PROCESSES OF ADAPTATION IN THE VERTEBRATE RETINA IN THE LIGHT OF RECENT PHOTOCHEMICAL AND ELECTROPHYSIOLOGICAL RESEARCH (1)

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INTRODUCTION

It is necessary at the outset to indicate the limits to which this review of recent research will be confined. In accordance with the title, photochemistry and electrophysiology will be treated only from the point of view of adaptation; for a complete history of the problems under discussion the reader is referred to reviews to be mentioned in the course of this paper. The large body of publications dealing with photochemical generalizations on the basis of sensory data had to be neglected *in toto* or else it would have been difficult to decide what to leave out and what to mention without unduly increasing the scope of this review.

From the practical point of view the relation between processes of adaptation and photochemistry concerns the properties of visual purple, this being the only substance known to possess both adaptability and sensory significance. For the sake of completeness the possible rôle of lactoflavin (vitamin B_2) will be briefly discussed. Apart from iodopsin (WALD, 1937) and the « Zapfensubstanz » of v. STUDNITZ (1932, 1937) no other substance, likely to be the photochemical mediator of sensations from the cone system, has been found, the well-known coloured globules, recently discussed by ROAF (1933) obviously belonging to another category of substances.

Adaptation in the cone system is therefore largely a question of electrophysiology. For this section I have had access to as yet unpublished data, obtained in the Helsingfors laboratory by Drs P. O. THERMAN and C. M. WREDE, to both of whom I am greatly indebted for permission to take advantage of the facts they have found.

The terms « rods » and « cones » will here be used in the only sense in which at present they can have physiological significance, the rods accordingly being defined as elements possessing some type of visual purple, the cones as elements failing to respond with the spectral distribution of sensitivity characterizing elements activated by visual purple.

As a process of adaptation will be regarded every adjustment of an eye or transformation within a substance in response to a change in the general level of illumination, be it then in the direction of an increase or a decrease of the stimulus. When at the moment it does not seem possible to interpret an electrical change photochemically, the latter will be mentioned among the pheno-

mena of « electro-adaptation », of which some at least can have very little to do with the nature of the photochemical substance initiating the reaction.

I. — VISUAL PURPLE

1. Historical and technical

On the whole recent work has confirmed and amplified the views held by Kühne (1879) in his well-known summary of the Heidelberger papers, subsequently taken over and defended by Garten (1907) who himself made some notable contributions to this subject. The chief point on which Kühne and Garten differed from Köttgen and Abelsdorff (1896), Nagel and Piper (1905) and Trendelenburg (1904) concerned the existence of visual yellow which the latter authors denied. The work to be reviewed has confirmed Kühne's standpoint. König (1903) who already in 1894 had seen visual yellow in an extract from a human eye went as far as to suggest that this substance may prove to be the « blue » substance postulated by the Young-Helmholtz theory. However, his work was continued by his pupils, Köttgen and Abelsdorff (1896), who did not subscribe to this view.

A brief summary of Kühne's generalizations still is as good an introduction as any to the problems which have been raised by those who to-day are engaged in research of this character. Kühne held that visual purple was decomposed by light to visual yellow and that the latter substance underwent further decomposition to visual white, a colourless final product showing greenish-white fluorescence. Visual purple regenerated both from the yellow and the white stage. The former process was faster and was found even in extracts of V.P. (visual purple) with bile salts, the latter slower and requiring contact with the pigment epithelium. Regeneration from visual white did not involve a yellow intermediary stage. It was held to be reformation of V.P. *de novo* and termed *neogenesis*, whereas regeneration from the yellow stage was held to be a recombination of the V.P. molecule from intermediary photoproducts and was called *anagenesis*.

Garten (1907) defended the formation of visual yellow during bleaching, on the basis of improved methods of measuring the absorption spectra. These Kühne could not measure with any degree of accuracy with the methods then available. To Kühne's observation that V.P. regeneration was enhanced by an increase in temperature he added the specification that regeneration from

the yellow stage took place after freezing and subsequent thawing of the retina and that it was very much faster than KÜHNE had thought. He also found that the decomposition of visual yellow to visual white was very much delayed by cooling the preparation.

The diagram of fig. 1 illustrates what may be called the KÜHNE-GARTEN concept of V.P. breakdown and regeneration. It is hardly necessary to emphasize that the diagram is hypothetical inasmuch

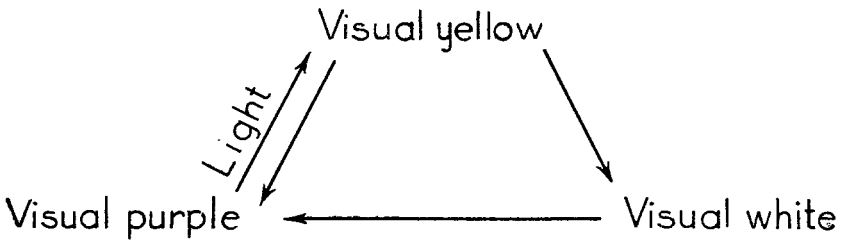

Fig. 1. — Diagrammatic representation of the views held by KÜHNE and GARTEN on visual purple decomposition and regeneration.

as the arrows are held to represent chemically identified transitions. The literature until 1934 has been summarized by KRAUSE (1934). There is also a recent brief review by LYTHGOE and GOODEVE (1937).

The recent research in this field has profited from the general advance in chemical and physical technique that has taken place since the beginning of this century. Absorption spectra can now be measured quickly and accurately with the aid of photoelectrical methods. The significance of the pH of a solution introduces concepts that were unknown to those who studied V.P. solutions during the last active era of research in this field. The general chemical technique of purification of minute amounts of known and unknown substances likewise has made great progress. Fifty years ago substances such as vitamins hardly stood a chance of being isolated and synthetized as is being done to-day. In view of such facts the photochemistry of the retina would seem to be full of promise for the near future.

2. VITAMIN A AND VISUAL PURPLE (1)

Owing to the central position of the carotenoids in modern biochemistry, the discovery made by FRIDERICIA and HOLM (1925)

(1) The most recent summaries of the general relation of vitamins and retina in this same volume. See the reviews by KARRER and by JUHÁSZ-SCHÄFFER.

(see also HOLM, 1925) that rats deprived of vitamin A had a delayed regeneration of V.P., has proved an important stimulus to research in this field. It was first shown in feeding experiments (HOLM, 1929; YUDKIN, 1930; YUDKIN, KRISS and SMITH, 1931; WALD, 1934-1935) that the retina is an active source of vitamin A or its pro-vitamin, carotene. Then followed TANSLEY's (1931) measuments of the curves for regeneration of V.P. in normal and A-deficient rats. Finally a number of authors (v. EULER and ADLER, 1933; v. EULER and HELLSTRÖM, 1933; HAUROWITZ, 1933; WALD, 1934-1935 and others) attacked these questions with chemical methods. The problems raised by the biochemical line of attack are best discussed in connexion with the work of WALD (1935-1937) and of KRAUSE (1937-1938) (see p. 20, below). TANSLEY's (1931) results were confirmed by CHARPENTIER (1936) with the electrophysiological method. In his paper and in a recent summary by v. EULER (1937) the greater part of the literature dealing with the connexion between vitamin A and night blindness may be found (1). v. EULER also discusses the possible significance of other vitamins in vision as well as some of the clinical work on hemeralopia as an early sign of A-deficiency. The practical application of the new knowledge for diagnostic purposes is a subject outside the scope of this review.

FRIDERICIA and HOLM (1925) and KUWANA (1934) measured the concentration of V.P. colorimetrically. The details of the method used by AMENOMIYA (1931) are not given. However, delayed regeneration of V.P. was always found in A-deficient animals. A more accurate photographic method of determining the density of the solutions of V.P. obtained from A-deficient and normal albino rats was used by TANSLEY (1931). The animals had previously been light adapted and were then killed in groups after certain standard periods of dark adaptation. Thus points along the whole curve of regeneration, shown in fig. 2, were obtained. The curve for the A-deficient animals, despite 20 hours of dark adaptation, remains below the normal values. She could therefore conclude that not only is regeneration of V.P. delayed but also the quantity, ultimately regenerated, less than in normal rats. Using direct measurements of the electrical responses of the eyes of living rats CHARPENTIER (1936) came to the same conclusion. He obtained curves that were almost identical with those of TANSLEY when reduced to the same ordinates (see below, p. 37).

The first attempts to use the antimony trichloride reaction of CARR-PRICE with retinal extracts (HAUROWITZ, 1933; v. EULER

and ADLER, 1933; v. EULER and HELLSTRÖM, 1933) showed conclusively that visual purple itself could not be identical with vitamin A. Some carotene, however, was found in the retina of cattle (v. EULER *et al.*; BRUNNER, BARONI and KLEINAU, 1935). Thus

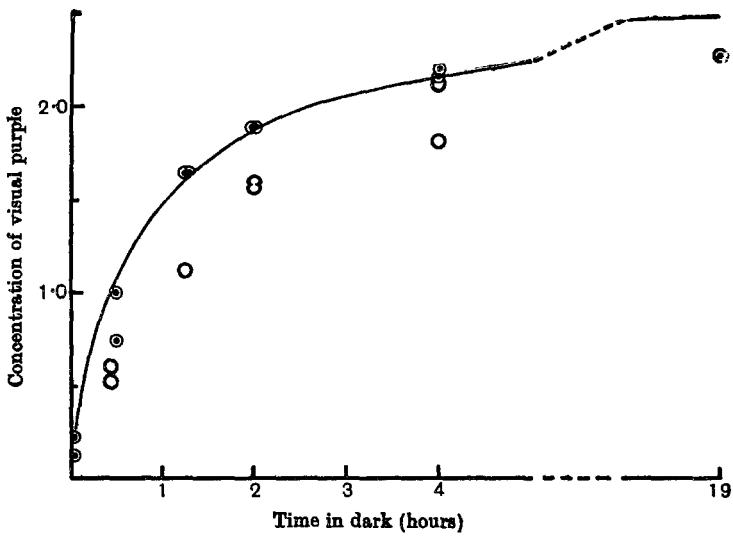

Fig. 2. — The regeneration of visual purple in animals kept in the dark for different periods. ⊙ Normal rats. O Vitamin A-deficient rats. The curve was calculated from the equation for a bimolecular reaction. TANSLEY (1931), *J. Physiol.*, 71, 442.

v. EULER and ADLER (1933) extracted 240 γ carotene from the pigment epithelium, and 95 γ carotene from the retinæ of 20 eyes, (1 γ = 1/1000 milligram). An absorption band at 0.623 μ was held to signify the presence of vitamin A. WALD (1934-1935) found neither carotene nor xantophyll in bovine retinæ. KRAUSE (1937 b) likewise found no carotene nor other carotenoids in the retinæ of cattle and seems to hold the carotene content of the eyes to be variable and dependent upon race and diet of the cattle. In frogs HAUROWITZ (1933) obtained a positive CARR-PRICE reaction with extracts from retinæ from which previously the V.P. had been removed. The colouration of the extract treated with antimony trichloride showed great seasonal variations. WALD (1934-1935) and KRAUSE and SIDWELL (1938) have isolated vitamin A from retinæ and WALD (1935-1937) has tried to find a definite place for vitamin A in the chain of reactions of visual purple. His conclusions are not supported by the work of KRAUSE (1937 b) and KRAUSE and SIDWELL (1938). These questions will be discus-

sed below (p. 21) in connexion with experiments on the bleaching and regeneration of V.P. Preliminarily we conclude that there are very obvious gaps in our knowledge about the nature and significance of the substances that have been extracted from the retina. Thus, for instance, LÖNNBERG (1935, 1936, 1937) has isolated xantophyll from eyes of various birds and fishes and his results have been confirmed by WALD (1935, 1936, a, b) in later work with the eyes of amphibians, but we have no knowledge whatsoever about the possible significance of this finding.

TANSLEY (1933 a) developed her work along anatomical lines and discovered that the formation of V.P. in embryonic rats coincided with the appearance of the outer limbs of the rods. There are according to TANSLEY four indications of an association between V.P. and the outer limbs of the rods : « First, both in direct observations of the living retina and in preparations suitably stained this substance is always found to be present in the outer limbs of the rods and nowhere else in the retina. Second, visual purple and the outer limbs seem to appear simultaneously in the developing retina. Third, visual purple is not produced in preparations in which the outer limbs do not develop ». The question of the influence of vitamin A on the development of the rods is complicated by the fact that A-deficiency is upsetting the mechanism of labour in the mothers. It is therefore difficult to obtain young rats seriously deficient at birth. TANSLEY's (1936) experimental results suggest that A-deficiency affects the production of V.P. directly rather than by interfering with the normal structure of the rods.

As an abnormality in developing retinæ of embryonic rats there are sometimes formed « rosettes », studied by TANSLEY (1933 a, b) in tissue cultures. Such rosettes are also found in retinæ developing *in vivo* after trephining the eyes at an early age. The rods in rosetted areas are found to possess both V.P. and outer limbs, despite lack of contact with the pigment epithelium. Tissue cultures of retinæ did neither form outer limbs nor V.P. although all the time in contact with the pigment epithelium. (TANSLEY, 1933).

The rôle played by the pigment epithelium would probably be more or less important according to whether the retina may be supposed to be primarily nourished through this layer, as *e.g.* in frogs, or primarily through the blood vessels as is suggested by the retinal circulation of mammals.

3. THE EXTRACTION OF VISUAL PURPLE FROM THE RETINA

The preparation of solutions of V.P. is discussed in a number of papers (TANSLEY, 1931; HOSOYA, 1933; HOSOYA and BAYERL, 1933; LYTHGOE, 1937; KRAUSE, 1937 b, 1938 and others). Very

detailed descriptions of the technique are given by LYTHGOE and KRAUSE. The influence of factors such as duration of extraction, centrifuging, and temperature have been studied by HOSOYA and SAITO (1935) and by CHASE (1936). Attention to detail is of great importance because of the necessity of avoiding opalescence as well as intermediary photoproducts derived from V.P. (see below). In LYTHGOE's method the first step is the preparation of a suspension of retinal rods, then follows extraction of lipoids with petrol-ether. As a third step some of the proteins are precipitated by treating the rods with an acid solution (pH 4.6). Finally the V.P. is extracted by a solution of digitonin. (1)

With solutions, less pure than those obtained by the methods of LYTHGOE and of KRAUSE, HOSOYA and BAYERL (1933) and HOSOYA (1933) came to the conclusion that during bleaching absorption increases in the short wave-lengths indicating the formation of a yellow substance. This result that soon was confirmed by HECHT and CHASE (1934) and by CHASE (1936) drew attention to the old controversy over visual yellow, referred to above. It had the further merit of reviving interest in the photochemistry of V.P. in solution.

Various stages of purification of the extracts are shown in fig. 3 from LYTHGOE's (1937) paper. The points around the top curve are from the paper by CHASE (1936). (2). Ordinates are density, defined as

$$D = \log_{10} \frac{I_o}{I_t}$$

where I_o is the intensity of the light incident on the solution, I_t the light transmitted to the recording instrument, nowadays chiefly a photoelectric cell connected to an electrometer.

With density 1, the solution transmits 10 p. c. and with density 2, 1 p. c. of the incident light. This expression may also be given the form

$$I_t = I_o 10^{-D}.$$

For some purposes the form $I_t = I_o e^{-\alpha cl}$ will be found useful (see below). In this expression α is the extinction coeffi-

(1) See *Addendum to proof* for a recent contribution to the technique of extraction.

(2) In a recent contribution CHASE (1938) has obtained purer solutions of V.P. See *Addendum to proof*.

cient per chromophoric group, c the number of chromophoric groups per c.c., l is the length of the optical cell, and e the base of the natural logarithms.

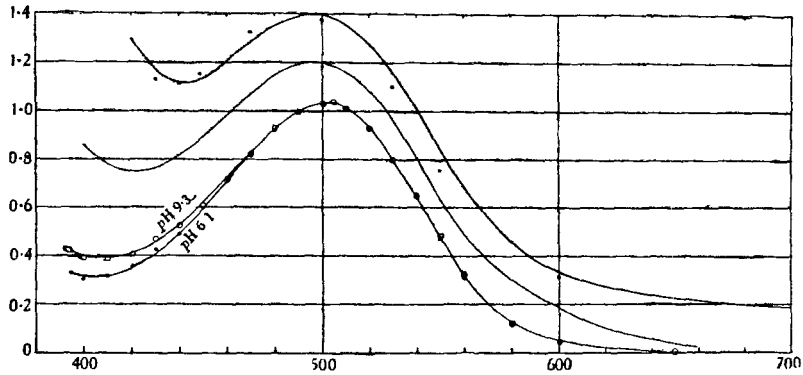

Fig. 3. — Absorption curves of unbleached retinal extracts showing the elimination of impurities at different stages of purification. Values corrected so that the density of the contained visual purple at 502 mμ is 1.00 exclusive of impurities. Top curve : Extraction of whole retina with 2 p.c. digitonin solution. Extraction with 4 p. c. bile salts gives values shown by the points of CHASE (1936). Middle curve : A suspension of « rods » was given a preliminary washing with a pH 4.6 buffer solution. Lower curve : Extracts used in Lythgoe's paper in which rods were also washed with petrol ether. Final solutions buffered to pH 6.1 (●—●) and pH 9.3 (o—o). The same preparation was used differing only in the added buffer. Abscissæ : wave-length (mμ). Ordinate : density. LYTHGOE (1937), *J. Physiol.*, 89, 331.

4. GENERAL COURSE OF BLEACHING OF VISUAL PURPLE IN SOLUTIONS

The newer work on the course of bleaching of V.P. in solution has explained why there could be some doubt as to whether yellow intermediary products are formed during this process. The main factors determining the density of visual yellow appearing in bleached solutions would seem to be the pH of the medium (NAKASHIMA, 1929; CHASE, 1936; LYTHGOE, 1937) and the temperature (HOSOYA and SAITO, 1935; CHASE, 1936). It has been suggested that summer and winter frogs behave differently with regard to the formation of visual yellow (HECHT and CHASE, 1934) but this difference may have been due to the difference in room temperature at the time of extraction (HOSOYA and SAITO, 1935; CHASE, 1936).

