), \( a \) the amount of visual purple after complete regeneration and \( x \) the amount regenerated in time \( t \). She pointed out that, in the process of adjusting \( a \) and \( k \) to give the best fit a very large range of values of \( a \) were found which did not involve marked inconsistencies in the calculation of \( k \). Actually she found that the equation for a monomolecular reaction fitted the experimental curve better.

In the course of his investigation of regeneration in the frog, Zewi measured the density of about 1,500 visual purple ex-
REGENERATION OF VISUAL PURPLE

tracts made from eyes at different stages of dark adaptation. He used the photoelectric method described by Granit, Holmberg, and Zewi (1938) and was able to reduce his experimental error to about 1 per cent.

Zewi especially examined the effect of temperature on regeneration, and the curves in Figure 106 are plotted from his results. In all cases the early part of the curve is practically a straight line showing that during the initial phase the amount

![Figure 107. Effect of removal of frog retina on regeneration of visual purple at 22.1°C.](image)

of visual purple produced is nearly proportional to the time during which regeneration has been proceeding. When he tried to fit these curves to the equation for a bimolecular reaction Zewi found that figures as high as 0.700-0.900 had to be allotted to $a$, whereas the actual densities observed in fully dark adapted animals were never more than 0.500-0.600. It was impossible to fit the equation to the curves for regeneration at temperatures between 7 and 17°C. whatever values were chosen for $a$ and $k$. It should also be pointed out that in spite of the fact that the
eyes were light adapted for one hour in a reflector illuminated by 20,000 m.c. the initial density of visual purple in the extracts was always about 0.040 and not zero.

In the course of these experiments Zewi made an important discovery. He found that, in excised opened frog eyes, regeneration is practically independent of temperature and that, for the first three hours of dark adaptation, the rate of production of visual purple under these conditions is the same as that found in intact animals at 7.2° C. (see Fig. 107). Thus, part of the regeneration process in the living frog must be susceptible to temperature and this part must be lacking, or nearly so, in excised eyes. Later (1940) Zewi obtained further evidence that normal regeneration can be divided into two processes. He did this by investigating the effect of temperature on the action of pilocarpine and atropine. Ayres and Kühne (1882, also Dreser, 1886) had shown that pilocarpine accelerates the production of visual purple in the dark, but Zewi found that neither this drug nor atropine has any effect on regeneration if the temperature is kept low, at 8° C., but that at 22.4° C. pilocarpine accelerates and atropine retards the process. He therefore concluded that the component of regeneration which is sensitive to temperature is the only one which can be influenced by these drugs. These results demonstrate the caution which should be exercised in fitting equations to curves illustrating the regeneration of visual purple since this is, apparently, by no means a homogeneous reaction (cf. Tansley, 1931).

In view of Zewi's observations it seems probable that the type of regeneration found in solution is the same as that obtained in excised eyes and in intact frogs at low temperatures. The amount of regeneration obtainable in solution is always less (about 15 per cent) than that in excised eyes (about 50 per cent), but this is only to be expected since Kühne's rhodophyl hypothesis certainly contains an essential element of truth. The energy necessary for this part of the regenerative process must be derived, partly at least, from the light used for bleaching (Chase, 1937; Chase and Smith, 1939; Zewi, 1939; Granit, Therman, and Wrede, 1938) while that used in the phase sensitive both to temperature and drugs is, apparently, obtained from living processes, probably taking place in the epithelial cells.
Figure 108. Effect of oxygen on regeneration of visual purple at 22.1°C. ◇ intact frogs (41 eyes); ● excised opened eyes in oxygen (8); ○ excised opened eyes in nitrogen (8). Light adaptation: 1 hr. to 20,000 m.c. Abscissae: time in dark (hours). Ordinates: density of visual purple. (Zewi, 1939, Acta Soc. Sci. Fenn., N.S.B., 2)

For regeneration to occur in excised eyes the presence of photoproducats, or other substances capable of storing and giving off the energy of light, is not enough; oxygen is also necessary before the visual purple molecule can be reconstituted. The curves in Figure 108 illustrate this point. Here the bottom curve represents the course of regeneration in excised open eyes kept in nitrogen and the middle one in similar eyes kept in oxygen. The top curve illustrates regeneration in the intact animal. Regeneration is greater in pure oxygen than in air and this need for a raised oxygen tension may help to explain the better regeneration found in the intact animal. So far no experi-
ments on the effect of oxygen on regeneration in solution have been reported, but it seems likely that part, at least, of the favourable effect of oxygen observed by Zewi is due to the requirements of cell respiration. It has been stated by some workers (Jongbloed and Noyons, 1936) that both the oxygen uptake and the carbon dioxide output of the retina are increased in the dark (cf. Lindeman, 1940) although others (Wald, 1934-5; Oguchi, 1934; Chase and Smith, 1939) deny that this is the case.

![Graph](image)

**Figure 109.** The effect of previous dark or light adaptation on the production of visual purple during illumination by 55 m.c. • previous dark adaptation (67 eyes); ○ previous light adaptation of 1 hr. to 20,000 m.c. (52 eyes). Intact frogs at 17° C. *Abscissae:* time of illumination (hours). *Ordinates:* density of visual purple (Zewi, 1939, Acta Soc. Sci. Fenn., N.S.B., 2)

Zewi found that regeneration in the isolated retina, whether removed before or after bleaching, is negligible and Kühne may, therefore, well have been right in suggesting that, where bleaching is taken to the final stage (as it was in all Zewi's experiments unless otherwise stated), the decomposition products are removed to the 'epithelial factory' where he supposed the neogenetic elaboration of visual purple to take place. The well-known photomechanical changes of the receptor and pigment cells of the lower vertebrates may, therefore, play an
important part in the whole complex of receptor-epithelial metabolism. Such changes do not occur in the mammalian eye where an adequate retinal circulation is provided.

