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With 5 figures in the text

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Selective Effects of Different Adapting Wave-Lengths on the Dark Adapted Frog's Retina¹

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(With 5 figures in the text)

Already at an early period of retinal electrophysiology experiments on bleaching the eye with monochromatic lights were undertaken in the hope of obtaining evidence for the existence of substances selectively sensitive to different wave-lengths. Such experiments have been carried out for instance by Waller (1903), Kahn and Löwenstein (1924), and Chaffee and Hampson (1924). The results were negative inasmuch as it was found that selective bleaching with any wave-length diminished the response to all wave-lengths, regardless of their locus within the spectrum. The effects of bleaching with different monochromatic lights depended only on the relative size of the response caused by these wave-lengths and not upon the quality of the light used.

These experiments are open to criticism on several grounds. However, beyond pointing out that the observations must have been limited in number and that the two states of adaptation were not properly separated, it is not our intention to enter into a detailed discussion of this work. We have obtained results, positive in the sense that under certain conditions, to be specified in this paper, definitely selective effects of bleaching may be obtained. It has even been possible to show that adapting the eye to monochromatic light may lead to an increase of the response elicited by another wave-length. This paper will be devoted to a description and preliminary analysis of this unexpected finding.

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Nature of the Problem

Consider the absorption curve for visual purple, determined *e. g.* by Köttgen and Abelsdorff (1896), Trendelenburg (1904), and recently by Lythgoe (1937). To an equal-energy low intensity spectrum the frog's eye reacts with a distribution of size of potential (*b*-wave of the electrical response) which can be proved to be determined by this absorption curve (Chaffee and Hampson, 1924; Granit and Munsterhjelm, 1937; cf. also Graham and Riggs, 1935 with rat's eyes).

The *b*-wave is maximal around 0.510μ and diminishes towards either side. However, size of *b*-wave at low intensities is proportional to the square root of the intensity (Chaffee and Hampson, 1924). As pointed out elsewhere (Granit, 1937), a comparison between the absorption curve for visual purple and the »physiological absorption curve«, given by the electrophysiological data, requires to be corrected for the lack of direct proportionality between size of potential and energy absorbed. Finally it is necessary to give the electrophysiological data in terms of a spectrum of equal quantum intensity rather than in one of equal energy (Dartnall and Goodeve, 1937). When this is done the absorption curve calculated from observations on the average size of *b*-wave in an equal energy spectrum fits the absorption curve for visual purple fairly closely (Granit, 1937). The latter curve is somewhat higher at the short wave-lengths, which probably is due to other absorbing substances (see Lythgoe and Goodeve, 1937).

Let us now choose 0.500μ as our test light and then bleach the well dark adapted eye for 5 minutes with some other wave-length from the equal energy spectrum. The facts just referred to imply that the effect of bleaching should be a depression of the response to wave-length 0.500μ , proportional to the amount of absorption of visual purple at the wave-lengths used for bleaching. Thus, for instance, if we choose two points on either side of 0.500μ , giving equipotential responses in the low-intensity spectrum, and use the corresponding wave-lengths as our bleaching or adapting lights, the latter should depress the *b*-wave to 0.500μ to the same extent. In this paper it will be shown that in general the long wave-lengths cause a considerably greater depression of the response to 0.500μ .

Technique and Procedure

The retinal action potential of excised opened frogs' eyes was recorded with the aid of a 4-stage push-pull amplifier and a cathode ray oscillograph. The stimulating devise was a Tutton monochro-

mator, set up as described by Granit and Munsterhjelm (1937). In this apparatus the spectrum is adjusted to equal energy between 0.650 and 0.450 μ . For details of this adjustment their paper should be consulted.

Hungarian frogs were dark-adapted over night, their eyes removed in red light, cut open, and put into a dark box on the sliding electrode devised by Therman (1938). At intervals of 2 minutes a number of preliminary 1 second exposures to 0.500 μ were taken. A level of spectral energy was used bringing in some cones in the long wave-lengths (Granit and Wrede, 1937), but the choice of 0.500 μ as the testing light should ensure a very limited participation of cones in the test *b*-wave.