In 1929, NAKASHIMA (1) made the important observation that bleached and unbleached extracts became deeper yellow in acid than in alkaline solutions and suggested that differences in hydrogen ion concentrations could explain the discrepancies in the old literature as to the reality of visual yellow. This suggestion was endorsed by KRAUSE (1934) in his well-known review. CHASE (1936) found an increase in absorption in the blue and violet only in acid solutions while no yellow colour appeared in alkaline-buffered solutions. He also found that a partially bleached solution could be made yellow merely by increasing its acidity without illuminating it any further. Owing to the use of a visual method his spectrum was limited to 0.430 μ. Employing photoelectrical methods HOSOYA and SAITO (1935) and LYTHGOE (1937) were able to conclude that illumination of solutions of V.P. always leads to an increased absorption in the short wave-lengths, LYTHGOE in particular showing that only in acid solutions this increase remained well within the visible part of the spectrum. He then proceeded to measure the absorption curves for this decomposition product of V.P. in media of different hydrogen ion concentration. His results, reproduced in fig. 4, show that the substance that is yellow in buffers of, say, pH 5-6, shifts its absorption spectrum towards the ultra-violet in alkaline solutions. This process is completely reversible. As visual yellow behaves like an acid-base indicator LYTHGOE suggested that it be called « indicator yellow ». The absorption spectrum of visual yellow as well as of further decomposition products of V.P. have since been followed by KRAUSE and SIDWELL (1938) into the ultra-violet (0.250 μ).

HOSOYA and SAITO (1935) and CHASE (1936) have pointed out that solutions of V.P. at different temperatures are bleached in

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(1) CHASE (1936) reports NAKASHIMA (1929) as having studied « the effects of a number of substances upon *un*bleached visual purple » and having found that in an acid medium a distinct yellow colour was produced. From not having himself found any decomposition of V.P. with buffers between pH 5.8 and 10.0 he concludes: « This would indicate that the hydrogen ion concentrations used by NAKASHIMA may have been outside of the physiological range so that use of his results to explain the discrepancies in the literature seems unwarranted. His conclusions, however, are supported by the data presented here (CHASE, 1936) ». The following quotation from NAKASHIMA's communication shows this criticism to be unnecessarily severe: « Mit der Pufferlösung kann man diese Differenz zwischen Säure und Base bei Ausbleichung durch Licht deutlich demonstrieren ». This quotation seems to me to indicate that different hydrogen ion concentrations were tried and to show that NAKASHIMA also studied the effect of acid and alkaline buffers on bleached solutions of visual purple. NAKASHIMA used frog's retinæ and solutions of bile salts for his extractions.

such a fashion as to make high and low temperature equivalent to respectively alkalinity and acidity as far as colour of the solutions is concerned.

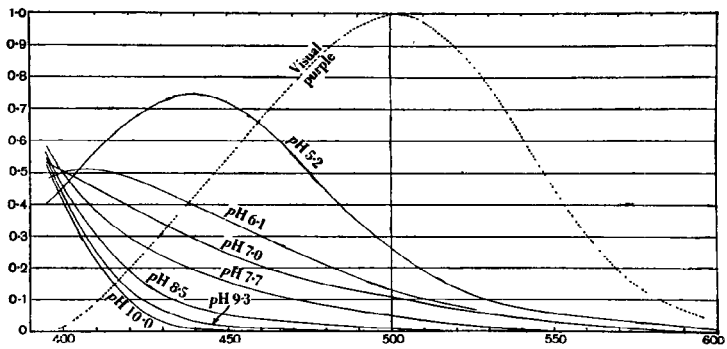

Fig. 4. — The absorption curves of indicator yellow alone for hydrogen-ion concentrations which do not destroy visual purple. The density of the parent visual purple would be 1.00 at 502 mμ and its absorption curve is shown by the dotted line. The curves for pH 5.2 and 6.1 have been corrected for fading. Abscissæ: Wave-length (mμ). Ordinate: Density. LYTHGOE (1937), *J. Physiol.*, 89, 331.

KÜHNE (1879) already knew that V.P. does not become yellow or white immediately upon bleaching with light. There are intermediate stages: reddish purple, pure red, orange, but KÜHNE held them to be due to the simultaneous presence of purple, yellow, and white molecules. LYTHGOE (1937) discovered a separate intermediate photoproduct, « transient orange », and bases this finding on the following experiment : « If two samples of alkaline visual purple, one of which has been cooled in ice and the other warmed, are placed in the light, the cooled solution bleaches to a vermilion-orange colour. On subsequent warming of the latter solution it loses its orange colour and becomes identical in colour with the solution which has been bleached in the warm ». This experiment suggests that only the breakdown from visual purple to the intermediary « transient orange » is a photochemical reaction; the rest of the chain of reactions have temperature coefficients indicating ordinary endothermic chemical processes not necessarily requiring light.

HOSOYA (1933) discovered that a partially bleached solution of V.P. in darkness continues to lose colour. Such a « dark » reaction or « Nachbleichung » (HOSOYA) was also seen by DARTNALL (1936), by CHASE (1936) in confirmation of a personal commu-

nication by WALD, and by LYTHGOE (1937) who suggested that it may mark the breakdown of his transient orange to the stabler indicator yellow.

WALD (1937 a) studied the changes taking place in a neutral solution of V.P. that had been illuminated for 1/2 min. with a bright light (700 ft. candles). The solutions were left in complete darkness and their spectra periodically remeasured. Of the total fall in density 31 p.c. at 0.500 μ and 42 p.c. at 0.480 μ was found to be due to « dark » processes. WALD distinguishes a slow general fall, to be identified with the « Nachbleichung » of HOSOYA, from a second « dark » component rapidly obliterating the initial maximum (after bleaching) at 0.480 μ. The latter process goes with a simultaneous increase in density below 0.435 μ indicating formation of visual yellow. The final product, a yellow residue, has the indicator properties of the « indicator yellow » analyzed by LYTHGOE (1937). Tested with antimony trichloride a chloroform extract of the yellow pigment gives the absorption band at 0.664 μ, characterizing the « retinene » of WALD, (see below p. 21).

LYTHGOE's (1937) tentative scheme for the bleaching of visual purple by light and by moderately strong acids and alkalis is shown in fig. 5. « Transient orange » is the only intermediary

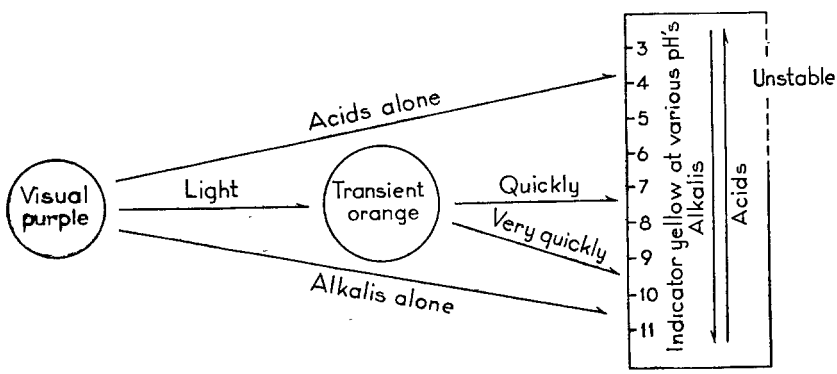

Fig. 5. — Lythgoe's tentative scheme for the bleaching of visual purple by light and by moderately strong acids and alkalis. LYTHGOE (1937), *J. Physiol.*, 89, 331.

substance in this scheme. According to the work of WALD, just referred to, other intermediary photoproducts may be formed.

To sum up: visual purple in solution is bleached by light in such a manner as to leave behind a transient orange-coloured photo-

product — and perhaps other intermediary photoproducts — which then without requiring light is thermally decomposed to indicator yellow or visual yellow. The further reactions in which these products of the decomposition of visual purple are engaged take place at rates which are greatly influenced by pH and temperature of the solutions. For details the original papers must be consulted.

Opinions still seem to differ as to whether visual yellow is sensitive to light. The work so far reviewed indicates little or no sensitivity to light, and WALD (1937 a) definitely states that his yellow pigment, retinene, « is the final product of bleaching in solution ». However, HOSOYA and SAITO (1935) find that solutions of V.P. exposed to bright sunlight become colourless (pH not given), and KRAUSE and SIDWELL (1938) report the same result with chloroform solutions of bovine « visual yellow pigment » (temperature ?). By following the absorption curve into ultraviolet they have reached the rising part of the absorption curve for visual white.

5. BLEACHING OF VISUAL PURPLE IN VIVO

There are at present no reasons for assuming the decomposition of V.P. in the retina to reach the yellow stage via routes other than those known from the work on the spectral properties of solutions. GARTEN (1907) and HOLM (1922) had found that strong stimuli are particularly apt to produce visual yellow. This substance is probably always formed in the course of bleaching of V.P., but only in bright light is the rate of breakdown of V.P. faster than the thermal breakdown of visual yellow. According to WALD (1935, 1936 a, b) the first effect of the light is to make the retina orange coloured. It can then be made yellow or colourless if treated with respectively strong acids or alkalis. The change is reversible but a colourless retina cannot be brought back to the original orange colour. The tissue therefore shows the indicator properties already mentioned. However, it is difficult to compare the results, obtained with solutions, with those of *in vivo*-experiments as the latter only are qualitative.

It has been known since the work of KÜHNE that visual yellow in the retina is decomposed to visual white. The process is thermal (GARTEN) but does not require light (WALD, 1936 a). Observations on pH and temperature have recently been reported by

WALD (1936 b) (1). His diagram showing the course of bleaching and regeneration of V.P. in the retina is reproduced in fig. 6. The reaction marked with a star does not occur in the isolated retina.

It is probable that also in the living eye there is an intermediate stage between visual purple and visual yellow. As it stands the diagram is very similar to that of fig. 1 in which I tried to

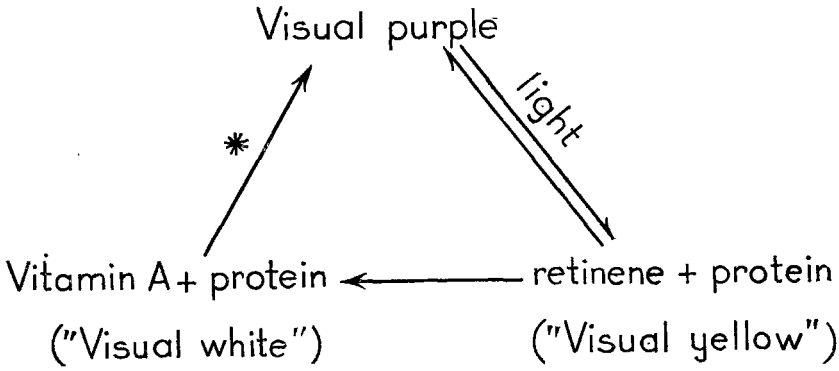

Fig. 6. — Diagrammatic representation of visual purple decomposition and regeneration according to WALD (1936), *J. Gen. Physiol.*, 20, 45.

summarize the KÜHNE-GARTEN concept of the same processes. WALD's chief contributions to these problems have been concerned with attempts to study the nature of the reacting substances and the transition between the stages reproduced in the figure. This work will be discussed in the next section.

6. THE DECOMPOSITION PRODUCTS OF VISUAL PURPLE

In a number of papers WALD (1934-1937) has followed the course of bleaching of V.P. with chemical methods and arrived at the general conclusion, summarized in fig. 6, that the processes concerned actually do form a cycle of the kind shown. The substances taking part in these reactions, inasmuch as they are known, are those indicated in the diagram of fig. 6.

Scotopic retinæ (frogs) contain visual purple and small amounts of vitamin A. Bleaching with light leads to formation of visual yellow. Extraction at this stage, of retinæ as well as of solutions, yields a substance with a characteristic absorption band in anti-

(1) A number of papers in Japanese have been published by AMENOMIYA (1930 a, b, 1931 a, b) and by KUNITA (1931) most of which are very incompletely reviewed in *Zentralbl. Ophth.* It is, to take an exemple, not even clear whether the latter used solutions or retinæ.

mony trichloride at 0.664 μ, called *retinene*, and held to be a new kind of carotenoid. If bleached solutions of V.P. are used, all the yellow colour may be extracted with benzine containing 1 p.c. ethanol. Tested in chloroform with antimony trichloride the yellow substance is found to be retinene. During the further decomposition of visual yellow to visual white (in retinæ) retinene is decomposed to another carotenoid, vitamin A. « The fading process (fish with same V.P. absorption spectrum as frog) converts retinene liberated in bleaching quantitatively to vitamin A (1936 b) ».

Chloroform which decolorizes the dark adapted retina also liberates retinene. Visual purple is held to be a carotenoid-protein with retinene as the prosthetic group. Any process which breaks this linkage, be it *e.g.* bleaching with light or chloroform, may discharge the colour of V.P. and allow the yellow colour due to retinene to appear. « The bright yellow, comparatively photostable material which is formed when the retina is treated with strong acids has long been employed as a test for visual purple. In reality it is a test for retinene or visual yellow. It is yielded by dark adapted and visual yellow retinas, but not by retinas which have been bleached and allowed to fade completely; that is, in which the retinene has been converted to vitamin A. (1936 b) ». The diagram of fig. 6 shows that according to WALD visual yellow is a combination retinene + protein. This may explain why WALD's visual yellow has the indicator properties of LYTHGOE's « indicator yellow » without having the solubility of the latter substance (cf. LYTHGOE, 1937). Fig. 7 from WALD (1936 a) shows the antimony trichloride reaction carried out with extracts of dark adapted and bleached retinæ.

KRAUSE (1937 b) and KRAUSE and SIDWELL (1938) have recently studied the decomposition of V.P. with chemical methods and arrive at conclusions which partly support and partly do not confirm those of WALD. Whereas the latter has worked chiefly with extracts from retinæ of amphibians and fishes in various stages of adaptation (a solution being used only in the preliminary report 1937 a) KRAUSE *et al.* have experimented with V.P. solutions obtained from large quantities of bovine retinæ. They conclude that V.P. is a lipoprotein composed of a protein conjugated with a coloured lipid with the properties of a carotenoid derivative. Exposed to light the V.P. splits into a protein and the coloured lipid, which separated from the rest gives a positive antimony trichloride reaction and seems to be identical with the

retinene of WALD. KRAUSE and SIDWELL (1938), however, do not seem prepared definitely to subscribe to the view that it actually is a carotenoid. But so far their work may be regarded as having confirmed the findings reported by WALD.

The disagreement, alluded to, refers to the change from visual yellow to visual white, which according to WALD was a quantitative conversion of retinene to vitamin A. This apparently does

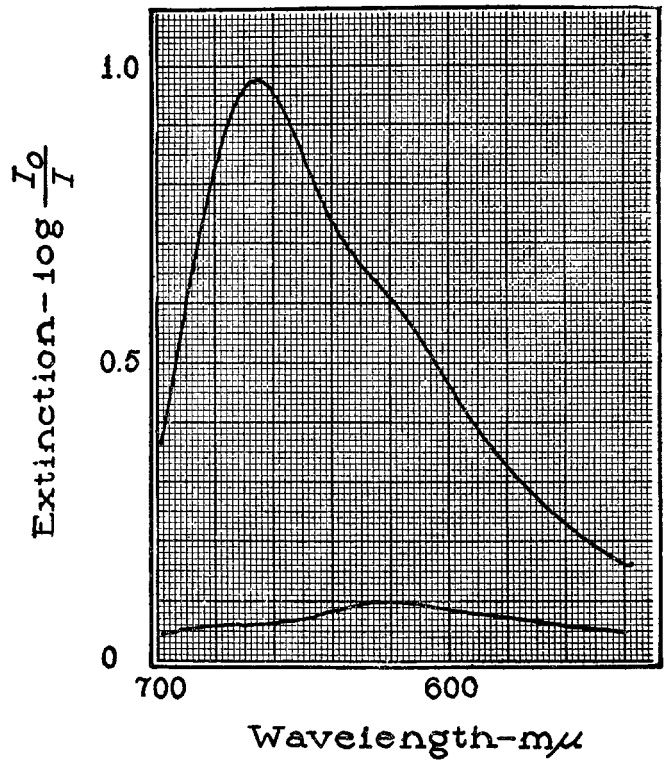

Fig. 7. — The liberation of retinene from visual purple by light. Spectra of antimony trichloride reactions with benzine extracts of dark adapted retinas (lower curve), and of the same retinas subsequently bleached to the visual yellow stage in bright light (upper curve). The lower curve was drawn 5 mμ too high in wave-length due to a fault in calibration wich was corrected before the upper curve was recorded. WALD (1936), *J. Gen. Physiol.*, 19, 781.

not take place in solutions of V.P. KRAUSE and SIDWELL (1938) present spectrophotometric measurements of bovine visual yellow bleached in solutions to visual white in such a manner as to cause disappearance of the absorption spectrum of the yellow indicator substance. The antimony trichloride reaction then is negative. Thus decomposition of visual purple in solution does not ultimately

lead to formation of vitamin A and it is difficult to understand how then the stages combined in WALD's diagram (fig. 6) could form a cycle in the chemical sense of the word. The rôle of vitamin A still seems elusive. Why is it found chiefly after bleaching to visual white (WALD) only when the retinæ themselves are extracted, not in solutions? There may be differences between eyes from different species. Thus, according to WALD (1934-1935) mammalian retinæ contain 22 γ, the frog's retina 400 γ, and the frog's pigmented layer almost 2 mg of vitamin A per gram of dry tissue. One could also point to the fact that the frog's pigmented layer, which is especially rich in vitamin A, tends to cling to the retina, thereby interfering with the measurements of the minute quantities concerned. However, the discrepancy will have to be solved experimentally.

In the meantime it is to be hoped that the successful attacks on the indicator yellow from different angles may be followed by attempts to explain the significance of the occurrence of transient orange in the frog's eye. There are great difficulties to be overcome, especially in proving that the appearance of one and the disappearance of another substance are quantitatively connected and not otherwise causally related or perhaps merely simultaneous. It cannot either be regarded as definitely proved that indicator yellow and retinene are identical substances.

Further contributions to the bleaching kinetics have been given by BRUNNER and KLEINAU (1936), STERN and SALOMON (1937), and WALD (1937 b). BRUNNER and KLEINAU find that the bleaching of V.P. in solutions follows a normal course in O_2-free air. STERN and SALOMON (1937) studying ovoverdin, held by them to be a pigment chemically related to V.P., calculate that the energy of the light in the effective wave-lengths does not suffice to disrupt the V.P. molecule with liberation of retinene on the idea that V.P. is a carotenoid-protein, a concept supported by HECHT, CHASE and SHLAER (1937) on the basis of attempts to determine its molecular weight from the diffusion coefficient. In a preliminary note WALD (1937 b) reports that the visual purple of certain fresh water fishes with maximal absorption around 0.540 μ (KÖTTGEN and ABELSDORFF, 1896; see also below p. 29) reproduces the behaviour of the V.P. system hitherto studied, but with quite different components (1).