Figure 109 illustrates an illuminating experiment of Zewi’s. The lower curve represents the production of visual purple in frogs during exposure to a light of 55 m.c. after one hour’s adaptation to 20,000 m.c., the other curve was also constructed from measurements made during exposure to 55 m.c., but in this case the frogs had been in complete darkness during the preceding twelve hours. It will be seen that the first group of frogs immediately began to regenerate visual purple at a considerable rate while the other group first lost visual purple as a result of stimulation and did not begin to regenerate it until much later and then only slowly. This experiment puts Bauer’s (1911) observation that illumination favours the production of visual purple on a quantitative basis. This effect of light on visual purple regeneration has, therefore, now been shown to occur in solution (Chase and Smith, 1939), in the intact frog (Zewi, 1939) and, indirectly, in excised eyes (Granit, Therman, and Wrede, 1938). Although, as will be shown later, the b-wave of the electroretinogram which Granit, Therman, and Wrede used as their index does not directly measure the concentration of visual purple in the retina, there is good reason for supposing that, in an eye where visual purple is being regenerated, large b-waves do in fact denote high concentrations (Granit, Munsterhjelm, and Zewi, 1939).

‘It seems possible that we have two methods for the regeneration of visual purple. Regeneration from its breakdown products almost certainly involves the addition of energy to the system, and this energy can be provided either by the absorption of light or by a chemical process needing oxygen’ (Lythgoe, 1940). Vitamin A and other carotenoids are probably involved in very complex catalysed ‘neogenetic’ reactions of which, so far, we know nothing. Accepting Wald’s view that the chromophoric group of the visual purple molecule is a carotenoid it is probable that vitamin A also provides real building material for the process of regeneration.

In the course of his work on regeneration Zewi (1939) found that short exposures to strong light are followed by a period during which the regenerative process remains at an absolute
standstill; this effect was more pronounced at low temperatures. Perhaps under these conditions some time must elapse before the photoproducts, which we assume to be the starting-point for regeneration, can accumulate in sufficient quantity.

At the time of his death Lythgoe was working on the problem of regeneration from transient orange and had reached a stage where he was prepared to state that this type of regeneration is particularly rapid and leads to the formation of a visual purple the absorption curve of which does not quite agree with that of the parent material (Lythgoe, 1940). This remarkable result, of which unfortunately we know no more, led him to suggest

that cone substances may be formed in this way. Such a statement indicates that the absorption curve of Lythgoe’s regenerated substance was shifted towards the long wavelengths. When we come to deal with colour reception in Section IV we shall see that there is also electrophysiological evidence to suggest that the visual purple molecule with its ten chromophoric groups linked to a protein nucleus may be the basic model for cone substances (cf. Granit, 1941, 1943).

The chain of reactions which make up the whole process of the decomposition and regeneration of visual purple is summarized in the diagram in Figure 110.

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1 In a paper, not available in this country at the time of writing, Peskin, 1942 (J. Gen. Physiol., 26, 27-47), reports that, in the living frog, he found this type of regeneration in most cases, whereas both Tansley (1931) and Zewi did not, as a rule, observe any initial delay.
Chapter XVI

THE PHOTOCHEMISTRY OF VISUAL PURPLE. THE SPECTRAL DISTRIBUTION OF ITS SENSITIVITY AND THE SCOTOPIC LUMINOSITY CURVE

The subject of visual purple photochemistry is still young; it is at the stage where results can be understood, although not criticized, by those without special training in the field. Before discussing the results it is necessary, however, to consider some elementary facts and definitions. The most important of these is the Grotthus-Draper Law which states that only those light wave-lengths which are absorbed by a photochemical system can produce a chemical effect in it. It is, therefore, also necessary to know the laws governing the absorption of light. The first of these is Beer's Law stating that the concentration in a coloured solution of constant depth is proportional to \( \log \frac{I_o}{I_t} \) where \( I_o \) is the intensity of the incident light and \( I_t \) that of the light transmitted. Although, for various reasons, there are sometimes deviations from this law, the work of Dartnall, Goodeve and Lythgoe (1936) has shown it to hold for visual purple solutions. Next we have Lambert's Law stating that, where the concentration is kept constant, the depth of a coloured solution is also proportional to \( \log \frac{I_o}{I_t} \). These two laws are generally combined so that:

\[
\log \frac{I_o}{I_t} = acl
\]

where \( a \) is the so-called molecular extinction coefficient, \( c \) the concentration and \( l \) the depth (in cm.) of the solution. When measurements are made of, for instance, the concentration or spectral absorption of a visual purple solution with an optical cell of constant length, decadic logarithms are usually employed and the product \( acl \) presented as the density (\( D \)) or extinction as in the equation:

\[
D = \log \frac{I_o}{I_t} \quad \text{(see p. 198)}
\]

This is the 'density' used in most of the curves illustrated.

227
Where the radiant energy used is of different wave-lengths, definitions in terms of energy are complicated by the phenomena dealt with by Planck’s quantum theory. According to this theory radiant energy is emitted and absorbed in minute quanta (q) or integer multiples of q and:

\[ q = h\nu \]

where \( \nu \) is the frequency of the radiation (and thus inversely proportional to the wave-length) and \( h \) the Planck constant. The energy of the quantum is \( h\nu \) ergs and is, therefore, a function of the wave-length. Thus, although two beams of different wave-length but of equal energy per second will heat a thermopile by exactly the same amount they do not emit the same number of quanta per second, for the beam of the shorter wave-length contains a smaller number of quanta each one of which represents more energy. Now the equal energy spectrum which is usually used in sensory work on the physiology of vision is expressed in terms of units of energy whereas the first stage in the bleaching of visual purple is the absorption of quanta of light. If one is going to compare the spectral distribution of the sensitivity of the dark adapted eye with that of the absorption curve of visual purple one must, as pointed out by Dartnall, Goodeve, and Lythgoe (1937), use the same units. Therefore, before such a comparison can be made the sensory data must be corrected to bring them into line with a spectrum with an equal quantum intensity at each wave-length, or, in other words, a spectrum of uniform quantum intensity.