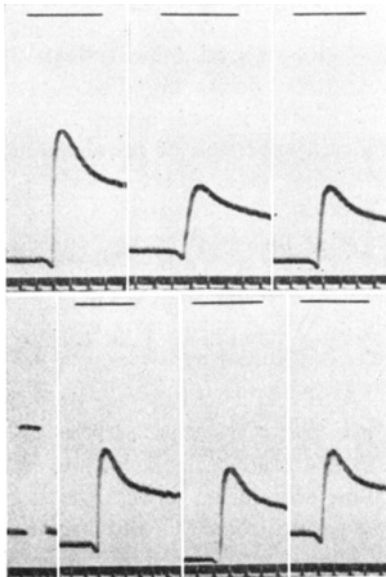


Fig. 1. Cathode ray oscillograph records of *b*-waves before and after adaptation to wave-lengths 0.585 and 0.430 μ . Light signal along upper edge of record, time in $\frac{1}{5}$ sec. below

Upper set of records: 1. Response to test light 0.500 μ before adaptation. 2. Same, 1 min. after cessation of adaptation to wave-length 0.585 μ . 3. Same after 2 min. of recovery

Lower set of records: 1. Calibration to 0.500 mV. 2. Response to test light 0.500 μ before adaptation, but later in the experiment when several adapting lights already have been tested. 3. Same 1 min. after cessation of adaptation to wave-length 0.430 μ . 4. Same after 2 min. — Note that the response 1 min. after adaptation to this wave-length is bigger than the response following after 2 min. of recovery

Then the level of energy was increased by 1.3 log units (removal of an extra density) and the eye adapted to the higher intensity for 5 minutes. Different wave-lengths were used for this process of *in vivo* bleaching of the retinal photosensitive substances. After the termination of the period of bleaching the extra density was replaced and the response to 0.500μ tested at 1, 2, 4, 6, and 8 minutes after the adaptation. A set of *b*-waves obtained in such an experiment is shown in fig. 1. The response is smaller to begin with and gradually increases in size as the eye recovers from the effects of adaptation.

Results

Analysis of an individual experiment

A typical experiment is illustrated diagrammatically in fig 2. Ordinates are millivolts, abscissae time after termination of bleaching. The values for the original responses to 0.500μ before adaptation have arbitrarily been plotted to zero abscissa. Before averaging a number of experiments, we shall follow this one in greater detail in order to show the principle of the analysis. It happened to be an experiment in which the eye lasted long enough to enable tests to be carried out with several adapting wave-lengths.

Curve 1 shows that the original response of 0.560 mV , elicited by 0.500μ , after adaptation to 0.540μ has declined to 0.320 mV . Within 8 minutes it rises to 0.490 mV . Next follows bleaching with 0.470μ (curve 2) starting from 0.490 mV . The *b*-wave drops only to 0.410 mV , and rises in 6 minutes to 0.485 mV from which bleaching with 0.585μ starts. Thus every new period of adaptation begins from the level of potential reached at the end of the previous period of recovery. Now it is interesting to note that after bleaching with 0.430μ , starting from 0.480 mV (curve 4), the first response to the test light instead of being smaller than before has risen to 0.540 mV . After 2 minutes the response has fallen slightly and then slowly reattains the value of 0.540 mV . Obviously here during adaptation two processes have been competing with each other, one trying to enhance, the other to depress the response to the test light at 0.500μ .