(1) Those who want further information about the biochemistry of the eye are referred to the monograph by KRAUSE (1934) as well as to further papers by the same author (KRAUSE, 1936, a, b, c, d, 1937 a).

7. The regeneration of visual purple

Small quantities of V.P. are formed in bleached solutions left in darkness as shown by Kühne, and confirmed by Hecht, Chase, Shlaer and Haig (1936) and by Lythgoe (1937). It is not yet known whether this process requires some catalyzing agent from the pigment epithelium, as would be the modern interpretation of some results of Kühne (1).

Some selective effects of wave-length, preliminarily reported by Hosoya (1935) and Chase (1937), have been found in experiments concerned with both bleaching and regeneration of V.P. in solution. Hosoya followed the absorption during 80 minutes of bleaching with either ultraviolet light (0.365, 0.313 and 0.302 μ) or with the wave-lengths 0.577 and 0.546 μ (wave-lengths from the mercury spectrum). The ultraviolet light caused increased absorption and by combining wave-lengths 0.577 and 0.365 μ he obtained perfect compensation of the two opposite effects so that during 60 min. the density of the solution remained constant. The result clearly depends on formation of visual yellow. Chase used a yellow and a blue filter representing equal absorption in terms of the properties of visual purple. Regeneration was faster after bleaching with the short wave-lengths. A yellow-bleached sample decreased in density if afterwards bleached by blue light. Chase interprets his results as having to do with an accessory photosensitive substance in V.P. regeneration and mentions flavin as a possibility (for comparison with electrophysiological results, see below p. 33).

In the retina regeneration is a thermal process, and, when starting from visual white, does not proceed via visual yellow (Kühne, 1879). The latter process, Kühne's neogenesis, he held to be slower than anagenesis or regeneration from the yellow stage. Garten (1907) found anagenesis to proceed even faster than Kühne had thought. The rôle of the pigment epithelium was emphasized by Kühne who seems to have held it to be relatively more important in neogenesis than in anagenesis. Observations of this type have been made by Wald (1936 a) whose final conclusions are in general agreement with those of the older workers. However, he has found that visual purple is both regenerated and broken down thermally from the yellow stage by processes not requiring contact with the pigment epithelium. Both decom-

(1) This prediction has since been confirmed. See *Addendum to proof*.

position and regeneration may take place simultaneously. Observations on various factors favouring or inhibiting regeneration have also been reported by AMENOMIYA (1930 a, b). JONGBLOED and NOYONS (1936) found an increase of both O_2-uptake and CO_2-production in darkness indicating that V.P. regeneration requires oxidative processes (cf. also KÖGEL, 1929).

If the essential facts are combined to illustrate in a general way the most probable course of events in the retina during bleaching and regeneration, the diagram of fig. 8 is obtained. It is a tentative scheme and merely serves to summarize the views to which impartial examination of the experimental evidence has led the reviewer.

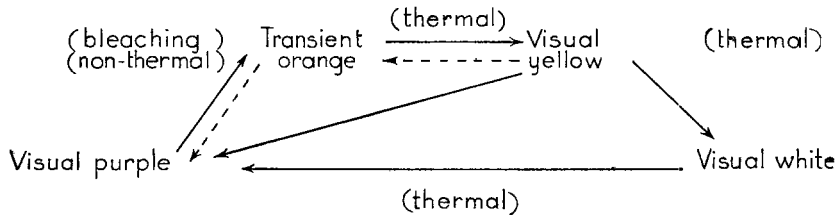

Fig. 8. — Chain of reactions of V.P. breakdown and regeneration in the retina according to present status of research. The term « visual yellow » has been used to cover both « retinene » (WALD) and « indicator yellow » (LYTHGOE) as the question as to whether these two substances are identical still must be held open. Broken lines illustrate alternative hypothetical path from visual yellow to visual purple.

8. THE TIME COURSE OF BLEACHING AND REGENERATION OF VISUAL PURPLE

The kinetics of bleaching of visual purple has been treated by HECHT (1921 a, b, 1924) in three papers in which a colorimetric method was used, the comparison scale being made up of mixtures of bleached and unbleached V.P. solutions. V.P. was found to bleach according to the equation for a monomolecular reaction and the velocity constant to be proportional to the intensity of the bleaching light. It is difficult to see what the first order equation means in terms of the complicated processes that have been found to accompany the bleaching of visual purple. CHASE (1936), discussing this question, presents spectrophotometric measurements of the course of bleaching and finds the isotherm of the monomolecular reaction to be obeyed with summer extractions but not with winter frogs. Acid and alkaline buffered solutions

behave like winter and summer solutions respectively. CHASE solves the issue by stating that the « real kinetics » of the disappearance of V.P. in solution is given by the summer and alkaline solutions. This is apparently because the final stage of bleaching then is definite and not complicated by the yellow colour, which again is due to his employing a spectrophotometric method and 0.500 μ as the wave-length of his measurements.

We have seen above that at low temperatures the « transient orange » phase between the initial V.P. and the indicator yellow becomes visible because the reactions proceed at a slower rate. This suggests that for the relatively slow spectrophotometric measurements relatively cold solutions would be more likely to show up the real kinetics of the complete bleaching process. On the other hand HECHT (1921 b) has found changes in temperature between 5.2 and 36.1° not to influence the rate of bleaching of V.P. in solution, concluding from his results that the bleaching reaction probably is a « simple photochemical transformation, consisting most probably of a single reaction (p. 289) ». LYTHGOE (1937) has pointed out that decomposition of the intermediary transient orange to visual yellow is greatly delayed by cooling the solution. WALD (see above p. 18) also has described intermediary photoproducts without yet reporting on their thermal properties. HECHT, when trying the effect of temperature, apparently did not see the intermediate photoproducts between V.P. and visual yellow. They do not either seem to have interfered with his colorimetric work which led to the conclusion, since confirmed by DARTNALL, GOODEVE and LYTHGOE (1938) (see, *Addendum to proof)*, that the rate of bleaching is independent of temperature.

Recently the course of bleaching of V.P. in solution has been followed quantitatively by DARTNALL, GOODEVE and LYTHGOE (1936) with a photoelectrical method. The authors set out by assuming (i) the V.P. solutions to obey BEER's and LAMBERT's laws (see above p. 14) and (ii) the quantum efficiency γ, a ratio defined as *number of chromophoric groups destroyed: number of quanta absorbed*, to be independent of concentration. On the basis of these assumptions equations were derived for the course of bleaching in an ideal case and for the case of solutions containing impurities serving as internal filters.

If I be the intensity in quanta per second, and I_t the intensity

transmitted at time t after absorption in the trough containing the V.P. solution, then, in an ideal case

$$\log_e \frac{I}{I - I_t}$$

plotted against t should give a straight line with a slope $\alpha \gamma I/A$ where A is the exposed area of the solution, the extinction coefficient α having been defined above (p. 14) and γ being the quantum efficiency. Actually, however, I_f, the intensity finally transmitted after complete bleaching, is not equal to I on account of the extra absorbing material present and formed during the course of bleaching. It was found necessary to determine experimentally the factor Φ with which to multiply $\alpha \gamma I/A$ in order to correct for the reduction in rate of decomposition of V.P. due to the internal filter effect of the impurities. In good solutions with $I_f = I$ the value of Φ should be 1. Quite commonly a value of 0.8 was found but with a great deal of foreign matter in the solutions values as low as 0.6 were obtained. When this was taken into account the final equation became

$$(3) \qquad \log_e \frac{I_t}{I_f - I_t} = \Phi . \frac{\alpha \gamma I}{A} . t + \text{const.}$$

The expression (3) reproduced the experimental determinations of the course of bleaching well enough to justify the assumptions made in deriving it. It is perhaps not necessary at this stage to enter into further details. It remains now to have the validity of this equation tested from the point of view of the factors that above have been found to influence the course of bleaching of V.P. in solution. DARTNALL, GOODEVE and LYTHGOE (1936) mention that $\alpha \gamma$ is independent of temperature and light intensity and point out that $\alpha \gamma I/A$ can be shown to appear as the « velocity constant » of a monomolecular reaction (1).

The only quantitative work on V.P. regeneration in the living eye is that of TANSLEY (1931). One of TANSLEY's curves was illustrated in fig. 2. She also tried to fit her data to the equation of a bimolecular reaction used by HECHT (1920) for describing the corresponding data obtained with the human eye. According to this equation

$$(4) \qquad k = \frac{1}{t} \cdot \frac{x}{a\,(a - x)} \cdot$$

where k is the velocity constant of regeneration, t the time in the dark (after bleaching), a the amount of V.P. after complete regeneration and x the amount of V.P. regenerated at time t. TANSLEY

(1) See *Addendum to proof.*

came to the conclusion that in the process of adjusting the two constants a and k to give the best fit a surprisingly great latitude of values for a were found that did not involve marked inconsistencies in the resulting velocity constant k. The difficulty is to secure experimentally a sufficiently accurate value for a, the amount of V.P. that represents final complete regeneration. Actually her results gave an even better fit with the equation for a monomolecular reaction. TANSLEY herself was not prepared to attach much significance to this fact as a contribution to the chemistry of the process of regeneration.

9. THE ABSORPTION CURVES OF DIFFERENT TYPES OF VISUAL PURPLE

It is well known from the work of KÖNIG (1903), published in 1894, KÖTTGEN and ABELSDORFF (1896) and TRENDELENBURG (1904) that the absorption spectrum of the visual purple *(rhodopsin)* of man and most laboratory animals is identical within the limits of experimental errors. Among the recent experimenters LYTHGOE (1937) has devoted particular care to determining the density of solutions of V.P. of frogs as a function of wavelength. His figures are given in Table 1. Earlier measurements

TABLE 1

Wave-length (mμ)	395	400	410	420	430	440
Visual purple absorption	0.001	0.016	0.103	0.196	0.306	0.416
Wave-length (mμ)	450	460	470	480	490	500
Visual purple absorption	0.530	0.661	0.780	0.878	0.953	0.997
Wave-length (mμ)	505	510	520	530	540	550
Visual purple absorption	0.993	0.975	0.899	0.780	0.622	0.460
Wave-length (mμ)	560	580	600	650		
Visual purple absorption	0.310	0.113	0.034	0.003		

have been summarized by KRAUSE (1934). I cannot here enter into the reasoning that has led LYTHGOE to correct his readings for the short wave-lengths. The difficulty of having to measure the absorption curve as a difference between the values of bleached and unbleached solutions can hardly be avoided. This introduces the whole problem of indicator yellow, requires control of the pH of the medium and assurance that other impurities have not been formed during the time the measurements have been carried out. There is the further difficulty of how to interpret a difference between bleached and unbleached solutions in a case where as a result of the bleaching new photoproducts have been formed.

Some of them may be photosensitive and thus add to the complexities. I shall return to these questions when comparing LYTHGOE's absorption curve with direct electrophysiological determinations of the absorption curve, as given by low-intensity retinal responses to an equal energy spectrum. As is well known TRENDELENBURG (1904) carried out a similar comparison with the human scotopic luminosity curve, and LYTHGOE's values have been used for this purpose by DARTNALL and GOODEVE (1937). They should be more accurate than any hitherto obtained.

The early work of KÖTTGEN and ABELSDORFF (1896) already had shown that apart from the substance isolated from the retinæ of *e.g.* frogs and rabbits with the absorption maximum around 0.500 μ (0.502 μ according to LYTHGOE, 1937), there is a different kind of V.P. giving maximal absorption around 0.540 μ. This question has recently been re-investigated by BAYLISS, LYTHGOE and TANSLEY (1936) with improved technique. A number of new forms of V.P. were found in a study confined to different species of sea fishes. Some examples of the absorption maxima found are: Mackerel *(Scomber scomber)* 0.505 μ; Plaice *(Pleuronectes platessa)* 0.520 μ; Pollack *(Gadus pollachius)* 0.530 μ; Tench *(Tinca vulgaris)* 0.537 μ; Trout *(Salmo fario)* 0.540 μ; Gurnard *(Trigla hirundo)* 0.545 μ. The absorption curves for the visual purple of three species is given in fig. 9. (For comparison with behaviour see GRUNDFEST, 1932 a, b).

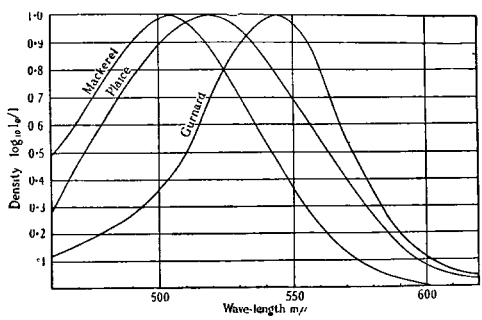

Fig. 9. — Wave-length absorption curves for the visual purple of three species. The actual readings are reduced to the same scale of ordinates. BAYLISS, LYTHGOE and TANSLEY (1936)., *Proc. Roy. Soc. B.*, 120, 95.

Thus visual *purple* is really a rather inadequate though for historical reasons still acceptable name for a number of pigments having the high light-sensitivity and general characteristics of

the purple substance rhodopsin to which hitherto we have confined our attention. WALD (1937 b) who has published a preliminary report of some work on the visual purple system in fresh-water fishes (see above p. 18) refers to the substance described by KÖTTGEN and ABELSDORFF (1896) as porphyropsin. It is not yet known whether the many substances with intermediate properties are mixtures of two forms only, as suggested by him and as also is quite probable (cf. the three curves of fig. 9). The possibility that these compounds may mediate cone vision is discussed in the brief review by LYTHGOE and GOODEVE (1937).

II. — CONE VISION

An old suggestion to explain cone vision in terms of a known and extractable substance was revived in 1921 by WEIGERT. He assumed photoreception in the cones to be mediated by highly diluted visual purple, basing this hypothesis on the supposition that a diluted solution of V.P. enclosed in a suitable optic medium might show photodichroism. This suggestion deserves to be mentioned because of attempts by WEIGERT (1929, 1930) and WEIGERT and NAKASHIMA (1929, 1930) to put it to a first test with the aid of « artificial retinæ » made by dissolving V.P. in gelatine. The films obtained by this method are almost colourless and possess dichrometric properties such as selective adaptability to sharply defined monochromatic bands, activation of antagonistic colours and after-effects. The latter indicate that the absorption spectrum of diluted V.P. in gelatine has shifted towards the long wave-lengths (0.530 μ). In order to be well marked the changes described require to be elicited by polarized light.

Considering the great number of papers that in the last five years have been devoted to the analysis of V.P., it is surprising that attempts to extract substances from the cones should have had such a small share of the general interest in photosensitive compounds. In 1932 v. STUDNITZ (1932, 1934) tried to measure directly the absorption of the isolated retina, put into the beam of the PULFRICH « Stufenphotometer ». During illumination absorption decreased, even in the cone retina of the European tortoise *(Testudo graeca)*. — Considering this result in the light of the work with V.P. we can hardly regard experiments of this general type as offering more than a hope of reward for those who are planning a fresh attack on cone photochemistry with the aid

of chemical methods of extraction. Recently v. STUDNITZ (1937) has made ether extracts of retinæ, preliminarily treated with hæmolytic substances (saponin, digitonin) rupturing the membranes of the receptor cells. If visual purple was present, as *e.g.* in the frog's eye, the layer containing V.P. was pipetted off and the rest extracted with ether. No V.P. was obtained from the retina of the tortoise. The ether extract of this eye contained a substance with an absorption maximum between 0.550-0.600 μ. The corresponding extract from the frog's eye, freed from V.P., had a second rise further out in the red. The oil droplets, likewise dissolved by this method (cf. as to their properties WALD and ZUSSMAN, 1937) gave a rise of absorption towards the short wave-lengths.

v. STUDNITZ (1937) holds the absorption maximum between 0.550 and 0.600 μ, which is due to a light sensitive and thus « adaptable » pigment, to represent his « Zapfensubstanz » of 1932. Its general properties and absorption curve are still inadequately analyzed, but this clue to cone vision certainly deserves to be followed up with improved spectroscopic technique.

In a preliminary communication WALD (1937 c) describes a photo-labile pigment, extracted from the chicken retina containing chiefly cones. This substance, however, normally enters the digitonin solution of visual purple from which it can be separated by bleaching with red light. This process selectively removes the new pigment, and the solution afterwards gives the difference spectrum of V.P. alone. The new pigment, by WALD named *iodopsin*, has its absorption maximum around 0.580 μ which is also the region of maximal sensitivity of the light-adapted chicken, according to an elaborate study by HONIGMANN (1921). Preliminary soaking of the retinæ in 4 p. c. alum makes digitonin extract V.P. alone.

Both v. STUDNITZ and WALD describe their photo-labile pigments as being relatively easily bleached. If either or both of these substances are to explain the properties of light-adapted eyes, there must then be found an extremely efficient mechanism of regeneration to explain the capacity of the cone retina to endure intense stimulation without excessive fatigue.

III. — LACTOFLAVIN

Lactoflavin is a yellow heterocyclic nitrogen containing compound, isolated in 1933 by ELLINGER and KOSCHARA (1933) and by KUHN, GYÖRGY and WAGNER-JAUREGG (1933), and synthetized

in 1935 by KUHN and his collaborators (1935) and by KARRER, SCHÖPP and BENZ (1935). For the literature concerning its chemical properties and vitamin B_2 activity the reader is referred to a monograph by VETTER (1936). I shall refer to the literature dealing with lactoflavin, only inasmuch as it suggests some relation to the physiology of adaptation in the retina.

Lactoflavin was first isolated from the retinæ of fishes by v. EULER and his collaborators (v. EULER and ADLER, 1933, 1934; ADLER and v. EULER, 1933; v. EULER, HELLSTRÖM and ADLER, 1935; KARRER, v. EULER and SCHÖPP, 1935), confirmed by BRUNNER and BARONI (1936), WALD (1936 a, b) and ZEWI (1937). (See also the review by v. EULER, 1937). It is very unevenly distributed over the types of eyes hitherto studied, even among fishes, which on the whole have eyes richly endowed with this substance. Thus, for instance, there is much lactoflavin in the pigment epithelium of perches and little in the corresponding tissue of pikes. This curious fact, despite attempts at correlation (rods or cones? habits of living?), is as yet unexplained.