According to the Einstein photochemical equivalence law the absorption of one quantum of radiation should produce a reaction in one molecule. This law only applies to the primary process whereby a molecule is excited; what happens later is a different matter. The excited molecule may lose its energy in collisions with other molecules or it may be decomposed. If it is decomposed in such a way that one molecule is destroyed for each quantum absorbed the quantum efficiency (\( \gamma \)) is said to be 1. If, however, the decomposition activates a series of secondary chain reactions the quantum efficiency may be much greater. It is clear, therefore, that the determination of the quantum efficiency of a photochemical reaction will give a great deal of information about its nature.
FIGURE 111. Course of bleaching of frog visual purple. A in solution; B in the eye. O intact animal at 18° C. (52 eyes); • intact animal at 6° C. (41 eyes); ● excised opened eyes (26). Illumination: 20,000 m.c. (Zewri, 1939, Acta Soc. Sci. Fenn., N.S.B., 2.)
Work on the photochemistry of visual purple breakdown is complicated by the appearance, during the process, of 'impurities' such as indicator yellow which act as 'internal filters', absorbing light without contributing to the reaction which is being studied. When visual purple is bleached in an absorption trough the amount of light transmitted increases as the reaction proceeds (Fig. 111) and, in an ideal case, would finally reach a value equal to the incident light ($I_o$). In actual fact, however, the amount of light transmitted is diminished by substances formed during bleaching so that the final value ($I_f$) is smaller than $I_o$. According to Dartnall, Goodeve, and Lythgoe (1936, 1938), the integrated relationship between the quantities involved in such measurements is:

$$\log_e \left( \frac{I_t}{I_f - I_t} \right) = \phi \frac{a\gamma I_o}{A} \cdot t + \text{const.}$$

where $A$ is the area of the solution exposed and $\phi$ the factor by which $a\gamma I_o$ must be multiplied to correct for the reduction in the rate of decomposition of visual purple due to internal filter effects. This factor can be determined from the values of $I_o$, $I_t$, and $I_f$ and in good solutions where $I_f = I_o$ its value would be 1. Dartnall, Goodeve, and Lythgoe often found a value of 0.8.

This particular equation is based on the assumption that visual purple solutions obey both Beer's and Lambert's Laws and that the quantum efficiency is independent of the concentration. Dartnall, Goodeve, and Lythgoe found that the results of their experiments were, in fact, quantitatively described by the equation and that these assumptions were, therefore, justified.

Hecht (1921 b, c; 1923) studied the course of bleaching of visual purple using a colorimetric method. He found that, when there was little absorption, the process could be mathematically described by the equation for a monomolecular reaction, that the velocity constant was directly proportional to the intensity of the incident light and that the process was independent of temperature over a range of 30°C. Dartnall, Goodeve, and Lythgoe showed that, using their notation and similar approximations, they could derive the equation:

$$\log \left( \frac{C_o}{C} \right) = \frac{a\gamma I_o}{A} \cdot t$$
where $C_0$ is the initial concentration and $C$ the concentration at the time $t$. This is, in fact, the equation for a monomolecular reaction in which $a^2/\lambda$ is equivalent to Hecht's constant $k$.

Dartnell, Goodeve, and Lythgoe found the quantum efficiency of the bleaching process to be independent of concentration, light intensity, temperature (from 20–60° C) and pH (from 6·8–9·2). The factor actually measured in their experiments was not really the quantum efficiency but the product $a\gamma$. However, even though $a$ (the molecular extinction coefficient) cannot be measured directly so long as the molecular weight of visual purple is unknown, it is possible to find approximate values for both $a$ and $\gamma$. Such values give $a = 9 \cdot 10^{-17}$ cm$^2$ molecules per quantum (for wave-length 0·506 $\mu$), corresponding in another notation to a decadic-molar extinction coefficient ($\varepsilon$) of $2·3 \cdot 10^4$, and $\gamma = 1$ or not much less.

A temperature coefficient of $i$ is characteristic of photochemical reactions. But, as already pointed out, the primary excitation of a molecule may be followed by chain reactions which are not independent of temperature and, if this is the case, a rise in temperature would increase the value of $\gamma$. The quantum yield under these conditions will be more than $i$ and will probably vary over a wide range. On the other hand, an inactivation of excited molecules by collision with molecules of the solvent will result in quantum yields below $i$ because the number of molecules permanently affected will be less than the number of quanta absorbed. The fact that $\gamma$ is approximately equal to $i$ for the bleaching of visual purple indicates that one molecule or one chromophoric group is bleached by each quantum of light energy absorbed. Free oxygen is not necessary for this process to take place (Brunner and Kleinau, 1936) nor is oxygen necessary for the later, thermal, process (Chase and Hogan, 1943).

Aqueous solutions only of visual purple were used for the work described above so that reactions at temperatures below 0° C. could not be investigated. Broda and Goodeve (1941), by

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1 On the basis of some work of which the original account is not available here, Hecht, Shlaer, and Pirenne, 1941 (Science, 93, 585), have come to the conclusion that a single quantum is sufficient to stimulate one rod and that at least six rods must be stimulated before a visual sensation can be produced. Add. Complete account since in J. Gen. Physiol., 1942, 6, 819–840.]
using a solvent made up of 75 per cent glycerol and 25 per cent water, were able to extend their investigations to temperatures as low as $-73^\circ$ C. Such solutions ‘assume glass-like consistency at the temperature of solid carbon dioxide and remain perfectly homogeneous and brilliantly clear’. Yet the quantum efficiency of bleaching was still of the order 1 and the velocity of the reaction was unchanged. At these low temperatures the photochemical change of visual purple to transient orange can be isolated since the thermal change to indicator yellow is prevented. Both visual purple and transient orange were shown to belong to that class of organic substances in which the absorption curve is sharpened and the peak shifted towards the long wave-lengths when the temperature is reduced. This shift is about 0.010$\mu$ for visual purple and 0.030$\mu$ for transient orange. This promising method of attack on transient orange by the use of low temperatures and glycerol-water solutions has not yet been followed up.