The favourable influence on the test response of bleaching the eye seems to be restricted to the short wave-lengths. Thus curve 5 shows the effect of bleaching with 0.560μ , starting from a response of 0.540 mV . Again the drop in size of the deflection caused by the test light is considerable: It falls to about 0.410 mV and rises after 6 minutes to 0.504 mV . Bleaching with 0.450μ (curve 6) does not change this potential in the least. Therefore 0.450μ has activated

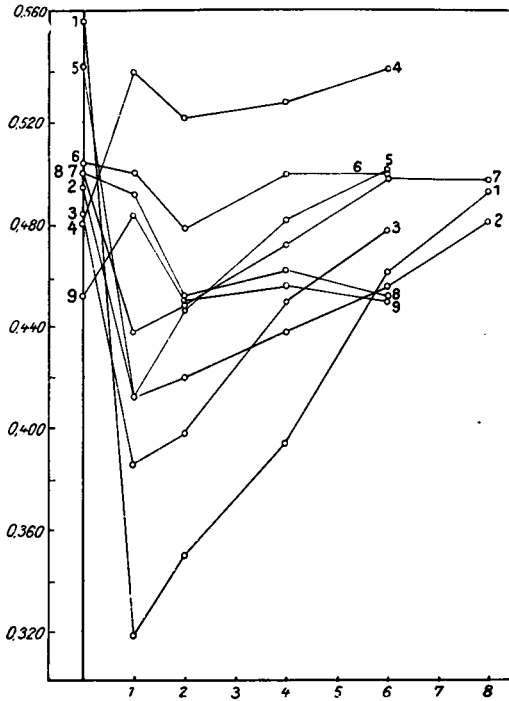


Fig. 2. Plot of values obtained in complete single experiment in which several adapting lights were used. Test light 0.500μ . Ordinates: size in millivolts of *b*-wave, the figures against the individual curves indicating the order in which bleaching with the various wave-lengths (see below) took place. Abscissae from 1 to 8 show minutes after cessation of bleaching. Size of *b*-wave before each period of adaptation plotted to zero abscissa

The adapting lights for the different curves are: (1) 0.540 , (2) 0.470 , (3) 0.585 , (4) 0.430 , (5) 0.560 , (6) 0.450 , (7) 0.500 , (8) 0.400 and (9) 0.450μ (a second time)

some other process tending to favour the response to the test light. This effect is transient to judge by the fact that the *b*-wave caused by the test light after 2 minutes is only 0.476 mV. At the end of the experiment (curve 9) bleaching with 0.450μ was repeated, this time starting from a smaller response to the test light (0.453 mV). The first response to the test light after 1 second has now risen to 0.484 mV. Again the value after 2 minutes is smaller, 0.453 . As the eye gradually deteriorates and the response to the test light grows less, the immediate after-effect of bleaching with certain short wave-lengths, *i.e.* the enhancement of the response to 0.500μ , alone is present. The slower secondary recovery process is absent, since after a preliminary rise, following upon bleaching with 0.450μ (curve 9),

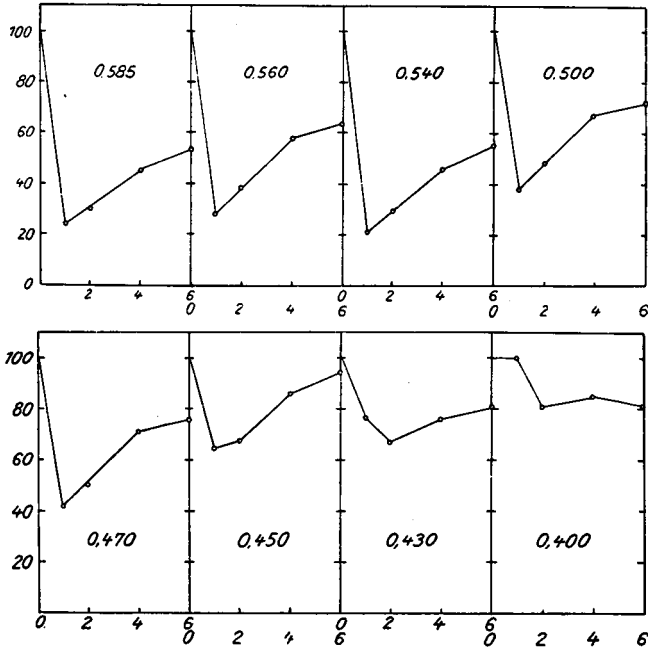


Fig. 3. The data of fig. 2 replotted to show relative effect of bleaching with different wave-lengths, and course of recovery. Adapting wave-lengths marked in the figure. Explanation of the curves in the text. Ordinates show reduction in response to test light from original value of 100 at zero abscissa. Abscissae from 1 to 8: time in min. from cessation of adaptation

the response remains practically constant. The remaining curves (7 and 8) illustrate the changes in the *b*-wave of the test light after bleaching with respectively 0.500 and 0.400 μ .