Lactoflavin absorbs maximally around 0.445 μ in the visible spectrum (KUHN, GYÖRGY and WAGNER-JAUREGG, 1933) and shows an intense greenish fluorescence, maximal in neutral solutions (KUHN and MORUZZI, 1934) of low concentration (KARRER and FRITZSCHE, 1935). In combination with protein and phosphoric acid lactoflavin forms the yellow oxidation catalyst of WARBURG and CHRISTIAN (1932) and then loses its fluorescence. This property has been utilized by v. EULER, HELLSTRÖM and ADLER (1935) to demonstrate microscopically that the retinal lactoflavin is *free* and concentrated in the pigment epithelium, as also shown by them and others by direct extraction (cf. BRUNNER and BARONI, 1936; WALD, 1936 a, b; ZEWI, 1937.)

Dissolved lactoflavin is sensitive to light. A number of papers (see *e.g.* KUHN *et al.*, 1933-1934; KARRER *et al.*, 1934-1936; KOSCHARA, 1934; THEORELL, 1935; ZEWI, 1937) deal with the chemistry of the various photoproducts obtained under different conditions. From the point of view of retinal physiology the bleaching of lactoflavin in neutral or weakly acid solution in the presence of oxygen is the case that in the first instance may be presumed to be of interest. Apparently the decomposition of the lactoflavin molecule is then a complicated process leading over an intermediary photoproduct (THEORELL, 1935) to the formation of lumichrome (KARRER, SALOMON, SCHÖPP, SCHLITTLER and FRITZSCHE, 1934; KUHN and RUDY, 1934 a, b).

At this stage of our knowledge it is hardly necessary to devote more space to the properties of lactoflavin, so ably analyzed by the biochemists, as there is very little physiological information with which to compare it. Apart from some facts obtained with the electrophysiological method, the rôle of lactoflavin in vision is largely a matter of speculation. Thus v. EULER and his collaborators have suggested, among other possibilities, that its fluorescence may serve to extend the visible spectrum towards the violet. In favour of this suggestion is the fact that the concentration of lactoflavin in the retina would fall within the region of optimal fluorescence, as pointed out by KARRER and FRITZSCHE (1935).

At present one hesitates to ascribe to lactoflavin the task of mediating sensations from the blue end of the spectrum. It is so obviously confined to the pigment epithelium and it is still possible that the lactoflavin in the retina itself merely has leaked over from the store in the pigmented tissue during extraction. This possibility is emphasized by v. EULER, HELLSTRÖM and ADLER (1935) and by ZEWI (1937).

As pointed out above, diluted solutions of lactoflavin are relatively easily bleached. Yet in the retina it has been found impossible to cause any diminution of the concentration of lactoflavin, despite exposure of excised, opened eyes to excessively strong stimulation (ZEWI, 1937). Several explanations of this fact could be offered.

The possibility that the chief task of lactoflavin in the eye is to serve as an oxidation catalyst is suggested by some experiments carried out in this laboratory with the aid of the electrophysiological method to be described below. THERMAN (1937) found that the size of the retinal electrical response (its *b*-wave) sometimes was increased when a drop of lactoflavin was pipetted directly into the excised, opened bulb of the frog's eye and that this effect was better marked towards the rod end of the spectrum. In the autumn of 1937 THERMAN then tried to follow regeneration of visual purple in terms of the increase in size of the electrical response (see below p. 38) in eyes, preliminarily bleached, and treated with glucose alone (isotonic with Ringer solution) or glucose + lactoflavin. Both eyes of a frog were excised, opened and bleached, and then allowed to regenerate V.P. in the dark. One eye served as control eye, and was given glucose alone, the other one glucose + lactoflavin. The increase of the electrical retinal

response to illumination during regeneration was furthered by lactoflavin, but the effect was insignificant.

IV. — THE ELECTROPHYSIOLOGY OF ADAPTATION

1. Introduction

If complications arose already with regard to the relatively simple question of bleaching and regeneration in a single substance such as visual purple, how many obstacles will not then have to be mastered before we have succeeded in elucidating the adaptive changes in the living retina in which the photochemical reaction is merely the initial stage in the series of events leading to a discharge through the optic nerve. The electrical retinal response (electroretinogram) undergoes a number of changes with changes in state of adaptation. Some of them may have to do with processes referable to visual purple, others with the switching-over from elements containing visual purple (rods) to elements lacking this substance (cones) or with electrical changes due, for instance, to electrotonic potentials. The sensory units themselves may be adaptable in a manner that need not necessarily depend upon the final state of balance between processes of decomposition and regeneration in a photochemical substance.

The author suggests that an elementary subdivision of the chapter dealing with the electrophysiology of adaptation first should be made on the basis of (i) whether a process is clearly referable to the breakdown of a photochemical substance such as visual purple or (ii) whether it cannot be thus understood. The latter phenomena will then be collected under the general heading Electroadaptation. We shall se below that some phenomena very obviously have to do with general properties of nervous tissue and then it would seem to be unnecessary to force a photochemical explanation upon them. On the other hand it is probable that processes which at the present moment can be described only in terms of electrophysiology in the future may prove to be due to properties of the retinal pigment, rod or cone movements, or photochemistry. There is no need to be dogmatic on this point.

Those interested in various aspects of the electrophysiology of the retina are referred to the reviews by Kohlrausch (1931), Graham (1934), Parsons (1936), and Granit (1936).

A. - Some correlations with visual purple

1. *General description of the nature of the electrophysiological evidence.*

The retina reacts to illumination with an electrical potential change, illustrated in fig. 10 for a dark-adapted cat's eye stimulated

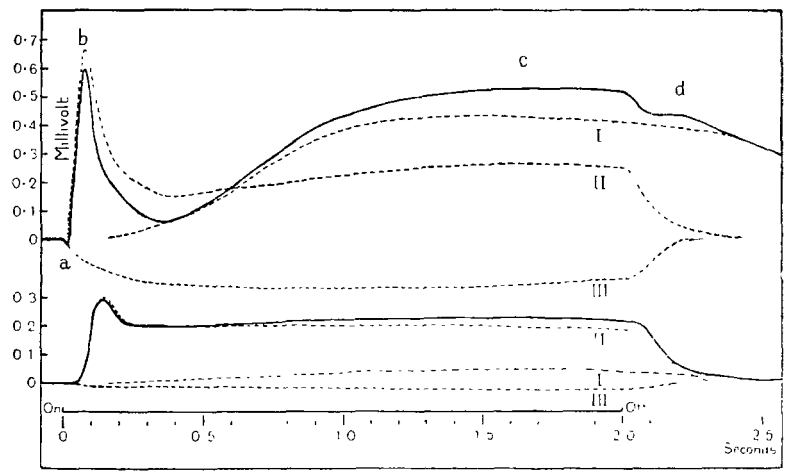

Fig. 10. — Analysis of composite retinal action potential at two intensities, 14 ml, and 0.14 ml, and area of 1661 sq. mm. viewed at a distance of 70 mm. Components: broken lines. Composite curve drawn in full. The *a*-wave is broadened slightly out of scale to show its derivation more clearly. Granit (1933), *J. Physiol.*, 77, 207.

at two intensities. We note that there are reasons for holding the complex response, drawn in full, to be made up of three components, labelled PI (Process I), PII and PIII respectively. PI and PII are positive, PIII is negative in the conventional manner of presentation. This analysis was suggested by the work of Piper (1911) and in particular by that of Granit (1933), the evidence for it being discussed in full in the latter paper. The rapid initial negative phase is called the *a*-wave, the fast rise the *b*-wave. The slow later rise is generally known as the secondary rise or *c*-wave, and the final plateau or small rise at cessation of illumination is the so-called off-effect or *d*-wave. This terminology was introduced by Einthoven and Jolly (1908).

In the frog's eye the off-effect is very much larger than in the cat's eye, as may be seen in fig. 19, but the same phases may be identified also in this eye, and, indeed, in all vertebrate retinæ.

This generalization is due to v. BRUECKE and GARTEN (1907).

It is also possible to record impulses from the optic nerve. Years ago this was tried with some success by KÜHNE and STEINER (1881), ISHIHARA (1906) and WESTERLUND (1912), but though deflections of the galvanometer were obtained upon illumination of the eye, the slow and relatively insensitive instruments then in use did not permit an analysis of the records. When then ADRIAN made his now well-known discovery of the general correlation between intensity of stimulation and the frequency of the discharge in nerves from sensory end-organs and neurones, he also together with R. MATTHEWS (1927 a, b, 1928) successfully tried recording from the optic nerve. Fig. 11 shows how the frequency of the

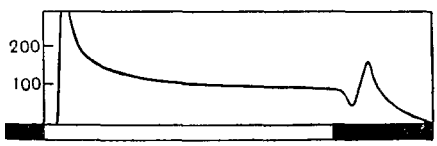

Fig. 11. — Ordinates: number of impulses per second. Abscissae: time of illumination (white). ADRIAN & MATTHEWS (1927), *J. Physiol.*, 63, 378.

discharge in the nerve, given as total number of impulses per second, depends upon illumination. There is a rapid initial outburst of impulses in the opticus, then some adaptation to the stimulus takes place, and, finally, there is a renewed discharge at « off ».

In 1933 GRANIT attempted to correlate processes in retina and nerve. He came to the conclusion, later developed and finally established in a series of three papers (CREED and GRANIT, 1933; GRANIT and RIDDELL, 1934; GRANIT and THERMAN, 1935) that the positive component PII accompanies the discharge through the nerve, that the latter disappears after selective removal of PII, and that PII roughly illustrates the integrated frequency of discharge curve, shown in fig. 11. The negative PIII was found to be somehow related to inhibition, definitely shown to exist in retina and nerve by GRANIT and THERMAN (1934, 1935). PI did not seem to be accompanied by clearly measurable changes in the optic nerve, but recent evidence obtained by THERMAN (1938) indicates that it coincides with changes of excitability to be discussed below.

At about the same time as this work was carried out HARTLINE and GRAHAM (1932) succeeded in isolating single fibres in the optic nerve of the horseshoe crab *(Limulus polyphemus)*. A record

of theirs is shown in fig. 12. The fibres were found to react with changes in frequency over a range of 1 million light units (meter candles). Finally HARTLINE (1935) isolated single fibres also in the optic nerve of the frog. We shall return below to this interesting contribution.

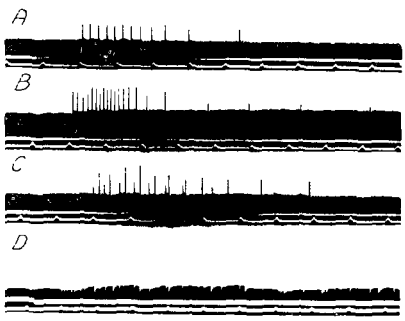

Fig. 12. — Action potentials from nerve strands containing several active fibers. A to C) From bundle containing two active fibers. A and B) Stimulation of respective end organs separately. Intensity, 0.1. C) Stimulation of both end organs simultaneously. Intensity, 0.03. D) Record showing discharge in three active fibers. Time in fifth of seconds. HARTLINE & GRAHAM (1932), *J. Cell. and Comp. Physiol.*, **1**, 277.

Very few of the papers dealing with the electrophysiology of the eye have been directly concerned with questions of adaptation. Most of the observations on adaptation have been made, as it were, in passing, and it is not easy to build up a coherent picture of retinal adaptation on the basis of the evidence hitherto obtained. Precisely for this reason it is an interesting task to attempt to review the material from this point of view in order to see where we stand and how to carry on most profitably. There should be a sufficient number of new discoveries and suggestions to make such an attempt interesting also to the non-specialist.

2. *Correlations with the phenomena of regeneration in the visual purple mechanism.*

Since HOLMGREN (1880) in 1865 discovered the electroretinogram (retinal current, retinal action potential) nearly everyone working in this field must have seen that in order to obtain large responses it was advisable to dark-adapt the experimental animal. But it is not until quite recently that anyone has tried to follow the time course of regeneration of visual purple by measuring size of *b*-wave as a function of time in the dark. The unknown

photosensory system of *Limulus* was studied from this point of view by HARTLINE (1930), but only three papers deal with regeneration in the visual purple mechanism (CHARPENTIER, 1936; RIGGS, 1937; WREDE, 1937). All of them consist of measurements of the rise in sensitivity after a period of adaptation to bright light.

Thus the type of visual purple experiment with which to correlate the electrophysiological results is the one carried out by TANSLEY (1931), showing dark adaptation as a rise in concentration (proportional to density) of V.P. after an initial period of illumination. Her results were given in fig. 2 of the section on photochemistry. The same experimental animal (albino rat) was used by CHARPENTIER (1936) who likewise had A-deficient as well as normal control rats. He measured the size of the *b*-wave of the electroretinogram as it rose during the time the rats remained in darkness after having been exposed for some hours to bright sunlight. We have already found the *b*-wave to be the retinal equivalent of the initial discharge in the optic nerve. It should therefore be a good index of excitability.

CHARPENTIER's most interesting result would seem to have been that after light adaptation there was no *b*-wave whatsoever to be had from the retina. As we shall see below, frog's eyes similarly treated react very well after exposure to sunlight, even when cornea and lens have been removed in order to provide maximal adaptation. The explanation of this difference must be the fact that the rat's eye is a practically pure rod-eye. The frog's retina has a very efficient cone system, the spectral properties of which will be discussed below. But in the albino rat bleaching of V.P. leads to a reduction in sensitivity that must render the photopic animal nearly blind. V.P. would therefore appear to be the real photochemical mediator of sensations arriving over the rods, and not only a secondary sensitizer of some less efficient photochemical system.

When the final point to which the *b*-wave rose during dark adaptation for 20 hours was given the value of 100, and when likewise TANSLEY's values for concentration of V.P. had been reduced to the same ordinates, her readings and those of CHARPENTIER fell on the same curve of recovery (see fig. 2). Similarly their readings for the A-deficient rats corresponded.

The significance of this correspondence must not be exaggerated. It has been shown by GRAHAM and RIGGS (1935) that in this animal size of *b*-wave is not proportional to concentration

of visual purple. But the precise nature of the relation between size of *b*-wave and concentration of V.P. would not assume significance except in the early rising phase of the curve of regeneration for which it is difficult to obtain accurate readings from living animals with electrical methods.

In this laboratory the work of CHARPENTIER has been continued by WREDE (1937 and personal communication) who has been using excised, opened frog's eyes. The animals were dark-adapted for a few hours, the eyes then removed, opened and exposed to daylight, avoiding direct sunshine, for 7-10 minutes. Immediately afterwards the eyes responded well to a strong stimulus but not at all to a weak one well above the rod threshold. The stimulus came from a monochromator, set at 0.500 or 0.450 μ.

However, after some minutes a small electroretinogram appeared. When for the initial period of dark adaptation size of *b*-wave was plotted against time in the dark, curves of the type shown in fig. 13 were obtained. The broken line shows the size of the off-effect for a three seconds exposure in a typical case.

There was often first to be seen an initial drift of base line in the galvanometer in the direction of the positive components of the electroretinogram. This altogether unexplained phenomenon rightly belongs to the section on electro-adaptation and cannot be discussed here. The facts to be noted are that the *b*-wave first rises slowly and then a great deal faster to high potentials. Individual eyes vary, and especially the fast phase has a very variable latent period. Sometimes it was found to appear after a delay of 75 minutes. If during the previous period of light adaptation the eye had been strongly stimulated, either because the adapting light had been too bright or the eye had been adapted for as long as 12 hours (overnight) and thus become extremely sensitive, the second phase often was found to be absent. Despite this the eye contained visual purple and reacted very well to stronger stimuli.

Facts like these do not quite fall in line with the generally accepted view, based on sensory work, that dark adaptation first proceeds rapidly and then slowly. Both slow and fast processes are found to be present. Quite different curves of regeneration are obtained with stronger stimuli, as also pointed out by RIGGS (1937), but then cone processes obviously must complicate the results still further. There is as yet no quantitative work on regeneration of V.P. in the frog's eye with which to compare these results. Some information on the significance of rod and cone movements for

the electroretinogram would also seem to be needed before we attempt to explain these results.

A comparison with V.P. regeneration also requires knowledge about the general relation between size of potential and energy

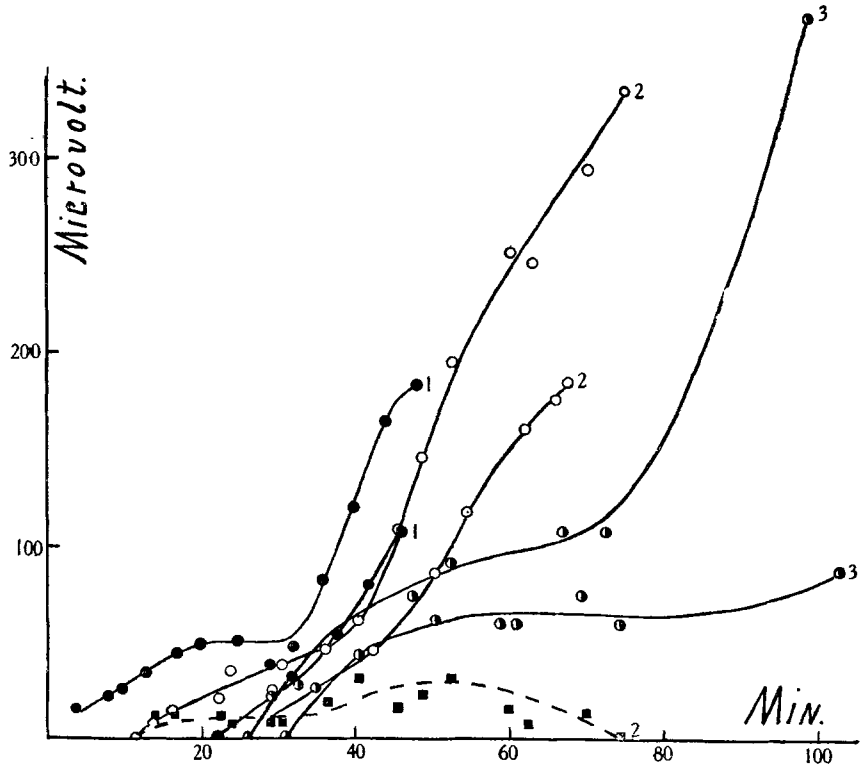

Fig. 13. — Plot of size of *b*-wave as a function of time in darkness after previous adaptation to daylight (avoiding direct sunshine). Excised, opened frog's eye. The dotted line shows size of off-effect.
All curves equally lettered are from the same eye, those reaching higher potential having been taken with monochromator set at 0.500 μ, the lower ones with monochromator set at 0.450 μ. WREDE (1937-1938). *Unpublished and partly published observations.*

of the stimulus, since energy absorbed would determine the response both within the absorption curve of V.P. as well as when at a given wave-length the intensity is increased. Knowing this relation from the latter case, we could calculate the rise in V.P. absorption during regeneration from the known size of the *b*-wave as a function of time in the dark. According to CHAFFEE, BOVIE and HAMPSON (1923) size of *b*-wave is proportional to the square root of the intensity, provided that low intensities within the rod region be used. Using this equation I have calculated from the curves

of fig. 13 the corresponding increases in the concentration of visual purple, assuming that the observed rise in the *b*-wave during dark adaptation actually is due to formation of V.P. The end point for all the curves has been given the value of 100 so as to make them directly comparable. The result of this computation is shown in fig. 14, which, accordingly, should illustrate V.P. regeneration in the cases analyzed.