Another method of approach to the problems of visual purple photochemistry has been used by Weigert and his collaborators (1929, 1940). Dried visual purple is a relatively stable substance, as Kühne discovered, and Weigert has succeeded in preparing ‘artificial retinae’ by dissolving the pigment in gelatine and preserving the solutions as gelatine films. These films are almost colourless but they have enough visual purple dissolved to give them certain interesting properties. Exposure of a film to light of a given wave-length increases its transparency to this wave-length while decreasing it to others. These changes are accompanied by molecular reorientation leading to relatively large changes in the preferential sensitivity to polarized radiation and they are, therefore, best studied with the aid of polarized light. White light produces a maximum transparency to a wave-length of 0.530$\mu$. Takamatsu (1933) has been able to reproduce some aspects of this work using visual purple in solution. Weigert regards these reactions of his films as models of cone phenomena and therefore holds that visual purple, present in the cones in a state similar to that in his ‘artificial retinae’, forms the different cone substances necessary to explain colour vision. We shall discuss the attempts to extract cone substances from the retina later.
PHOTOCHEMISTRY

Photosensitivity of Visual Purple and the Rod Luminosity Curve

König was the first to show by actual experiment that the absorption curve of visual purple is strikingly similar to the curve illustrating the normal spectral distribution of sensitivity in the dark adapted human eye, the so-called scotopic luminosity curve (see chap. XVIII). Trendelenburg (1904) also made a direct experimental comparison between this curve and one representing the effectiveness of different spectral wave-lengths in bleaching visual purple. In 1922 Hecht and Williams made new measurements of the scotopic luminosity curve and compared their results with those of König and Trendelenburg, the most accurate data on visual purple absorption known at that time. They came to the conclusion that the scotopic luminosity curve is shifted towards the long wave-lengths compared with the curve for visual purple absorption. In Fig. 112 different workers' measurements of scotopic sensitivity (those of Hecht and Williams and of Weaver, 1937, being based on the largest number of observations) are compared with Lythgoe's corrected visual purple absorption curve.
In considering this discrepancy between the sensory and photo-chemical data, the first question is clearly whether visual purple absorption (α) does actually correspond to its photosensitivity (αγ). Schneider, Goodeve, and Lythgoe (1939) have shown that this is the case. Then, the sensory data must be recalculated on the basis of an equal quantum intensity spectrum instead of an equal energy spectrum. In Figure 113 Abney and Watson's (1916) sensory curve recalculated in this way by Dartnall and Goodeve (1937) is compared to the curve for the photosensitivity of visual purple obtained by Schneider, Goodeve, and Lythgoe (the squares and filled circles show Trendelenburg's original values). While the maxima of these two curves are at practically the same wave-length, the photosensitivity of visual purple to short wave-lengths is clearly greater than is necessary to account for human scotopic sensitivity. Ludvig and McCarthy (1938) concluded that this difference in the curves is due to the absorption of short wave-lengths in the refractive media of the eye, an absorption which they attempted to measure (cf. also Roggenbau and Wetthauer, 1927). Using their results Ludvig (1938) produced the curve shown in Figure 114 (continuous line) which is a scotopic luminosity curve based on a spectrum of equal quantum intensity and corrected for selective absorption in the eye. In Figure 114 this curve is compared with Lythgoe's (1937) uncorrected visual purple absorption curve (broken line) obtained by direct
measurements on the purest visual purple solutions he could produce. In Section IV I shall show that the scotopic sensitivity curve, obtained by electrical recording from the optic nerve of eyes from which the cornea and lens had been removed agrees exactly with this visual purple absorption curve.

Measurements of the spectral distribution of visual purple photosensitivity have since been extended into the far ultra-violet by Goodeve, Lythgoe, and Schneider (1941). In Figure 115 these measurements are compared with those for visual purple absorption down to a wave-length as short as 0.250 μ. The absorption (α) rises considerably in the ultra-violet quite apart from that due to the protein component of the complex visual purple molecule (Krause and Sidwell, 1938). The maximum at 0.360 μ and the minimum at 0.400 μ are common to both curves. The rise in photosensitivity (αγ) at 0.360 μ appears to be closely connected with the reactions of the chromophoric group of visual purple and ‘corresponds to a transition to a higher electronic level than that for the band in the visible’. This second rise also occurs in the scotopic luminosity curve of

![Figure 114. Comparison of Lythgoe's visual purple absorption curve with Ludvigh's retinal scotopic luminosity curve. (Ludvigh, 1938, Nature, 140)](image-url)
an observer with an aphakic eye (lacking a lens) examined by Goodeve, Lythgoe, and Schneider (1941), so that here too the photosensitivity of visual purple corresponds to the sensitivity of the dark adapted eye. Goodeve, Lythgoe, and Schneider, quoting Graham (1922), state that the lens and cornea are completely transparent to all wavelengths down to $0.365\mu$ (cf. Ludvigh and McCarthy, 1938) and that the absence of the lens cannot, therefore, account for the abnormal sensitivity curve of this aphakic eye. They conclude that their observer must lack some yellow pigment which is normally present. Broda (1941) has calculated that the phospholipins studied by him would give the necessary extra absorption of short wave-lengths.

Goodeve, Lythgoe, and Schneider also measured the limits of vision in the ultra-violet and found them to be between $0.311$ and $0.309\mu$ for normal subjects and as low as $0.300\mu$ for their aphakic observer. This difference is probably due to absorption by the lens which, according to Graham (1922) begins at about $0.313\mu$. Absorption by the cornea does not start until $0.298\mu$ while the aqueous and vitreous humours are transparent to still shorter wave-lengths.

![Figure 115: Comparison of photosensitivity ($\alpha\gamma$) curve of visual purple with its absorption curve extended into the far ultra-violet. (Goodeve, Lythgoe and Schneider, 1942, Proc. roy. Soc. A., 130)]
This is, perhaps, a good point at which to draw attention to some work on the effects of radium and X-rays on the electroretinogram (Himstedt and Nagel, 1902; Thier, 1933). Fluorescence seems to play a part in stimulation by ultra-violet light and Becquerel rays but not in stimulation by X-rays. According to Thier, beta and gamma rays cause all parts of the eye to fluoresce if they are used in combination but gamma rays alone do not have this effect. Gamma rays however can elicit an electroretinogram even when they are used alone and, therefore, probably cause direct excitation of the receptors.
CHAPTER XVII

LIGHT AND DARK ADAPTATION

We have already discussed certain aspects of light and dark adaptation in Section II in connexion with the question of the transition from cone to rod vision, and shall do so again in Section IV in dealing with the dependence of the colour sensitivity of the receptors on their state of adaptation. In view of the primary importance of visual purple as the photochemical agent of the rods, it has seemed to me that this is the best place for a full discussion of adaptation.

The General Problem of Adaptation

In every highly developed sense organ it is possible to distinguish a primary mechanism specially developed for the actual reception of the appropriate stimulus, and a secondary ‘generator’ mechanism for stimulating the nerve or ganglion cells in contact with the receptor. The primary mechanism in the case of the rods of the retina is, undoubtedly, the photochemically active substance visual purple. The nature of the cone mechanism is still unknown (see below p. 307). We have assumed that the secondary mechanism in both cases is of the nature of an electrical ‘generator’ potential which depolarizes the neighbouring nervous structures and so activates the autorhythmic mechanism of the nerve in the manner described in Chapters I and VI. True adaptation to the stimulus may occur either in the primary mechanism of reception, in the ‘generator’ mechanism or in the nerve fibres, ganglia or synapses attached more or less directly to the receptors. In visual research the term ‘dark adaptation’ (Aubert, 1865) is a well-established misnomer for the process of recovery in the absence of stimulation.

There have been very few systematic studies of light adaptation by electrophysiological methods. This is probably due to the fact that such studies require experiments with isolated receptors involving a difficult technique for which there have been more interesting applications. Yet, in a sense, all the records hitherto published deal with light adaptation, for these,
almost without exception, are concerned with the relation between the duration of a light stimulus and either the amount of retinal potential developed or the frequency of the discharge in the optic nerve. Actually, in the vertebrate eye, the frequency of the discharge in a single nerve fibre is an unsatisfactory index of the reactions of either the primary photochemical or the secondary generator mechanism. The frequency is so much influenced by the complex interactions between excitation and inhibition, described in Section I, that the properties of the primary and secondary mechanisms hardly ever determine the final effect in the nerve.

In the eye of Limulus, on the other hand, where conditions are simpler, it can be shown (Figure 116, Riggs and Graham, 1940) that the frequency of the optic nerve discharge falls during continued stimulation in the manner which Adrian's (1928, 1932) work on other receptors has led us to expect. In Figure 116 we can see how, although the initial frequencies for the four intensities of illumination differ widely, they all fall to nearly the same value during illumination. This finding
would also be true of the vertebrate optic nerve discharge if an average response (i.e. that from a large number of fibres) were recorded. Working on visual sensations, Wright (1934, 1937) found that a constant level of brightness was attained during continued stimulation in spite of considerable variation in the intensity of the illuminant. Figure 117 illustrates the changes in the frequency of the discharge in an isolated retinal element of the dark adapted cat. The curve shows a rapid initial fall and then flattens out. This element was of the type which responds so long as illumination is continued; it gave a very small off-effect.

Rather a remarkable adaptive effect was noted by Riggs and Graham (1940) in Limulus. If the stimulus is strong enough there is a 'silent period' (Fig. 116, two top curves; cf. Fig. 17) in the optic nerve discharge, (like that seen when a nerve is stimulated by a slowly rising electrical potential (Fig. 10)), followed by an increase in sensitivity which can be demonstrated by testing with a superimposed flash. This increase of sensitivity can occur
at intensities of stimulation too low to produce a 'silent period' which is visible on the records. It is not known whether this temporary increase is due to the initial photochemical mechanism, the secondary generator mechanism or the nerve. On the whole a nerve mechanism seems the most probable.

Riggs (1940) made another experiment on the eye of Limulus in which he was probably able to exclude the initial receptor mechanism altogether. He recorded the impulses from a single fibre preparation during continuous illumination and at the same time tested its sensitivity with a higher intensity flash superimposed after the frequency of the discharge had fallen to a low value. If the superimposed flash was timed to elicit an impulse of its own just after one caused by the adapting light, it either had no effect or produced a delayed extra impulse, whereas, with a longer interval, the flash was more effective, producing two or more extra responses with a shorter latent period. Owing to the long period of illumination it is probable that the photochemical process was in a semi-stationary equilibrium before the test light was used and that the 'refractoriness' which followed the discharge of an impulse was therefore due either to the secondary generator mechanism or to the autonomic mechanism of the nerve fibre itself. This type of behaviour is always found in structures showing rhythmic activity and would probably also occur in an isolated optic nerve fibre (separated from its receptor) stimulated electrically with a slowly rising current (cf. Figs. 5–13, Chap. 1) and tested with a superimposed shock. As Riggs points out, his results are consistent with the view (inherent in Hill's excitation theory and elaborated by Katz, 1936) that rhythmic activity is determined by the refractory period and that the more excitable a structure the shorter will the refractory period be (see Chap. 1).

General Aspects of Dark Adaptation

Far more time and thought has been devoted to dark adaptation. Thanks to such knowledge as we have of the regeneration of visual purple we have a foundation for studying dark adaptation which is lacking in the case of light adaptation, although it is only recently (Granit, Holmberg, and Zewi, 1938; Granit, Munsterhjelm and Zewi, 1939), that any attempt has been made to correlate the amount of visual purple in a retina with
its electrical reactions. Other aspects of the dark recovery process have already been dealt with in Chapters VIII–X.

The standard experiment on dark adaptation was introduced by Aubert in 1865 and consists—for work on visual sensation as well as on the electrical reactions of the eye—in measuring the fall of the threshold during a period in the dark. The result of such an experiment on the human eye (Kohlrusch, 1922) is shown in Figure 118. In this case the index used was the absolute threshold of sensation expressed as the logarithm in microlux. It will be seen that there are two phases of the fall in threshold. The eye was light adapted before dark adaptation was begun and it is, of course, essential that both the duration and intensity of the preliminary light adaptation should be strictly controlled.

The same experiment can be made on a frog's eye by using the amount of light energy required to produce a discharge in the optic nerve as a criterion. Some curves derived from such an experiment are shown in Figure 119 where the threshold is
calculated in terms of energy units \( E \), \( \log E \) and the reciprocal of \( E \) (generally called sensitivity). The function \( \log E \) plotted against the time in the dark, in minutes, gives the most satisfactory curve from the point of view of presentation (cf. Kohlrausch, 1931) and is also a way in which physiological results are commonly presented nowadays (but from other points of view it is more correct to use the sensitivity, \( l_E \)). When

![Graph of light and dark adaptation](https://via.placeholder.com/150)