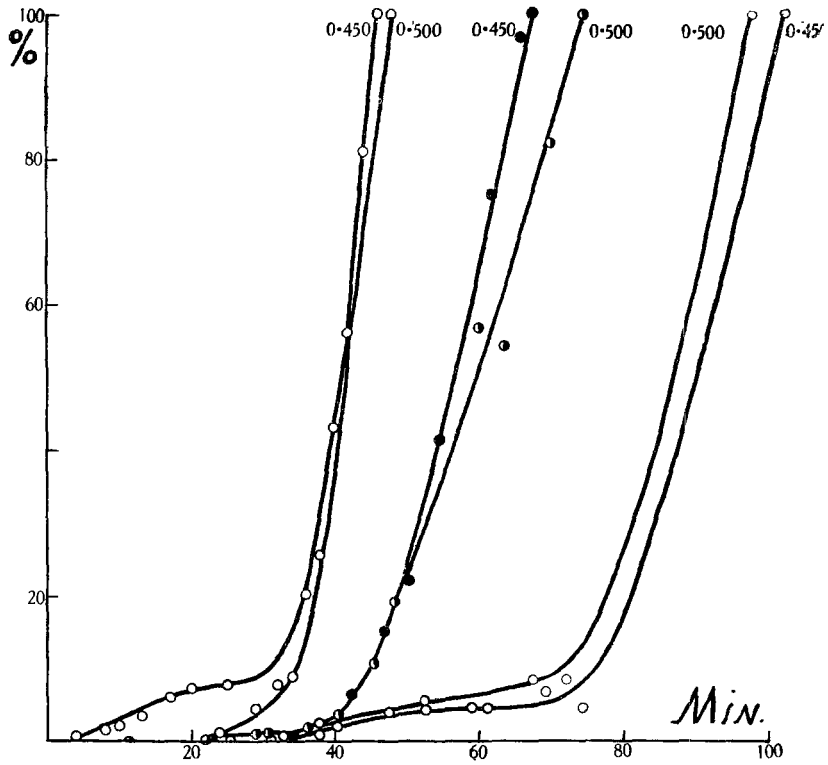

Fig. 14. — Same data as in fig. 13 squared, after which the end points of the individual curves have been given the value of 100 and the curves accordingly corrected.

The same end result may also be obtained by measuring the amount of energy necessary for a constant response, as is generally done in sensory work. HARTLINE (1930) has applied this principle to the eye of *Limulus* which apparently has a very uniform set of receptors (GRAHAM and HARTLINE, 1935) and in addition is a very constant preparation. In order to find the amount of energy necessary for a constant response the eye must be light adapted and re-adapted to darkness several times. During the increase in sensitivity, which takes place during regeneration, less and less

energy will be needed for the constant response. But precisely what amount is needed can only be found out by trying a number of stimuli of different strength for each point on the abscissa giving time in the dark. This is why repetition of the process of light- and dark-adaptation is necessary. This method could therefore only be applied to the intact animal, if frogs were used. The excised eye could hardly be expected to react with a constant response for a period exceeding 3-4 hours.

RIGGS (1937) has tried this method with the curarized frog and plotted a curve showing the decrease in energy necessary for a constant response for various times in the dark. His curve shows a relatively rapid initial fall of threshold and a later slower fall (see, fig. 15), and, as pointed out by himself, reminds one of the

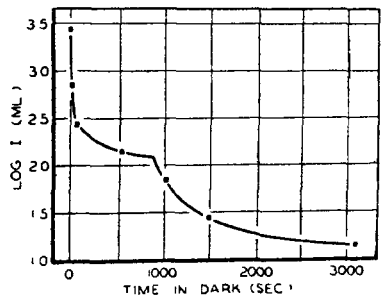

Fig. 15. — Plot of log brightness of flash required to elicit a constant height of 0.10 mV of response as a function of time in dark. Diameter of field 9°10'. RIGGS (1937), *J. Cell. Comp. Physiol.*, 9, 491.

well-known sensory curves for dark-adaptation in the human eye. There the threshold is used as the constant index of sensitivity, and evidence, which here need not be discussed, has shown fairly definitely that the first phase is due to cones, the second phase to rods. As in the early stage of dark-adaptation in the frog's eye visual purple still is lacking, the choice of a constant response above the threshold as an index of sensitivity means that this index necessarily is an index of cone sensitivity to begin with, and later, during continued dark-adaptation gradually begins to signify participation of the now activated rods.

RIGGS also finds dark adaptation to proceed at a faster rate after a shorter period of initial light adaptation, a fact noted several times also in this laboratory. There is a great deal to be done in this field both with photochemical and electrical methods before such facts can be taken as evidence for regeneration from visual yellow as against regeneration from visual white, when the pre-

ceding period of light adaptation has lasted longer. There is as yet no quantitative work on regeneration of V.P. in the frog's eye with photochemical methods, with which to compare the electrophysiological results.

The course of bleaching of V.P. in the frog's eye has not yet been analyzed with the aid of the electroretinogram.

3. *The absorption curve of visual purple and the distribution of sensitivity in the scotopic eye.*

The simplest way in which to analyze the spectral properties of the retina is to use an equal energy spectrum and measure size of *b*-wave as a function of wave-length. Assuming then that a low intensity be used and that V.P. is the only substance mediating low-intensity responses, we can expect size of potential to be distributed in the spectrum as the absorption curve of V.P. However, the two curves need not cover the same area because of the fact, pointed out above, that the relation between size of *b*-wave and energy absorbed is not one of direct proportionality. The great amount of energy absorbed at the top of the V.P. absorption curve causes a relatively smaller potential than, say, a tenth of this amount at the bottom of the curve would cause. A correction would have to be introduced, and, as in the previous section, this for the frog's eye would be provided by the data of CHAFFEE, BOVIE and HAMPSON (1923).

We may also measure « size of response » - « intensity » curves for a number of wave-lengths, choose all the points on our curves where irrespectively of wave-length the responses are equal and from the obtained energy values derive a « visibility curve ». This procedure is based on the principle that the greater the absorption at a given wave-length, the less the amount of energy needed for the constant response, and the greater accordingly the « visibility ».

Both methods have advantages as well as disadvantages. The former method enables a great number of readings to be taken at every wave-length wanted and thus is the method to be used when standards are wanted for an analysis of colour vision with the aid of the electroretinogram. Any variation in the experimental conditions can then be rapidly analyzed by taking a number of readings at a few wave-lengths and comparing the results with the standards. The second method has the advantage of giving energy directly, but the number of wave-lengths to be selected is limited by the circumstance that several intensities

have to be tested at each wave-length. Assuming, for instance, that an eye containing both rods and cones has to be kept light adapted; then a pure cone distribution of sensitivity can be obtained only for a relatively short time after light adaptation. Soon V.P. regeneration sets in and the experiment, that was concerned with the spectral properties of the cones, is spoilt by rod activity in the short wave-lengths. Thus repetition of the process of light adaptation becomes necessary, and it is quite possible that the final response-energy curves do not provide a more accurate basis of comparison with the properties of photochemical substances than those deduced from general equations correlating size of *b*-wave with energy, applied in the manner suggested by the first of the methods discussed. The ideal procedure obviously is to use both methods in parallell.

Both methods of determining the spectral properties of rods and cones have been used in the past. In the old work spectra of unknown energy distribution were used or, at least, the results not given in terms of the equal energy standard. However, reference to the standards then in use had given the following general results: (i) size of the *b*-waves elicited by relatively weak stimuli was distributed in the spectrum in such a manner as roughly to indicate that V.P. was the photochemical mediator of the response (HIMSTEDT and NAGEL, 1901; PIPER, 1904, 1905; BROSSA and KOHLRAUSCH, 1913), (ii) the maximum of sensitivity shifted towards the red end after bleaching of the eye with a bright light (HIMSTEDT and NAGEL, 1901; PIPER, 1904, 1905; BROSSA and KOHLRAUSCH, 1913). This particularly interesting fact, to be theoretically useful, would have required knowledge of the equal energy spectrum.

In 1924 CHAFFEE and HAMPSON studied the properties of the dark adapted frog's eye with a spectrum of known energy distribution, however, without trying to isolate cones from rods with the aid of light adaptation. But using the second of the two methods, discussed above, they calculated what may be called the electrophysiologically determined absorption curve and compared it with the human scotopic luminosity curve, as measured by HECHT and WILLIAMS (1922). Owing to the fact that the method of equal responses was used, the number of points determining the curve necessarily had to be limited. The maximum fell at 0.516 μ and the curve was somewhat wider than the human scotopic luminosity curve.

GRAHAM and RIGGS (1935) likewise used the method of equal

responses, their preparation being the rat. They found a distribution of sensitivity that was in fairly good agreement with the V.P. absorption curve, determined by KÖTTGEN and ABELSDORFF (1896). The wave-lengths were limited to 0.640, 0.610, 0.575, 0.530, 0.490, and 0.440 μ. Now V.P. absorption at 0.575 μ already is as low as to be roughly at 20 p. c. of the maximum so that by far the most important region of V.P. absorption is represented by three points on the curve, 0.530,0.490, and 0.440 μ. Considering in addition that the determination of V.P. absorption curves is beset of difficulties, too much reliance should not be placed on the precise correspondence of the two sets of data, as would also seem to be realized by the authors themselves.

As to number of measurements and points in the spectrum the data of GRANIT and MUNSTERHJELM (1937) and GRANIT and WREDE (1937) should be fairly complete. Also the two states of adaptation were separated. However, they only used the simple method of measuring size and rate of rise of *b*-wave and off-effect for an equal energy spectrum. Their aim was to obtain standard curves for a large experimental material and for easily repeatable conditions, enabling a further analysis of colour vision as a retinal property with the aid of modifications of these conditions. GRANIT *et al.* in this work used the frog's eye (i) dark adapted and stimulated at a low level of intensity, (ii) light adapted and stimulated at a high level of intensity. The two curves for distribution of size of *b*-wave in the spectrum in the two states of adaptation are shown in fig. 16. The lability of the curves measured in the scotopic state necessitated separate averaging of readings for eyes giving respectively broad and narrow distribution curves. Hence the double contour of the low-intensity scotopic curve. The photopic responses were maximal around 0.560 μ. The curve would have been symmetrical, were it not for the fact that readings beyond 0.490 μ in the blue and violet had been relatively too large. Later we have found the unsymmetrical « appendix » of the cone curve to be curiously labile.

The results of fig. 16 obviously mean that bleaching of the V.P. of the frog's eye enables another high-threshold element with a different distribution of sensitivity to come to the fore. By definition these elements are cones. (Cf. the different behaviour of the white rat, mentioned above, CHARPENTIER, 1936). The frog's eye therefore shows a PURKINJE effect (cf. HIMSTEDT and NAGEL, 1901).

According to CHAFFEE, BOVIE and HAMPSON (1923) the low-

intensity response of the photopic eye is proportional to the square root of the intensity. Using this equation GRANIT (1937) has corrected the common averages of the 801 low-intensity responses recorded by GRANIT and MUNSTERHJELM (1937) with the scotopic

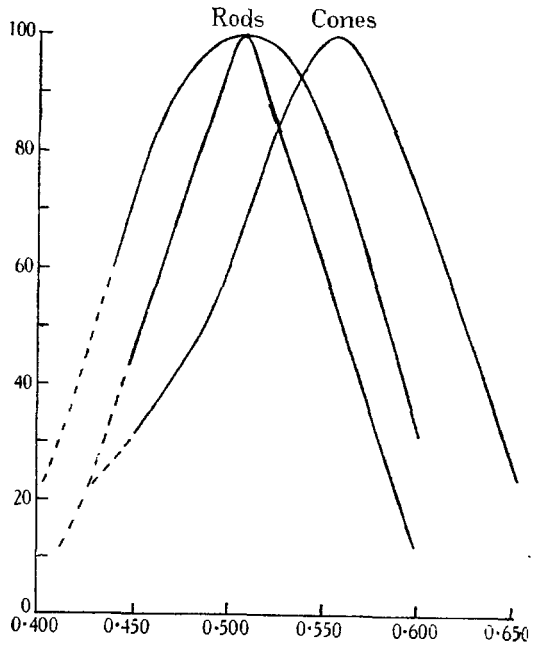

Fig. 16. — Rod and cone curves for size of *b*-wave against wave-length. Rod curve with double contour and maximum at 0.507 μ from GRANIT and MUNSTERHJELM (1937). Cone curve has maximum at 0.556 μ. Broken lines in region where energy of spectrum uncorrected and stimuli thus relatively too weak. GRANIT & WREDE (1937), *J. Physiol.*, 89, 239.

eye. Finally, following a suggestion by DARTNALL and GOODEVE (1937), he has given the « physiological absorption curve », thus obtained, in terms of a spectrum of equal quantum intensity rather than in terms of equal energy. The final result is the curve shown in fig. 17 (dots). The circles around it refer to LYTHGOE's (1937) absorption curve for V.P. in solution (Table 1). The hump around 0.550-0.560 μ probably indicates that some low threshold cones (cf. maximum of cone curve in fig. 16) have participated in the reaction. However, the main result is obviously that there is reasonably good agreement in the long wave-lengths but that LYTHGOE's « corrected » absorption curve for visual purple, used for comparison, is somewhat higher in the short wave-lengths. It remains for further work to explain the reason for this discrepancy.

In this connexion it is only natural to enquire into the significance of the intermediary photoproducts formed during bleaching of V.P. Unfortunately there is as yet no definite answer to what may be held as being the central question : are they merely to be

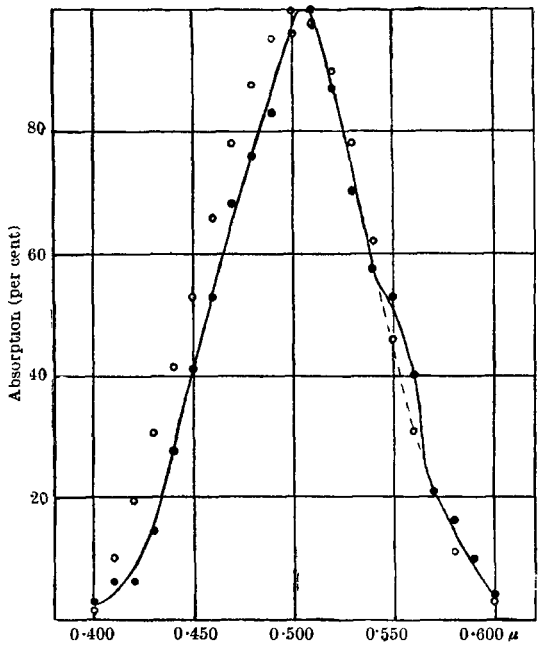

Fig. 17. — Dots : ab orption curve of visual purple, computed from the electrophysiological data of GRANIT und MUNSTERHJELM (1937). Circles : absorption curve of visual purple determined by LYTHGOE (1937), his table 1, column 2. GRANIT (1937), *Nature*, 140, 972.

regarded as internal filters reducing the sensitivity of the eye in the spectral region where they absorb or does this absorption initiate reactions ending in a discharge of impulses through the optic nerve?

LYTHGOE (1937) seems to have counted chiefly with the first possibility and visual yellow may, indeed, be merely an internal filter. More doubtful is the position of « transient orange » (LYTHGOE) and of other possible intermediary stages between V.P. and visual yellow (WALD). Definite evidence in favour of either alternative can best be obtained with the aid of the electroretinogram. So far our experiments in this laboratory have not given conclusive results beyond showing fairly definitely that it is difficult to understand the scotopic curves on the basis of a single absorbent with the spectral properties of V.P. It is possible that the lability

of the low intensity curves, shown in fig. 16, is satisfactorily explained by variations in concentration of V.P., variable amounts of intermediary photoproducts, and participation of cone activity to various degrees. But at higher intensities the scotopic curves expand inordinately also towards the violet (CHAFFEE and HAMPSON, 1924; SMIT, 1934; GRANIT and MUNSTERHJELM, 1937) and may give humps in the region of 0.450-0.480 μ (GRANIT and MUNSTERHJELM, 1937). There is also the above mentioned « appendix » of the cone curve (GRANIT and WREDE, 1937) in the same region to be considered. For a discussion of these intricate questions the reader is referred to the original papers.

B. - ELECTRO-ADAPTATION

1. *Elements of different adaptability.*

The positive phases of the retinal response of the frog's eye present a smooth contour only when recorded with a non-specific corneal lead, and even then, only if the stimulus is a good deal above threshold strength. Using localized electrodes on the retina CHAFFEE, BOVIE and HAMPSON (1923) saw the initial *b*-wave split up into a number of different *b*-maxima (cf. also RIGGS, 1937), and GRANIT and MUNSTERHJELM (1937) obtained the same result at low intensities when leading off from the unopened bulb. A number of authors (cf. GOTCH, 1903; EINTHOVEN and JOLLY, 1908; SMIT, 1934; GRANIT and RIDDELL, 1934; JOLLY, 1936) have noted small humps on the *b*-wave, in most cases probably to be explained as synchronous firing of the retinal units (GRANIT and THERMAN, 1935). From these cases must be distinguished the typical polyphasic low-intensity response, a specimen of which is shown in fig. 18 (curve A). Slow and fast phases of the off-effect (fig. 18, curves D) have likewise been observed (SMIT, 1934; GRANIT and WREDE, 1937). It was noted by GRANIT and MUNSTERHJELM that the different *b*-maxima belonged to independent component *b*-waves. Thus a series of responses to a constant stimulus showed a curious switching-over from one type of response to another. Records showing a typical « switchboard effect » are likewise given in fig. 18 (B and C). This peculiar, though at higher intensities not very common lability of the response as well as the fact that the component *b*-waves may be selectively sensitive to repetition of the stimulus at relatively short intervals, proved that the corresponding elements are to some extent independent.