**Figure 119.** Course of dark adaptation in the frog eye as measured by the micro-electrode technique with a stimulus of 0.500\( \mu \). Previous light adaptation: 20,000 m.c. **Ordinates:** (left) percentage energy necessary to produce a threshold response (curve \( E \)), reciprocal of this value in percentage of final value (curve 1/\( E \)); (right) \( \log E \). Cf. Fig. 107 (Granit, 1941, Acta Physiol. Scand., 3)

plotted as it is in Figures 118 and 119 the dark adaptation curve of both man and the frog falls into two distinct parts. That this is true of the frog was first shown by Wrede (1937), and Riggs (1937), who both used the electroretinogram as a measure of sensitivity; Riggs, in particular, showed that a typical curve can be obtained by using the appearance of a \( b \)-wave of constant size as an index in place of the threshold. Kohlrausch (1922) was the first to demonstrate the phenomenon on man.
Kohlerausch also pointed out that the first phase of the curve is due to adaptation of the cones, previously separately measured by Hecht (1921a) and the second to adaptation of the rods. The second phase is missing in night blind subjects (Dieter, 1931), if the fovea only is tested or if red light of too long a wave-length to affect visual purple is used (Kohlerausch, 1922; cf. also Kohlerausch, 1931b and, for later work, Hecht’s review, 1937). The dual nature of dark adaptation is, thus, another of the many manifestations of the duplex nature of the visual process.

Figure 144 (p. 285) illustrates an experiment on the frog in which the changes in the threshold to red, green, and blue wave-lengths during dark adaptation were compared. It will be seen that, just as in the human eye, the threshold for a red light of long wave-length never falls to the level of the second phase of dark adaptation. For this second phase, which we shall call ‘dark adaptation proper’, the primary receptor process must, therefore, depend on the photosensitivity of visual purple and be a rod phenomenon. We shall see later (Section IV) that the spectral distribution of photosensitivity does actually shift from that characteristic of cone dominance to one indicating rod dominance (visual purple) during dark adaptation proper. In the Limulus eye, on the other hand, dark adaptation as measured by the size of the electroretinogram (Hartline, 1930) follows a simple course, the curve for which can be fitted to the equation for a monomolecular reaction.

Dark Adaptation and Visual Purple

A number of questions are raised as a result of the discovery of these two phases of dark adaptation. What is the explanation of the initial delay before the rods begin to adapt? Is this delay only due to the removal of visual purple by the preceding light adaptation so that the sensitivity of the rods is depressed far below that of the cones? How far are dark adaptation and sensitivity correlated with the amount of visual purple present in the eye? What information can we obtain from the reactions of the single optic nerve fibres of pure rod, mixed and pure cone retinas? This last question has already been discussed from another aspect in Chapter IX.

Using Zewi’s (1939) modification of Tansley’s (1931) method for the extraction of visual purple it has been possible to com-
pare the size of the electroretinogram with the amount of photosensitive substance present in an eye at any given stage of adaptation. The natural expectation was that there would be a good agreement between the two values, not only because of the common belief that, in scotopic vision, sensitivity bears some very simple relation to the amount of visual purple present, but also because Tansley's visual purple regeneration curves do, in fact, agree fairly well with Charpentier's (1936) curves for the increase in the $b$-wave during dark adaptation; both workers used the same animal, the albino rat. However, Charpentier was unable to follow the first part of the curve by his method because, in this eye, the $b$-wave is so small at the beginning of dark adaptation that it is lost in the base-line irregularities of the amplifier. The excised frog eye or the decerebrate cat are better preparations for such experiments.

We (Granit, Holmberg, and Zewi, 1938) have found that bleaching of the frog eye by various monochromatic lights is followed by a considerable reduction in the size of the electroretinogram elicited by a wave-length of $0.500\mu$, but that this reduction is not accompanied by any reduction in the amount of visual purple that can be extracted. That this is due to a failure to destroy the visual purple and not to its regeneration during the experiment is shown by the fact that the bleaching lights used were too weak to decolorize a visual purple solution by more than 1 or 2 per cent. The large reduction in the size of the electroretinogram (of the order of 30—70 per cent) cannot, therefore, be explained on any hypothesis that the quantity of visual purple present is the sole determinant of the size of the $b$-wave during dark adaptation. It can, however, be explained if one assumes that a layer of visual purple molecules in combination with receptor molecules forms an excitable structure on the surface of the rod which is easily destroyed by illumination. But, if this surmise is correct, what is the function of the rest of the visual purple inside the rod? Is it merely a reservoir of inactive material kept to provide for the rebuilding of the surface structure?

A further study of these problems was made by measuring the $b$-wave of frogs after certain standard periods in the dark and comparing them with the amount of visual purple which could be extracted immediately after the test. Some of the results
obtained are illustrated in Figure 120. In this figure the two curves with an initial horizontal course represent the changes in the size of the b-wave for a stimulating light of two intensities, while the third curve is Zewi's standard for visual purple regeneration in frogs under similar conditions. The empty circles give the values for the actual amounts of visual purple obtained in the experiments. It is clear that, while, under the conditions used, the concentration of visual purple begins to rise as soon as dark adaptation begins, the electrical response remains con-

![Graph](https://via.placeholder.com/150)

**Figure 120.** Comparison of changes in size of b-wave and visual purple concentration during dark adaptation in frogs. ○ density of visual purple (curve is Zewi's standard visual purple regeneration curve at 16° C.); ○ size of b-wave with a low intensity stimulus; (dotted line) size of b-wave with a stimulus 20 times greater than ○. Light adaptation: 20,000 m.c. (Granit, Munsterhjelm and Zewi, 1939, J. Physiol., 96)

stant for about an hour after which it increases rapidly. There is, thus, a long delay before an average response, such as the b-wave, of the electoretinogram, "feels" the increased concentration of visual purple. If, instead of measuring the size of the response to a constant amount of energy as here, one measures the amount of energy necessary to evoke a constant response (Fig. 119) one can recognize what we have called dark adaptation proper after only twenty minutes.

When the frogs were cooled so as to delay their visual purple
regeneration the increase in the size of the \( b \)-wave was also delayed. It was found that, at normal as well as at low temperatures, the \( b \)-wave does not begin to show a marked increase in size until the visual purple concentration has reached about 50 per cent of its maximal value, indicating that there is some relation between the electrical response and the amount of visual purple present. Neither the addition of a short bleaching period under a strong light to delay the beginning of visual purple regeneration (see p. 225) nor the substitution of a weak adapting light of 330 m.c. for the usual one of 20,000 m.c. had any effect on this result—there was always a delay before the size of the electroretinogram increased, and this increase always began when the visual purple concentration had reached about 50 per cent of its maximum.