A satisfactory explanation of the *b*-components was found in the earlier demonstration by HARTLINE (1935) that the discharge from single fibres of the frog's optic nerve did not show the uni-

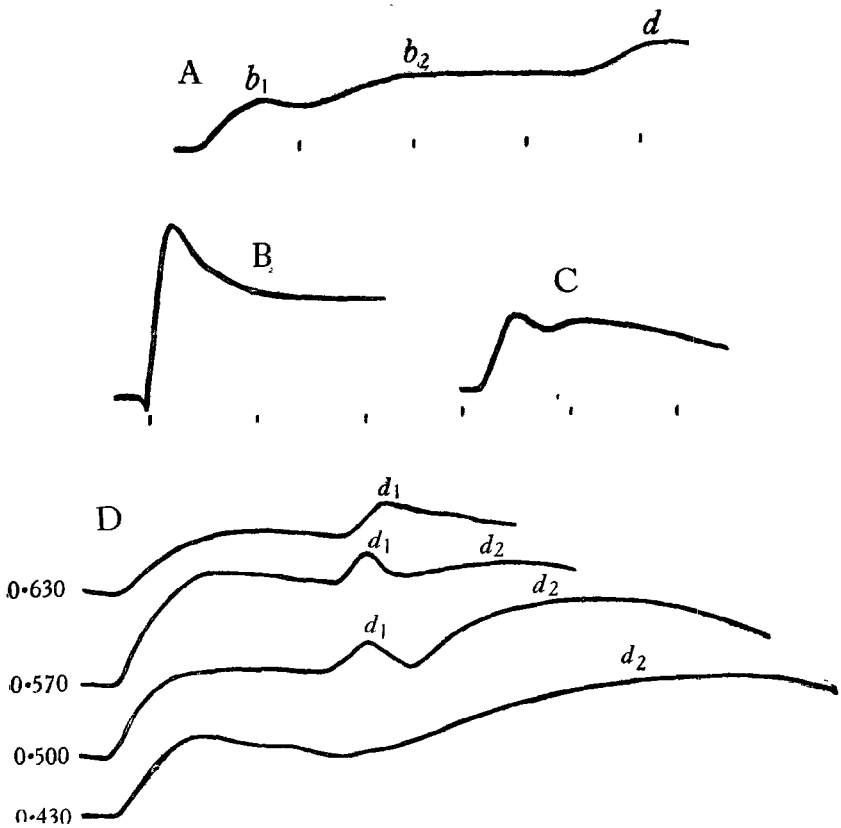

Fig. 18. — *A* : Typical diphasic low intensity response showing first *b*-wave (b), second *b*-wave (b_2) and off-effect (d). — *B* and *C* are two responses at 0.510 μ obtained in the same experiment with an interval of a few minutes between them; so-called « switchboard effect ». Experiments with frog's eye. GRANIT & MUNSTERHJELM (1937), *J. Physiol.*, 88, 436. D : Series of four responses to 1 sec. flashes, illustrating diphasic off-effects. *Note* : Only first phase of off-effect at 0.630 μ (uppermost curve), second slow phase already a great deal larger at 0.500 μ; only second phase visible at 0.430 μ, illustrating that the slow second phase belongs to rod spectrum. Time below 1 sec. Experiment with frog's eye. GRANIT & WREDE (1937), *J. Physiol.*, 89, 239.

formity of behaviour characterizing the ommatidia and nerve fibres of *Limulus* studied by HARTLINE and GRAHAM (1932). Some fibres were found to give a rapid discharge at the onset of stimulation, adapting themselves then to a constant slow rate of discharge. Other fibres reacted *only* at the onset of the light,

and then became silent, to discharge once more upon interruption of stimulation. There were also fibres that slowly built up their discharge to a certain level during continued stimulation. These fibres likewise contributed to the off-effect. Finally one group of fibres merely reacted with a brisk discharge of impulses at cessation of illumination. In these fibres the impulses often were found to be synchronized to periods of rest and activity.

It would seem to be futile to attempt to explain these differences of adaptability, so clearly illustrated by HARTLINE's interesting observations, with the aid of photochemical concepts. Behind these properties of the different fibres must be retinal processes intimately connected with the structure and organization of the neural elements themselves.

Considering that changes in the positive component PII, responsible for the *b*-wave, are reflected by corresponding changes in the optic nerve (GRANIT and THERMAN, 1935), the results of HARTLINE and those reported above, referring to the multiple nature of the *b*-wave, are in excellent agreement. The unitary analysis has shown with greater clarity than measurements of the whole response could have done that the positive PII is a sum total of component potentials with different adaptability and different time constants in general. « It is, of course, not necessary to represent the functional mosaic of different types of nerve responses or electrical component potentials as a pattern of stable units with different properties (among them adaptability). The secret of the problem may lie in the (retinal) switchboard effect being itself capable of providing different types of responses by suitably coupling units differing very little from one another » (GRANIT and MUNSTERHJELM, 1937). However, from the point of view of adaptation the net result of whatever mechanism be conceived must be the same.

2. *General change of the electroretinogram during adaptation.*

In general one could say that whatever happens in the dark adapted eye, be it then an effect at « on » or at « off », it will always be a far more drawn-out affair than in the light adapted state. Likewise there is in the dark adapted eye the slow secondary rise or *c*-wave which greatly diminishes after light adaptation, as has been noted by several observers after v. BRUECKE and GARTEN (1907) who first studied the effects of state of adaptation on the

electrical retinal response. These observers also found that the off-effect increases after light adaptation.

Reference to fig. 19 makes this clear. The frog's eye and a strong stimulus of about 1.800 Lux has been used. In the first

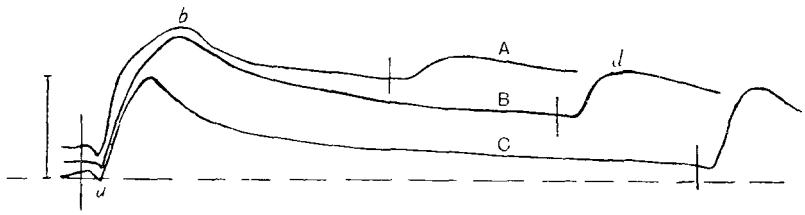

Fig. 19. — The short vertical lines show the beginning and end of the stimuli: 1 sec. in A; 1.5 sec. in B; 2 sec. in C. Explanation in text. In this figure the vertical line to the left shows calibration to 0.673 mV. GRANIT & RIDDELL (1934), *J. Physiol.*. 81, 1.

record, A, the off-effect is small, but it has only been necessary to increase the duration of the stimulus from 1.0 to 1.5 seconds in order to get the larger off-effect shown in curve B. Finally the eye has been light adapted for some time to the stimulus. As soon as the off-effect after the period of light adaptation has passed away, the eye is re-illuminated with the same light. This gives record C. The off-effect is now very much larger and its rate of rise faster than in the other electroretinograms. The intermediate positivity between *b*-wave and off-effect, which is so marked a feature of records A and B, is about to disappear in record C. Quite often the electroretinogram elicited by a bright light in a light adapted eye chiefly consists of *b*-wave and off-effect. In accordance with these facts electroretinograms of the type A and B are accompanied by a heavy nervous discharge between the initial and the end phase, whereas in the well light adapted eye with the low level of PII during stimulation *b*-wave and off-effect are the outstanding features of the record from the optic nerve (GRANIT and THERMAN, 1935).

The curves A and B from the dark adapted eye are taken at a relatively fast rate after short exposures and thus do not show the slow secondary rise which in the frog's eye has a latent period of 6-8 seconds. We shall return to the question of adaptation and *c*-wave below. Here remains to be mentioned that in the eye of the pigeon, containing a great number of cones, the *c*-wave does not seem to diminish after light adaptation. KOHLRAUSCH (1918) even finds it to be absent in the dark adapted state, but

this was not confirmed by GRANIT (1935) who only found the secondary rise slightly enhanced after light adaptation. GRAHAM, KEMP and RIGGS (1935) working with the pigeon's eye from the point of view of the spectral distribution of sensitivity in relation to a possible specific effect of wave-length do not report any observations bearing on this question.

The off-effect in the cat's eye, though hardly more than a retardation in the drop of potential towards the base line at cessation of illumination, is likewise enhanced by an increased duration of the stimulus. The eyes of rabbits and rats also belong to this type (E-retina). But it should be noted that despite the smallness of the off-effect in the retina, there is a brisk off-discharge of long duration in the optic nerve, as found by the author (1933) with the cat's opticus.

3. *Adaptation and the b-wave.*

It was shown by PIPER (1911) and confirmed by CREED and GRANIT (1933) that the first *b*-wave elicited by a series of flashes is very much larger than the following ones. There is, as it were, an initial explosion leaving a long « refractory period » in its wake. Now, if two lights be used, the one serving as the « adapting light », the other one as « test light » (being of the nature of a short flash), some interesting changes may be observed (WREDE, 1937). Let us assume that the eye (frog) to begin with is dark adapted and that adapting light and test light are of the same strength. The two stimuli are well above the cone threshold. The test light pitted against the background of excitation kept up by the adapting light is to begin with ineffective. It lasts some minutes before it is capable of eliciting a *b*-wave of its own. But when finally the test light succeeds in causing a small and fast *b*-wave, this means that some kind of an adaptive change has taken place. It can be shown that this change has made the eye faster. To this end it is only necessary to turn out the adapting light for a moment and then repeat the experiment with test light following adapting light. In the latter case the test light elicits a fast and transient *b*-wave as soon as the *b*-wave of the adapting light is over.

Thus, whereas the scotopic retina was sluggish and incapable of discriminating between test light and the general level of excitation set up by the adapting light, some light adaptation sufficed to enable the eye to record the added amount of energy as a separate *b*-wave, signifying a fresh outburst of impulses through the

optic nerve. Obviously some visual purple has been bleached during adaptation, but what else can have happened? Have new elements entered the scene of activity or have the properties of the already activated elements changed from fast to slow? We shall meet the same question again in connexion with the negative component PIII, « flicker », and the off-effect. Whatever suggestion is held to be most reasonable in one case will probably fit the other cases too, and therefore the discussion of this problem will be postponed until we have become acquainted with all its aspects.

4. *The a-wave and the negative component PIII.*

As shown in the analysis of the cat's retinal response in fig. 10, the *a*-wave is the initial phase of the negative component PIII. THERMAN (1937) has recently found that very active isolated negative components can be obtained by pipetting a drop of an 0.5 p. c. solution of potassium chloride into the opened excised eye of the frog. Some negative responses for different times of exposure to the full light of a monochromator set at 0.560 μ (the apparatus described by GRANIT and MUNSTERHJELM, 1937) are reproduced in fig. 20. The practically pure PIII first drops rapidly, then more slowly towards an almost stationary negative value. On cessation of illumination there is an off-effect on the negative side of the base line of the oscillograph (cathode ray). Like off-effects in normal responses it increases when longer exposures are used. The rapid off-effect proper is followed by a slow return towards the base line. The effect of a flash on the off-effect, a so-called « negative notch », is shown in each record. In dark-adapted negatively reacting eyes the fast phase of the off-effect is absent (GRANIT and THERMAN, 1938).

It is rather difficult to state definitely whether K-ions act by merely removing positive PII or whether in addition they actively stimulate the negative PIII, as at first sight the results would indicate. It is therefore of some interest to study another case (GRANIT and RIDDELL, 1934) in which a largely negative response was obtained by the simple expedient of rubbing the excised bulb between the finger tips. This procedure was first advocated by WALLER (1909), and is one example among many all of which go to show that PIII is the most resistant part of the retinal reaction to light. The records of fig. 21 have been obtained after massage. PII is only represented by the *b*-wave. A is a relati-

vely light adapted eye, it having been illuminated for some time with the stimulating light the strength of which was 1.800 Lux. Record B is the response to the same stimulus, the eye in the meantime having been left in the dark box for 5 minutes to recover. This has led to a diminution of the negative phase and the off-effect, the latter in addition being very much slower than in record A. Finally the eye was adapted to the stimulus for 2 minu-

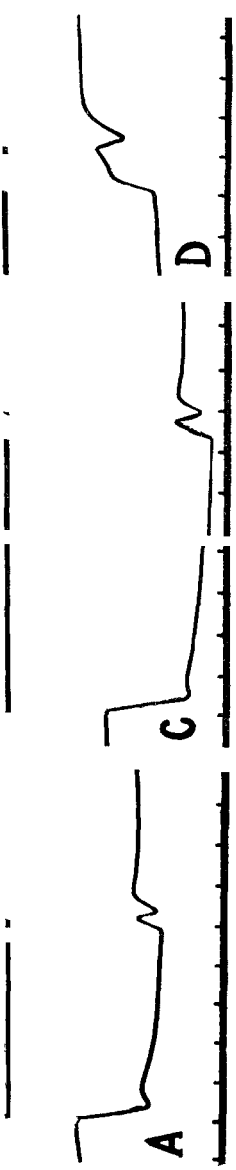

Fig. 20. — Original photograph (cathode ray oscillograph) of electrical responses of frog's retina after treatment with potassium. *A*: exposure of about 4.8 sec.; *B*: 7.2 sec.; *C*: about 12 sec.; *D*: off-effect alone after exposure of 60 sec.
Rate of recovery followed in B, in which two intervals of 5 sec. each have been cut away from the film. From film C likewise part of the electrical response cut out.
Time in seconds below. Upper line is photograph of stimulus.
Note. In record A small positive deflection clearly visible. Off-effect increases with length of exposure. In each case a flash on top of it causes a large negative deflection (« negative notch ») which does not reach the maximal level of negativity of PIII. Early in the same experiment it did reach it. GRANIT & THERMAN (1938). *Unpublished observations.*

tes. As soon as the off-effect of the period of adaptation had died away, the eye was exposed again to the same stimulus. This gave record C in which off-effect and negative phase again have

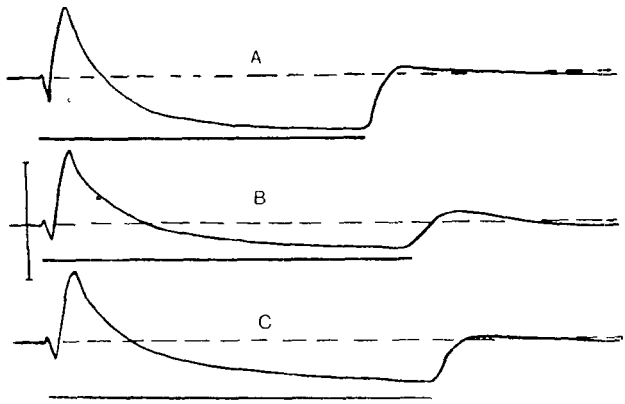

Fig. 21. — Unbroken horizontal lines show duration of the stimuli: 1.8 sec. in A; 2 sec. in B; 2.1 sec in C. Broken horizontal lines show respective base lines of the galvanometer. Explanation in text. GRANIT & RIDDELL (1934). *J. Physiol.*, 81, 1.

increased. The off-effect likewise rises at a faster rate than in curve B. Throughout the experiment the *b*-wave has remained practically constant.

Somehow light adaptation of a few minutes duration has succeeded in changing the type of reaction of the eye. It seems to have activated a fast and large off-effect and favoured the negative PIII. As in the previous section we must ask whether new elements have been brought in or whether those already operating have changed their properties.

In addition the results show that PIII actually may increase during the exposure to light. In this respect it differs from PII and from the curve for frequency of discharge against time of exposure, shown in fig. 11. It can hardly be doubted that here is a process of some significance for the performance of the eye, and that, in particular, the properties of PIII are of interest for an understanding of the phenomena of electro-adaptation. Such evidence as we possess (GRANIT, 1933; GRANIT and RIDDELL, 1934; GRANIT and THERMAN, 1935) indicates that PIII marks the development of an inhibitory process in the retina. If our conclusions are correct light adaptation would involve a re-distribution of the balance between excitation and inhibition represented by respectively PII and PIII.

5. The evidence for inhibition and the off-effect.

It is impossible to continue our discussion of the changes that take place during light adaptation without presenting and considering the evidence for inhibition in the retina. In 1933 (GRANIT, 1933), when working on the analysis of the retinal electrical response, I drew attention to the fact that the off-effect was strikingly similar to « reflex rebound » in the central nervous system (SHERRINGTON, 1905, 1906; GRAHAM-BROWN, 1911; CREED, DENNY-BROWN, ECCLES, LIDDELL and SHERRINGTON, 1932). This phenomenon may be described as the appearance of an extra volley of impulses after cessation of stimulation of a mixed afferent nerve. This leads to an additional contraction, a « rebound » of the reflexly active muscle at a moment when normally its activity should die away. The rebound contractions are held to be due to a release from an inhibition covered by excitatory effects on the muscle during stimulation.

As the retinal off-effect was found even in responses consisting of a pure negative PIII (cf. fig. 20), it was suggested that PIII was associated with the development of an inhibitory process and that the return of PIII towards the base line was the release mechanism giving rise to the off-effect. The next step in this line of work

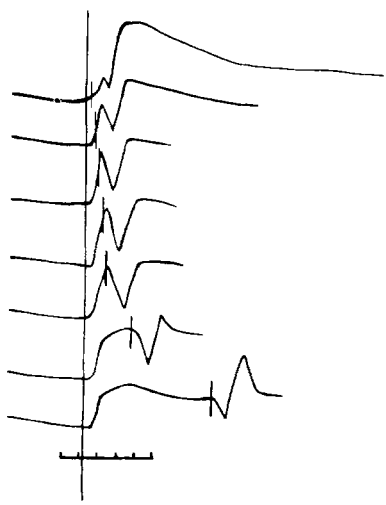

Fig. 22. — Vertical line through all curves marks cessation of illumination. Small vertical line on individual off-effects shows the onset of a flash of 0.040 sec. Explanation in text. GRANIT & RIDDELL (1934), *J. Physiol.*, 81, 1.

was taken by GRANIT and RIDDELL (1934) who systematically analyzed the large negative *a*-waves seen occasionally already by EINTHOVEN and JOLLY (1908) with repeated stimuli. GRANIT and RIDDELL showed that a flash thrown on top of the off-effect of the retinal response, depending upon the interval between cessation of illumination and the flash, gave the set of responses shown in fig. 22. The figure illustrates the « negative notch » in the record, caused by the flash, and identified by them with an *a*-wave and thus with the negative component PIII. These excessively large *a*-waves are followed by *b*-waves increasing in size as the flash is moved further out on the off-effect.