When experiments such as those described above are done on frog eyes the movement of pigment is always a potential source of error and for this reason it was considered advisable to perform similar experiments on the decerebrate cat. The curves in Figure 121 illustrate the effect of varying the period of a preceding light adaptation to an intensity of 3,900 m.c. on the course of the increase in the \( b \)-wave during dark adaptation.
With longer periods of light adaptation (10—30 min.) there is a marked delay before the b-wave begins to increase in size, but if the light adaptation lasts no longer than one minute the curve begins to rise immediately. In these experiments the first electroretinogram records after the termination of light adaptation were always negative, indicating that at this point PI11 has become the dominating potential. It may be objected that 3,900 m.c. is not a very strong light to use for adaptation, but

![Graph showing the size of b-wave and visual purple concentration in per cent of maximum over time in the dark (min.).](image)

**Figure 122.** Comparison of changes in the size of the b-wave with those in visual purple concentration during dark adaptation in the cat. ● density of visual purple; ○ size of b-wave. Light adaptation: 3,900 m.c. for 20 mins. Note absence of a measurable b-wave directly after light adaptation in spite of the presence of 40 per cent of end concentration of visual purple. (Granit, Munsterhjelm and Zewi, 1939, J. Physiol., 96)

one must not forget that, in these experiments, the pupil was fully dilated whereas, in life, it would be a mere slit under this illumination. It is probable that the amount of light reaching the retina is actually above the usual maximum.

With cats separate animals had to be used for the estimations of visual purple regeneration during dark adaptation. For this purpose one eye was kept in the dark as a control while the other was subjected to the light and dark adaptations used for
the electrical experiments. The retinas were hardened in a 4 per cent alum solution before the visual purple was extracted and the concentration in the experimental eye calculated as a percentage of that in the fully dark adapted eye. In Figure 122 a regeneration curve (filled circles) is compared to a dark adaptation curve, the mean of two experiments, obtained by measuring the change in size of the b-wave under similar conditions. In this case, too, the size of the b-wave does not begin to increase until the visual purple concentration has reached 50 per cent of its maximum—even when the concentration is as high as 40 per cent the b-wave is almost too small to record.

The question arises as to how far these results may have been distorted by regeneration during the experiment. Any errors due to this cause may safely be regarded as insignificant, for since the amount of visual purple found in a frog eye under a great variety of conditions faithfully reflects the duration and strength of the preceding light adaptation, it is clear that regeneration can only produce a slight increase in the values found.

The results of all these experiments agree in demonstrating that, in eyes with a large rod population, large b-waves are associated with large quantities of visual purple but that, at the same time, a relatively small reduction in the amount of visual purple present produces an excessive diminution of the b-wave. I am convinced that subjection of the cat’s eye to light adaptation for so short a time as one minute can only have a very insignificant effect on the amount of visual purple present, yet, under these circumstances (cf. Fig. 121), the b-wave is reduced to a small hump on the record. If the electrical response were directly dependent on the amount of visual purple present such a reduction in the b-wave would involve an 80 per cent reduction in the visual purple concentration. In this particular case the b-wave recovers quickly but a longer light adaptation delays its recovery in spite of the absence of any corresponding delay in visual purple regeneration. The size of the b-wave is a good expression of the average excitability of a large number of retinal elements and we must therefore conclude that average excitability and average visual purple content can, under certain conditions, be largely independent of one another and that the increase of rod excitability lags behind the increase in visual purple concentration during dark adaptation. In the mammalian eye the b-wave
can be depressed to the extent of not appearing on the record in spite of the presence of considerable quantities of visual purple.

It may be concluded, therefore, that there must be an intermediate process between retinal sensitivity as measured by a given size of b-wave and the sensitizing effect of the visual purple which can produce such a b-wave if given time enough. This process might be concerned in the combination of visual purple with some part of the rod surface (see above), or it might be something of the nature of a reorganization of the neural coupling of the retina as suggested by Lythgoe (1940). He thought that some such process must be the cause of the change in retinal properties which shows itself in the shift from differentiation to integration during dark adaptation and which has been discussed in Chapter VIII under the term 'differentiation velocity'. This change is certainly of neural origin and it is clear that nervous factors must play a part in dark adaptation.

In addition Lythgoe (1940) presented a formidable array of arguments showing that the theoretically simplest photochemical explanation of the increase in sensitivity in the dark cannot be correct. According to this explanation the product of visual purple concentration (provided this is not too high) and illumination should give a constant value directly proportional to the sensory constant which we call 'threshold'. As he points out, this is not the case. Comparatively small variations in visual purple concentration may be accompanied by enormously larger variations of the visual threshold.

But, if the visual purple present at a given stage of light adaptation does not contribute to sensitivity in proportion to its concentration, what does the surplus do? Does it merely represent an inactive store or does it still somehow mediate the reception of light?

One aspect of this problem was attacked in the course of an investigation of the differential sensitivities to wave-length of single retinal elements in various stages of adaptation which will be described in detail in Section IV. For our present purpose it is only necessary to mention that a number of elements were found which, in the dark adapted state, showed a spectral distribution of sensitivity corresponding to that of visual purple and whose sensitivity curve was not altered by adaptation to
amounts of light capable of suppressing most of the $b$-wave (actually the sensitivity curve of these elements did not agree precisely with that of visual purple, it was, rather, a somewhat narrower visual purple curve). It was found, moreover, that the level of sensitivity of these elements when light adapted was, in every respect, comparable to that of other elements with a cone sensitivity curve (‘dominators’ and ‘red-modulators’ see p. 277). Thus, these experiments showed very clearly that, in the cat for instance, light adaptation capable of producing a Purkinje shift in a large number of elements by no means excludes the apparent dominance of visual purple in many others.

We must, therefore, believe that, at the low concentrations found in light adapted eyes, visual purple is responsible for a sensitivity of the low level associated with the cone substances, whatever these may be. After some time in the dark the well-known second phase of increasing sensitivity begins—dark adaptation proper. This phase is associated with a broadening of the sensitivity curve in those elements which have a narrow visual purple band in the light. At the same time those elements with spectral distributions of sensitivity indicating cone substances show a Purkinje shift, producing typical visual purple curves. It must be remembered that all these records are taken from isolated optic nerve fibres on the inner surface of the retina, and that in the animals used, both rods and cones may be connected to a given nerve fibre. This means that, after dark adaptation, the rods (and visual purple) will finally determine the response in the fibre because, although the sensitivity due to cone substances does rise in the dark, it remains far below that due to visual purple.

It will be necessary to consult Section IV for a full understanding of these changes but it is already evident that rods are not necessarily inactivated but can, on the contrary, function as cones under conditions of moderate light adaptation.

The Dark Adaptation of Isolated Elements

Hartline and his collaborators (1941), working on the dark adaptation of single visual cells in the frog, found that recovery of activity can be retarded by prolonging the time or increasing the intensity of light adaptation (cf. Fig. 121). They reached the same conclusion as Granit and Riddell (1934) and Lythgoe
(1940), that dark adaptation involves more than just changes due to the increasing concentration of visual purple.

In this connexion it is interesting to compare the dark adaptation of single elements in the cat and in the guinea pig. Cats show a Purkinje shift in sensitivity, guinea pigs have a pure rod eye and no shift. The two upper curves in Figure 123 follow the change in threshold of single elements in the cat to red (top curve and dotted line) and white light during dark adaptation.

Figure 123. Course of dark adaptation in some isolated elements of the cat retina. ○ (upper curve and dotted line) with red stimulus, (lower curve) same element with white stimulus; ● and □ responses of other isolated elements (Granit, 1944, Acta Physiol. Scand., 7.)

The red response is reminiscent of the dark adaptation curve of the human fovea, the white response shows dark adaptation proper after an initial delay of nearly twenty minutes. The two lower curves illustrate characteristic variations in the course of dark adaptation of different isolated elements of the cat retina. In neither of these is there any initial delay, dark adaptation proper begins immediately.

Examination of the results of many experiments with isolated elements of the guinea pig (pure rod) retina showed that, as
far as their reactions during dark adaptation are concerned, these behave in exactly the same way as the mixed receptors (rods and cones) of the cat. Dark adaptation may proceed smoothly or in stages, there may be an initial delay or dark adaptation proper may begin at once.

I conclude that there must be two different types of rod, one reacting like cones so far as adaptation is concerned, the other like an 'ideal' rod reflecting, fairly accurately, the course of visual purple regeneration. These types are shown diagrammatically in Figure 124, but the fact that the first have been drawn with a short cone-like structure and the second with an elongated rod-like one is not meant to suggest that they are actually of different shapes. The differences are probably wholly at the photochemical level. However, by combining different proportions of two such types on to one 'final common path' different dark adaptation curves can be obtained. For instance, if the number of 'ideal' rods were too small to affect the sensitivity until they have had enough time to regenerate a considerable proportion of their visual purple, dark adaptation proper would be delayed.

At present it is best to admit that we don't know why different elements of a pure rod retina have different sensitivities or to what properties of visual purple (if any) this may be due. More evidence that some rods can behave like cones will be given in Section IV and so one hesitates to ascribe the different adaptabilities found to differences in the experimental conditions such as pressure due to the electrodes. In any case it is not clear
how the differences between the curves of Figure 123 could be due to such a cause since all the elements reached practically the same level of final sensitivity and showed no sign of deterioration.

I suggest, then, that the cone-like rods are light resistant, recover quickly to a semi-stationary level of sensitivity of relatively high threshold and probably contain a visual purple which is chemically slightly different from the usual form and is capable of mediating responses in the photopic state. These are the only rods which remain active after light adaptation. The real or 'ideal' rods are only able to influence the response (either of the whole retina or of one nerve fibre) after a period of dark adaptation long enough to allow sufficient visual purple to be formed. It is the sudden addition of these rods to the active retinal elements that causes the 'kink' in the dark adaptation curve. In this way the initial delay in dark adaptation as well as the lack of correspondence between 'sensitivity' and visual purple concentration can be explained on the basis of our experimental results. These definitely suggest the presence of receptors intermediate between rods and cones. Such a hypothesis neither excludes nor confirms the suggestions already made both by ourselves (cf. above p. 250) and by Lythgoe (1940), since it provides no explanation of the ultimate difference between the two types of rod. It does, however, follow that 'ideal' rods are particularly responsible for the positive component potential of the electroretinogram, PII, which is the cause of the b-wave (see Karpe, 1945 and Appendix II).

Since this chapter was written I have seen a paper by Wulff (1943) in which, as a result of work on the grasshopper eye, he claims that the electroretinogram gives a direct expression of the concentration of photosensitive material, not only in this but also in the vertebrate eye. Wulff misrepresents our work when he states that our attempts to correlate visual purple concentration with the size of the b-wave 'on the whole, were negative'. As I have pointed out above, such correlations are very obviously positive in some types of experiment and altogether lacking in others. For instance, in the cat's eye the b-wave may be so small as to be negligible after some minutes' adaptation to 3,900 m.c., but no one with any experience of making visual purple extracts could possibly believe—as apparently Wulff does—that the amount of visual purple present is also negligible.
LIGHT AND DARK ADAPTATION

(see p. 248). On the other hand, the b-wave and visual purple regeneration both react in the same manner to changes in temperature. Again, in experiments with single retinal elements, it was found that the behaviour of certain of these appeared to reflect the regeneration of visual purple while that of others did not, in spite of the fact that they must ultimately have produced visual purple because their final sensitivity curves were of the kind which is determined by this substance. Having stated his conviction that the electroretinogram of the vertebrate eye (of which he has no experience) should reflect the concentration of visual purple, Wulff gets out of the difficulty caused by the fact that it often does not by suggesting that our visual purple measurements were at fault (apparently he has no experience of these either). In our investigation this part of the work was done by Dr. Zewi whose experience, in his own work alone, is based on over 1,500 individual extractions. His regeneration curves for high temperatures on intact frogs are in fundamental agreement with those obtained by Tansley (1931). (For discussions of the accuracy of his method, see Zewi, 1939, Granit, Holmberg and Zewi, 1939, and above, p. 221.) Finally it is worth pointing out that the b-wave (PII of the vertebrate electroretinogram) is not homologous with the receptor electroretinogram of the grasshopper nor has this retina ever been shown to contain visual purple.