Here then was a case of selective activation of the negative component PIII at a time when the optic nerve was discharging impulses (off-effect in the nerve). What would happen to these impulses during the phase of negativity? GRANIT and THERMAN (1934, 1935) studied this question by recording alternately from retina and nerve. They found that the frequency of the discharge in the optic nerve rapidly diminished when a flash was thrown on top of it as in the experiments presented in fig. 22. In fig. 23

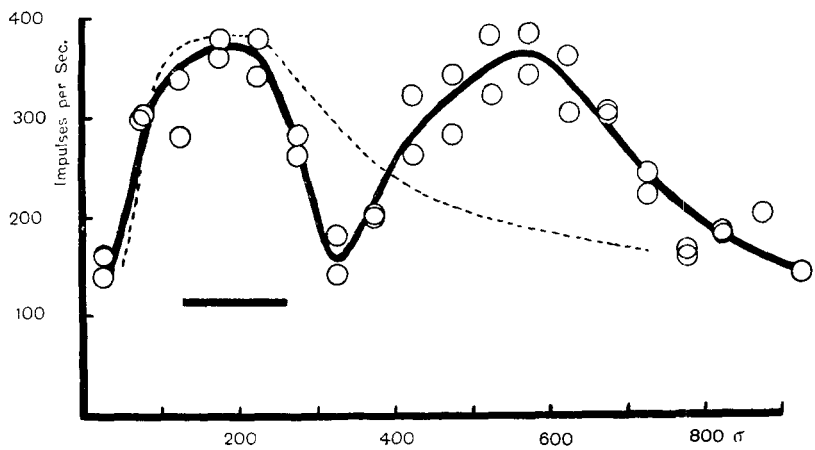

Fig. 23. — Number of impulses per sec. during off-effect. Normal off-effect control drawn in broken lines. Off-effect suppressed by flash (to the left in the figure) drawn in full. GRANIT & THERMAN (1935), *J. Physiol.*, 83, 359.

the broken line shows the frequency of the impulses in the optic nerve during the off-effect. Some minutes later, in the same experiment, a flash (black horizontal line to the left in the figure) was superimposed upon the off-effect. The line, drawn in full between the experimental data, illustrates the onset of inhibition

following the flash. The off-effect therefore could be inhibited by a flash on top of it. In 1935 HARTLINE (1935) found that the optic nerve fibres which reacted only with an off-effect on cessation of illumination stopped their discharge when the retina (frog) was re-illuminated. Likewise in *Pecten* there are receptors which only discharge at « off » and are easily inhibited by re-illumination (HARTLINE, 1937).

At this stage of our discussion of inhibition in the retina it is important so sum up the facts before returning to the hypothesis relating inhibition to the component PIII of the retinal response. Thus we may conclude that inhibition in the retina is a fact and that it coincides with selective activation of an *a*-wave of negative PIII (frog's eye). We are likewise entitled to describe the off-effect as a release from the inhibition developed by the stimulus, since the inhibitable elements obviously are inhibited by light and set free by darkness. — Fig. 20 shows that an eye reacting with a pure negative PIII (after treatment with K-ions) gives an off-effect on the negative side of the base-line and that this off-effect, just like normal ones, increases in size and rate of rise when the duration of the exposure is lengthened. A flash superimposed upon the off-effect of the negatively reacting eye causes a negative notch that to begin with completely suppresses the off-effect, but later in the experiment (as in fig. 20) only succeeds in removing part of the rise that has taken place. Finally GRANIT and THERMAN (1938) have found that in a completely dark adapted eye, treated with potassium, the negative response very slowly returns towards the base line, whereas in the light adapted eye it returns rapidly. Also in this respect the return to the base line of the negative PIII at « off » reacts in a manner that imitates the behaviour of the normal off-effect of the complete response. Potassium, however, kills the nerve long before the retina has begun reacting with purely negative responses. It has therefore not been possible to correlate the retinal events with processes in the nerve in an eye treated with potassium.

Returning now to the hypothesis connecting PIII with inhibition, we must admit that it has been exceedingly useful and that it has helped us to discover a great number of facts all of which on the whole support it, but that no evidence definitely puts it beyond criticism. We can hardly doubt that PIII is the electrical sign of a fundamental and important process, especially important from the point of view of adaptation to light and darkness. It is also obvious that many facts that otherwise must remain

coincidences are easily explained on the view that inhibition is causally related to PIII. Nevertheless this causal relationship is hypothetical.

It would be a mistake to infer from the facts mentioned that in every type of vertebrate retina flashes superimposed upon the off-effect cause « negative notches » followed by *b*-waves. As a matter of fact there are eyes (cat, rabbit) in which a flash thrown on top of the off-effect only elicits a *b*-wave (PIPER, 1911 ; CREED and GRANIT, 1933 ; GRANIT, 1935). It is not yet known whether off-effects in the nerves of these eyes are inhibited as easily as in the frog's optic nerve. Such eyes are said to have E-retinæ, whereas the frog is said to have an I-retina. The effect of reillumination after a gap of darkness giving rise to the off-effect is shown in fig. 24 for an E-retina (cat). The corresponding experiment

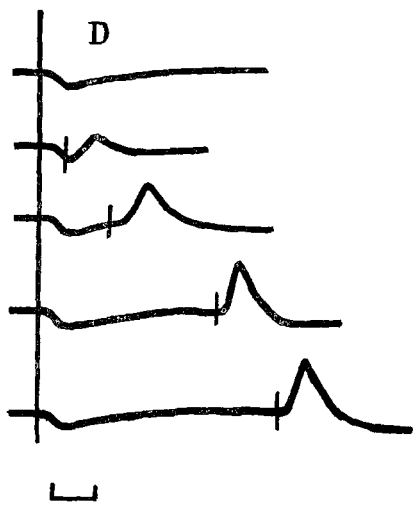

Fig. 24. — Off-effect of cat's eye. Long vertical line marks cessation of stimulation with intensity 620 metre-candles. The short vertical on the individual curves marks reappearance of stimulus ; the interval between them duration of gap of darkness (cf. fig. 22). Time 1/10th sec. GRANIT (1935), *J. Physiol.*, 85, 421.

with the I-retina was shown in fig. 22. On account of the connexion between the negative notch and inhibition, I-retinæ have been held to utilize inhibition to a greater extent than E-retinæ (GRANIT, 1935). Other differences between the two types of retinæ, summarized by the author in 1935, will be pointed out below.

6. *Off-effect and adaptation.*

A very direct correlation between state of adaptation and the « negative notch », caused by a flash on the off-effect of the I-retina, was found in the type of experiment illustrated in fig. 25. A and B show the effect of a flash on top of the off-effect of a

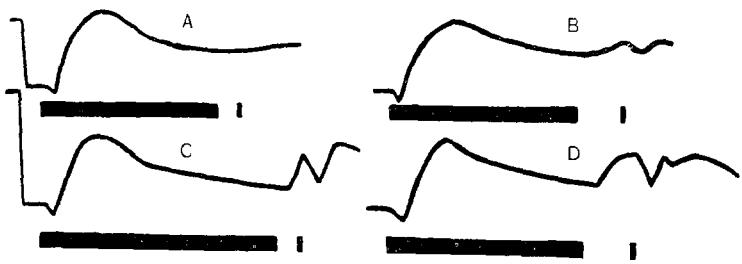

Fig. 25. — Stimuli shown by thick lines. A, initial stimulus lasts 1.3 sec. and is followed at an interval of 120 σ by a flash of 35 σ; B, corresponding values for stimulus and interval are 1.3 sec. and 270 σ; C, corresponding values 1.6 sec. and 120 σ; D, corresponding values 1.3 sec. and 290 σ. GRANIT & RIDDELL (1934). *J. Physiol.*, 81, 1.

thoroughly dark adapted eye, C and D the corresponding experiment with the same eye after it had been illuminated by the bright stimulus (1.800 Lux) for some time. It is seen that after adaptation not only the off-effect has become faster and larger, as several times pointed out above, but that the negative notch has undergone a similar change. The same result has been obtained with the isolated PIII (GRANIT and THERMAN, 1938), shown for the light adapted eye in fig. 20. In the dark adapted eye PIII returns very slowly towards the base line and a flash on top of it has a negligible effect. After light adaptation cessation of stimulation is followed by a rapid swing-back of PIII and this off-effect reacts to a flash with the typical deep and fast negative notch.

Here again we are facing the question that has been raised twice before. Have new elements been stimulated to activity by light-adaptation or have the elements, which acted in the scotopic state, changed their properties? This question will be finally dealt with in the next section.

Another property of the off-effect is likewise related to state of adaptation. It was seen by WREDE (1937) in the experiments illustrated in fig. 13 in which he followed the return of the electroretinogram after light adaptation of frog's eyes. As shown by fig. 13 the *b*-wave rises during dark adaptation but the off-effect

(broken lines) first rises and then falls. The same phenomenon was still better marked in some experiments by THERMAN (1938), probably owing to the fact that the eyes were treated with glucose after light adaptation. Fig. 26 is from one of THERMAN's

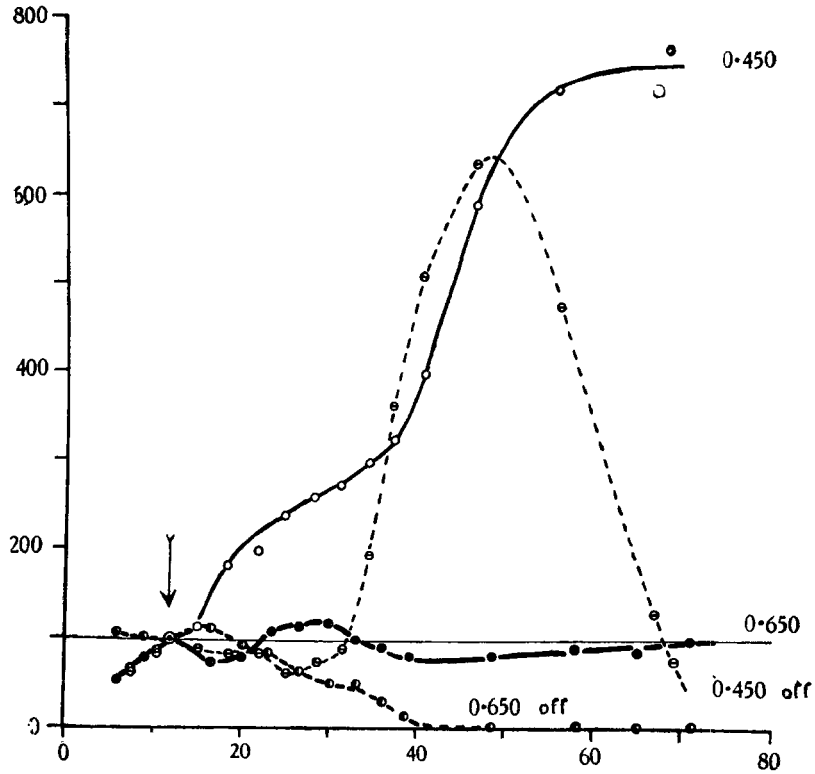

Fig. 26. — Regeneration in darkness of previously light adapted excised, opened frog's eye. Size of both *b*-waves and off-effects (broken lines) recorded as a function of time in the dark. Stimulus from monochromator set at 0.450 μ or 0.650 μ. Abscissæ minutes, ordinates arbitrarily given value of 100 at the moment when glucose is pipetted into the eye, shown by arrow in figure. See text. THERMAN (1938).

experiments in which size of both *b*-wave and off-effect was followed during dark adaptation with stimuli from a monochromator alternately set at 0.450 and 0.650 μ. At 0.650 μ only cones may be supposed to react, at 0.450 μ chiefly or exclusively rods — at the slit-widths used by THERMAN and known in relation to the frog spectrum from the work of GRANIT and MUNSTERHJELM (1937) with the same apparatus. When the *b*-wave at 0.450 μ enters the fast rising phase of adaptation, the off-effect follows it for a while and then drops down to low values. It should be noted

that the electroretinogram at 0.650 μ does not participate in the characteristic adaptive changes of the rod spectrum.

An effect of this type could easily be explained by assuming that the off-elements of the well dark adapted eye are sharing the pathways with the on-elements giving the *b*-wave. A large *b*-wave would then leave « refractoriness » for an off-effect in the same elements.

Experimental results in a certain measure support this suggestion. There is, for instance, the fact, discovered by HARTLINE (1935). that some fibres discharge both at « on » and « off » whereas others merely discharge at « off ». There are also some results by GRANIT and THERMAN (1937) which are of interest in this connexion. In their experiments the retina was kept illuminated by one light, every now and then interrupted to give an off-effect. A 1/100th second flash from another light source was made to precede the interruption of the adapting light or to fall on top of the off-effect. as in the experiments described above. When the flash preceded the off-effect, the latter was reduced by a certain amount, indicating that some of the elements reacting at « off » also had responded to the flash and therefore were incapable of delivering an off-effect immediatly afterwards. However, the whole off-effect could not be removed in this way and the size of the negative notch on top of the remainder was independent of whether a flash had preceded the off-effect or not. Thus there are two components of the off-effect, one that disappears when it falls into the wake of « refractoriness » left by a preceding *b*-wave, and another one that cannot be made to disappear in this way, and the latter reacts to the flash with a negative notch of PIII. The latter component is found also in negatively responding eyes, the former only in eyes in which the positive component PII is present. GRANIT and THERMAN (1937) suggest that this component of the off-effect should be identified with the elements that in HARTLINE's experiments with single fibres reacted at both « on » and « off ». In elements of this type a large *b*-wave may exclude an off-effect. The curious behaviour of the off-effect during adaptation to darkness could perhaps be explained in this manner. — The other component of the off-effect may be the inhibitable off-elements of GRANIT and THERMAN (1935) and the fibres (HARTLINE) merely responding with an off-effect.

7. *Nature of the transition from dark adaptation to light adaptation.*

Before finally taking up for discussion the nature of the electro-adaptive changes which, as we have seen, lead to the marked difference between scotopic and photopic electroretinograms,

let us consider an experiment with intermittent illumination in which these changes are conveniently summarized. It is illustrated in fig. 27. The flashes of the individual stimuli have been photographed on the film and are seen as dark oblongs alongside the lower edge of the individual records.

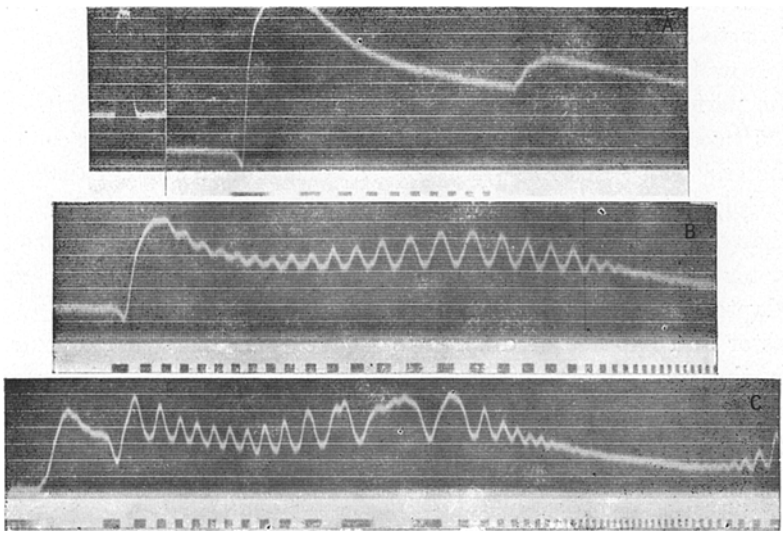

Fig. 27. — Original photograph of retinal response of frog's eye elicited by intermittent illumination. Uppermost record, *A*: completely dark adapted eye, *B* and *C* after light adaptation. Calibration to 0.673 millivolts. Time: Tuning fork with period 100. Intensity 1.800 Lux. Fully explained in text. GRANIT & RIDDELL (1934), *J. Physiol.*, 81, 1.

In the uppermost curve (*A*) the well dark-adapted frog's eye is incapable of following the rhythm of stimulation effectively. Individual wavelets are just visible in the middle of the record. After 5 minutes of adaptation to the stimulus (1.800 Lux) there is a complete change in the general behaviour of the eye (curve *B*). It almost looks as if a retina from another animal had been put into the apparatus. There are now brisk wavelets synchronized with a very much faster rate of stimulation than in curve *A*. Finally, in *C*, the record begins with the off-effect of a previous period of illumination after which follows intermittent stimulation with the same light. The first flash causes the typical negative notch coinciding with inhibition in the optic nerve; the whole curve swings up and down dependent upon the rate of stimulation; and the fusion frequency now is 18 flashes per second. In the same experiment the eye was then dark adapted for 15 minutes after

which the fusion frequency fell to 7 flashes per second. Readaptation to light brought it back to 18. — The facts described were discovered by GRANIT and RIDDELL in 1934 (see also RIDDELL, 1935), but this change in fusion frequency with state of adaptation is well-known from the work of SCHATERNIKOW (1902), LYTHGOE and TANSLEY (1929), and ENROTH and WERNER (1936) on sensations of flicker. Obviously therefore a similar transition from slow to fast takes place in the human eye.

Now this experiment with intermittent light shows, as it were, the performance as a unit of the whole machinery that hitherto has been described bit by bit. It is here seen fulfilling one of the essential tasks for which it was constructed, that of discriminating between light and darkness. The dark adapted eye is highly sensitive and slow just as a ballistically recording galvanometer integrating the total quantity of energy reaching it. After adaptation to light the retina is less sensitive but very much faster and capable of differentiating between light and darkness. In the records taken after light adaptation is recognized: the faster *b*-waves; the rapid negative notch signifying inhibition; the fast and large off-effects, and the shift in general level of potential dependent upon rate of stimulation and caused, chiefly, by the component PIII. Both excitation and inhibition are taking part in the flicker. The record *C* shows that the flicker begins with a negative notch of inhibition which serves the obvious purpose of cutting down the long afterdischarges left by the off-effects, which otherwise would prevent the eye from working quickly and efficiently. All these changes, brought about by light adaptation, had already been described separately and analyzed in detail in the previous chapters. But their significance should be better understood now when we have seen them cooperating as parts of a whole.

We have now to decide how to explain this central fact in electro-adaptation, the transition from scotopic to photopic vision culminating in making the slow integrating mechanism a fast differential instrument. Two fundamentally different lines of thought have been suggested: either the active elements in some curious way must alter their general properties under the influence of light adaptation, or stimulation somehow must facilitate the appearance of a new set of elements.

From many points of view (discussed by GRANIT, 1935) the latter explanation would seem to offer greater satisfaction. It is also supported by the fact that the properties which characterize

the mixed retinæ of *e.g.* frogs and owls after light adaptation in the relatively pure cone eye of the pigeon are constant and independent of state of adaptation. This retina always reacts like an I-eye : there is always a relatively large and fast off-effect, a negative notch, and the fusion frequency is fast and practically independent of state of adaptation (GRANIT, 1935). Some experiments by MESERVEY and CHAFFEE (1927) with different types of cold-blooded eyes indicate that those possessing chiefly cones are faster and have larger off-effects than mixed retinæ. But an analysis of the kind carried out with frogs, owls, pigeons, and cats has not yet been completed with these eyes. The cat and the rat, which have relatively pure rod retinæ (especially the rat), not only belong to the E-type with slow flicker, low fusion frequency, and no negative notch (when a flash is thrown on the off-effect), but, if heavily light adapted, either do not show a marked rise of the fusion frequency or the latter even may fall (CHARPENTIER, 1936, with the rat's eye).

These results indicate that in mixed I-retinæ light adaptation really paves the way for the appearance of new elements, identical with the ones that are found in relatively pure cone retinæ of the same type. Accepting this view, how are we then to explain that these elements refrained from participating in the reactions of the dark adapted eye. As pointed out several times above, intensity of stimulation has been constant; in most experiments the same light even has been used as the adapting light. Why and where have the fast elements been « in hiding » during dark adaptation?

Elsewhere (GRANIT, 1935, 1936) I have emphasized that the slow elements (the rods) in the dark adapted eye must have inhibited the fast elements (the cones). Only if this view be accepted can these phenomena of electro-adaptation be fitted into the general scheme of the duplicity theory; otherwise we have to seek for explanations along other lines. But as rightly pointed out by GASSER (1936-1937) inhibition as a general term may mean any of several possible mechanisms. And there is certainly no reason for identifying the inhibitory effect exerted by the rods upon the cones with the active process that cuts down the off-effect when the eye is reilluminated. In order to explain the predominance of one element over another the simplest assumption has seemed to me to be the idea of a competition of both elements for a « final common path » (SHERRINGTON), be it then a fibre or some other neurone. Once the paths are mobilized by the momentarily more active element — as during dark adaptation

by the highly sensitized rods — the other ones are excluded.

Such a state of affairs has actually been found to exist in another sense organ. It was found in the ear and auditory nerve by DAVIS and his collaborators (DAVIS, 1935; DERBYSHIRE and DAVIS, 1935) who use it for explaining the well-known « auditory masking ». They have aptly termed this phenomenon the « line-busy » effect. « The interference of two stimuli simultaneously arriving at the ear evidently depends upon competition for the same fibre tracts. It resembles the « occlusion » of SHERRINGTON in spinal centres » (DERBYSHIRE and DAVIS, 1935). « Whichever stimulus first succeeds in setting up impulses in the fibres has right of way, for the second stimulus finds these fibres refractory and therefore sets up no impulses » (DAVIS, 1935).

In this way the rods may be assumed to inhibit the cones and the electro-adaptation be simply explained. GRANIT and WREDE (1937) have likewise published some observations indicating a reversal of this « line-busy » effect in the light adapted eye; active cones suppressing the rod spectrum even though some dark adaptation has taken place.

8. *Adaptation and the secondary rise (PI or c-wave).*

Some observations relating to the *c*-wave or secondary rise as a function of state of adaptation have already been described (p. 51). On the whole very little is known about this slow rise of the electroretinogram found to be due to a separate component PI (GRANIT, 1933). It does not seem to be directly correlated with the frequency of discharge in the optic nerve (GRANIT, 1933; GRANIT and THERMAN, 1935), and in the eyes of *e.g.* frogs and cats it diminishes after light adaptation. But curiously enough a slow secondary rise may be obtained in the light adapted frog's eye, when some adrenaline has been pipetted into the opened bulb (THERMAN, 1938).

An experiment by THERMAN (1938) with adrenaline is illustrated in fig. 28. Two lights of relative strength 100 and 1 have been used. The eye is kept illuminated with light 1 and during adaptation to this stimulus flashes are thrown in from the light source of strength 100. The upper curve *A* shows the behaviour of the eye before adrenaline. The first flash in front of the calibration is delivered with light 100 and shows its effect before adaptation to light 1. The latter is turned on at the beginning of the black line below the record. This light elicits the *b*-wave after

the calibration mark. The following flashes are all delivered with light 100 breaking through the level of potential kept up by light 1. All *b*-waves rise to the same height of potential. Then the experiment is repeated after adrenaline (curve *B*). There is now a large secondary rise and the flashes are seen to increase in size during the rising phase of the *c*-wave.

At the moment a result of this type is difficult to explain but it

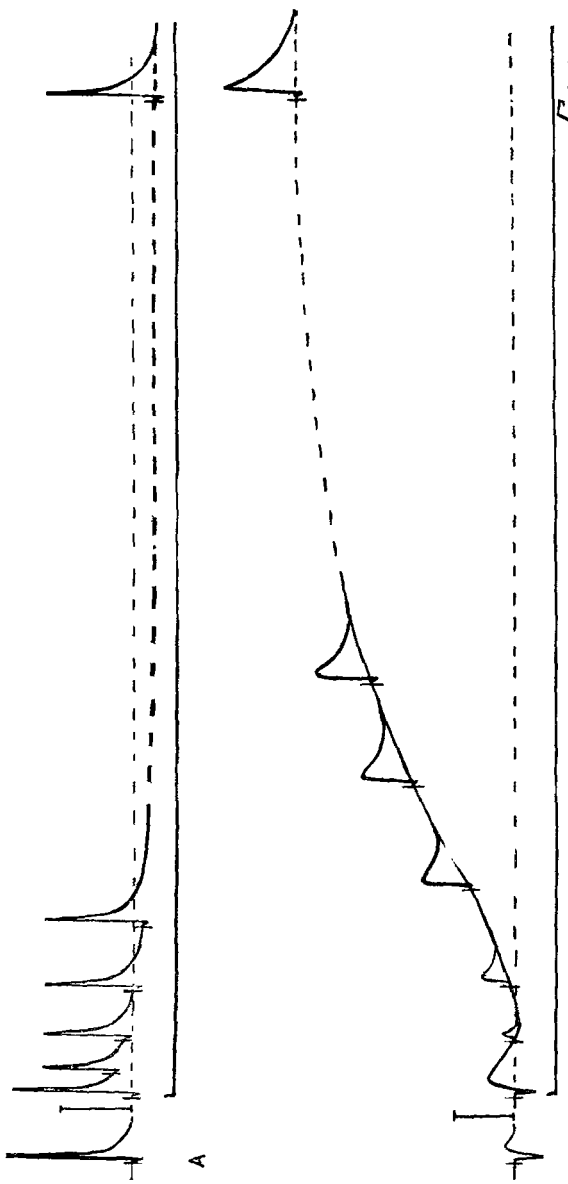

Fig. 28. — *A* : Lowermost line shows continuous illumination of excised, opened frog's eye with intensity 1. This causes a typical initial *a*- and *b*-wave preceded by calibration to 0.100 mV. The following deflections are all caused by flashes (0.01 sec.) of intensity 100, superimposed upon the continuous effect of the adapting light of strength 1. The flashes alone (without simultaneous adapting light) elicit a deflection shown by initial control preceding calibration. — *B* : The same experiment as shown in A, repeated some minutes later, the eye in the meantime having received a drop of 0.01 p. c. adrenaline in glucose.

Note. Control flash at intensity 100 preceding calibration elicits very little positive potential. The initial response caused by intensity 1 is larger than deflection caused by flash of strength 100. But the flash, when superimposed upon the secondary rise, caused by the continuous adapting light, increases during the rising phase of the *c*-wave. THEBMAN (1938).

indicates that the *c*-wave after adrenaline may have something to do with excitability, and thus with adaptation.

9. *Some less known phenomena of adaptation.*

For the sake of completeness I must mention selective adaptability and refer briefly to the review by KOHLRAUSCH (1931) for a discussion of its different aspects. It is obvious that if a retina contains a number of photochemical substances mediating sensations, it should be theoretically possible to obtain selective « fatigue » by bleaching with wave-lengths representing the absorption maxima of these substances. Experiments of this type would therefore offer a means of penetrating electrophysiologically into the nature of the colour sensitive mechanisms of a retina, as has been done by WRIGHT (1934, 1937) in his well-known work on selective adaptation of the human sensory apparatus. This possibility naturally occurred to the physiologists who studied the electroretinogram (see the review by KOHLRAUSCH, 1931) but the results were negative. The last contribution to this problem was made by CHAFFEE and HAMPSON (1924). The following statement from their paper summarizes the present status of this question: « Several experiments were made in which the eye was exposed constantly to one colour and the responses measured for flashes of another colour. These experiments were designed to determine if totally different mechanisms are active for different colours. The results confirm the observations of WALLER in that apparently so far as the response curves show there is no selective fatigue due to exposure to a single colour and that the fatigue due to one colour affects the responses of all colours ».

In criticism of the old work I must add that in my opinion experiments of this type presuppose that we know the standard curves for distribution of size of *b*-wave in the spectrum for the two states of adaptation and that these are based on a sufficiently large material. Otherwise, if selective effects be small as in the human eye according to the work of WRIGHT (1934, 1937), they cannot show up against the background of chance variations. For this reason I have re-opened this seemingly hopeless way of attacking colour problems without yet having had time to accumulate a sufficiently large number of measurements of this type. The preliminary work of standardization, however, was completed (see the papers by GRANIT and MUNSTERHJELM, 1937; GRANIT and WREDE, 1937).

Three effects of selective adaptation have so far been definitely established in this laboratory: with the *light adapted* frog's eye it was found that selective adaptation to green (0.530 μ Ilford spectral filter) depressed the whole curve symmetrically, but that during adaptation with the Ilford red filter the red end of the spectrum was selectively depressed, that is, the *b*-waves were relatively smaller in the red end than in the blue end of the light adapted frog's spectrum. In all these experiments flashes from the equal energy spectrum were superimposed upon the continuous illumination through an Ilford filter. With the dark adapted eye I have tried bleaching with 0.585 and 0.430 μ (from the monochromator) which give practically equal responses in terms of the low intensity equal energy spectrum and hence represent equal absorption in terms of visual purple. Wave-length 0.585 μ depresses the electrical response considerably more than does 0.430 μ. This effect is very easily demonstrated with an eye in good condition. It may have to do with the similar effects of regeneration after bleaching with short and long wave-length, described by CHASE (1937).

To the category of less well-known effects also belongs the « amplifier effect » described by the author (1937 a).

10. *Remarks on the components of the electroretinogram.*

From the electrophysiological work on motor and sensory neurones there has gradually emerged the not improbable generalization that, in the words of ECCLES (1936), « negativity of the soma of any nerve cell relative to its axon is associated with an increased excitability and consequent tendency to discharge impulses, while a positivity is associated with a diminished excitability ». One line of work partly supporting this conclusion has been followed by GASSER and his collaborators (HUGHES and GASSER, 1934 a, b; GASSER and GRAHAM, 1933; HUGHES, MCCOUCH and STEWART, 1937; GASSER, 1936-1937; see also ERLANGER and GASSER, 1937), their preparation being the spinal cord and peripheral nerve. ECCLES (1935 a, b, c; 1936 a, b, c) has contributed to this view a great number of experiments on the *superior cervical ganglion*, and we have come to this generalization from the work with the retina (see the papers quoted, especially GRANIT, 1933 and GRANIT and THERMAN, 1935). It is therefore of some interest to end this review of adaptation with some comments on the components of the electroretinogram.

In the old literature on retinal currents (see the review by KOHLRAUSCH, 1931) it was often emphasized that with the simple eye of cephalopods the electrical response upon illumination indicated negativity of the cornea in the lead cornea-bulb whereas with the vertebrate eye the normal electroretinogram means that the cornea has become positive relative to the back part of the bulb. The likewise simple monophasic electroretinogram of *Limulus* indicates negativity of the layer of receptors (HARTLINE, 1925; HARTLINE and GRAHAM, 1932) just as in cephalopods. The different behaviour of the vertebrate retina was explained by the fact that in this eye the retina is inverted so that the receptors are lying towards the back part of the bulb.

When proposing my analysis of the retinal response in 1933 I accepted this view. In terms of the analysis, adhered to in this paper, it means that the component PII is to be identified with the whole electrical response of *Limulus* and thus represents negativity of the receptors relative to the rest of the retina although for historical reasons PII is plotted upwards in this and in all other papers dealing with the vertebrate eye. It is thus merely a convention that has led to positivity of the corneal lead being normative rather than the simultaneous negativity of the back part of the bulb. Other facts also support the conclusion that PII is very directly concerned with the discharge in the optic nerve. In the cat's eye PII and the impulses disappear simultaneously when the animal is asphyxiated by applying pressure to the carotid (GRANIT, 1933). A number of other facts have been pointed out by GRANIT and THERMAN (1935). As to PIII, which in the standard lead and plot of the electrical response is negative, I suggested that it represented positivity of some elements of the retina relative to the cornea. The evidence for associating PIII with inhibition has already been discussed.

As long as it was difficult to remove PII selectively from the frog's retina it was likewise difficult to attack this problem with experimental methods. But THERMAN (1937) having found the selective effect of potassium ions upon PII leading to an electroretinogram containing only PIII, we proceeded to study the frog's electrical response in different leads. These experiments have given further support to the view that PII and PIII represent respectively negativity and positivity of the elements towards the receptors relative to the rest of the retina. If the retina is removed from the eye it may still react with a relatively large component PIII for some time. In this preparation PIII also repre-

sents positivity of the receptorial side of the retina relative to the ganglion cells. THERMAN (1938) also has found that when simultaneous records are taken from retina and nerve, the nerve ceases to discharge a few minutes after treatment with potassium ions at a time when PII still is very little influenced by this treatment. This observation shows that the impulses in the optic nerve contribute very little to the electroretinogram. On the other hand, if one lead is on the nerve and the other one on the cornea, the electrical response is completely disfigured by the activity of a process that immediately disappears after treatment with potassium (GRANIT and THERMAN, 1938) and hence must represent the spikes of the impulses starting in the ganglion cells or electrotonic spread from the latter.

We have therefore every reason to remain on the standpoint taken up in 1933 that PII and PIII represent respectively negativity and positivity relative to the ganglion cells in — probably — parallell elements lying towards the receptorial side of the retina and associated with respectively excitation and inhibition. Naturally we have considered other alternatives but not yet seen any reasons for accepting them. Most important among those alternatives is the possibility of PIII likewise representing negativity but in elements with a different orientation relative to the electrodes. Inhibition could then be explained on the « classical » interference theory of excitatory paths, recently revitalized by GASSER (1936-1937).

ADDENDUM TO PROOF

VISUAL PURPLE. — A method of purification of visual purple extracts is described by SAITO *(Tohoku J. Exp. Med.*, 1938, 32, 432). The rods are separated from the rest of the retina by shaking with a 40-45 p. c. sugar solution. In this work and in a paper by CHASE & HAIG *(J. gen. Physiol.*, 1938, 21, 411) the same standard of purification of visual purple has been reached as in the work of LYTHGOE (1937). His absorption curve is confirmed. SAITO in his paper also describes the visual purple system of carps.

HOSOYA and SATAKI *(Tohoku J. Exp. Physiol.*, 1938, 32, 447) confirm the reports of regeneration of small amounts of V.P. in solution and demonstrate the significance of the pigment epithelium in this process.

The rate of formation of visual yellow has been studied by HOSOYA and SAITO *(Tohoku J. Exp. Med.*, 1938, 32, 399).

The effect of temperature on the photochemical bleaching of visual purple in solution and the quantum efficiency of the bleaching process have been analyzed by DARTNALL, GOODEVE and LYTHGOE *(Proc. Roy. Soc. A.*, 1938, 164, 216). Their theoretical equation (see above p. 27) is confirmed

and the product $\alpha\,\gamma$ *(extinction coefficient* times *quantum efficiency)* is found to be independent of temperature (except possibly at low temperatures below 5°) up to 60° and of pH over a range of from 6.8 to 9.2. In addition both α and γ are independent of temperature and pH. The value of their product for wave-length 0.566 μ is about 9×10^{-17} cm^2 molecules per quantum. The quantum efficiency is not greater than unity. This means that each quantum absorbed bleaches one molecule of visual purple. This fact together with the absence of an effect of temperature on the rate of bleaching supports the conclusion that no chain reactions are involved in the bleaching of V.P.

A comparison between V.P. absorption and rod vision has been published by H. F. BLUM *(Science N. Y.*, 1938, 87, 193). The facts presented have already been fully discussed above and BLUM's conclusions are completely covered by the earlier work of DARTNALL and GOODEVE (1937).

CONE VISION. — CHASE *(Science N. Y.*, 1938, 87, 238) reports properties of extracts from frog's eyes indicating the presence of another not yet purified substance the maximum of which preliminarily is placed in 0.530 μ.

LACTOFLAVIN. — ADLER and VON EULER *(Nature*, 1938, 141, 791) finally establish that the retinal lactoflavin is free and not in the form of its phosphoric ester which likewise gives fluorescence. This suggests that the flavin phosphate of the food is dephosphorylated before deposited in the eye.

ELECTROPHYSIOLOGY. — HARTLINE *(Amer. J. Physiol.*, 1938, 121, 400) has published part of his work on single fibres in the frog's optic nerve. The results, known to the reviewer from the preliminary note quoted and from correspondence, had already been incorporated in this review inasmuch as they concern questions under discussion.

GRANIT, THERMAN and WREDE have reported their results on selective effects of adaptation to different wave-lengths in frog's eyes in *Skand. Arch. Physiol.*, 1938, in course of publication. Some of the work on selective effects in light-adapted eyes has preliminarily been reported in a review by WRIGHT and GRANIT discussing correlations between sensory and physiological facts *(Brit. J. Ophth. Monograph supplement n° 9.* in course of publication).

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