Disregarding for the moment changes in the size of the response to the test light owing to previous bleaching as well as to a possibly accumulating effect on the enhancement of the recovery caused by bleaching with short wave-lengths, let us reduce all initial readings for the test light before adaptation to a common ordinate. We then proceed to correct all values for visual purple absorption on the assumptions that

(i) the response to the test light at every moment is determined by the amount of visual purple available and

(ii) the size of the response to the test light is proportional to the square root of the energy absorbed by visual purple (Chaffee and Hampson, 1924 (Granit, 1937).

The curves of fig. 2 are then replotted in fig. 3 to show directly changes in concentration of visual purple immediately before and at various times after bleaching. The curves for the long wave-lengths, 0.540, 0.585 and 0.560 μ , tend to show a linear rate of recovery, those for the short wave-lengths illustrate the complications already indicated. We need not here describe them again as the figure is self-explanatory.

Significance of previous adaptations and wave-length of test light

It was noted with some surprise that bleaching with certain wave-lengths actually increased the size of the response to the test light. Hardly ever was this effect to be seen when a given preparation was bleached for the first time. The first bleaching tends to be relatively more effective in diminishing the response than later ones. However, that the wave-length of the adapting light is of relatively greater importance than the order in which adaptation is carried out is shown by Table I in which the effects of the bleaching lights 0.430 μ and 0.585 μ have been tried a number of times with the two eyes of the same frog. In one eye (to the left) bleaching was begun with 0.430 μ , then followed 0.585 μ , and then 0.430 μ a second time. In the other eye bleaching with 0.585 μ preceded adaptation to 0.430 μ . In both cases the two adapting lights caused their typical effects regardless of the order in which they were used. These two bleaching lights should give approximately equipotential responses with a dark adapted frog's eye and a low intensity spectrum. A similar pair is 0.540 and 0.470 μ . Most experiments were carried out with these two pairs in such a manner that for a given eye either pair was used at a given occasion.

Table I

Bleaching of the two eyes of a frog with wave-lengths 0.430 and 0.585 μ
Original value 100

		Left eye				Right eye			
Minutes after adaptation		1	2	4	6	1	2	4	6
Number of bleaching	Wave-length in μ								
1st bleaching	0.430	71	75	88	89	28	43	66	61
2nd bleaching	0.585	38	47	59	62	98	90	108	110
3rd bleaching	0.430	90	85	101	102				

A limited number of experiments were performed with wave-lengths 0.400 , 0.430 , 0.450 , and 0.580μ as test lights. Our main finding, *i. e.* that the short wave-lengths depressed the initial responses to these test lights far less than the long ones, was confirmed. Particularly instructive were the experiments carried out with 0.450μ as test light and 0.585 and 0.430μ as bleaching lights, as they were sufficiently numerous to establish beyond doubt the far greater bleaching effect of 0.585μ .

On rare occasions we have seen the response to the test light 0.500μ favoured by bleaching with light from the yellow region of the spectrum. This effect may therefore be potentially present in the long wave-lengths, though less marked there, and hence generally overpowered by the diminution in size of the test light caused by adaptation. Bleaching lights outside 0.590μ have not been used.

Averaged observations

The result of 70 bleaching experiments performed with 24 eyes of winter frogs have been averaged in fig. 4, based on the same principle as fig. 3. Great accuracy cannot be expected from averaging in this way readings obtained with different eyes at different times and with the adapting periods following in different order. But some idea about the processes concerned may still be obtained from this and the following figure.

Thus, within the first 4 minutes the recovery curves are linear for wave-lengths 0.585 — 0.470μ , suggesting a chemical reaction proceeding at a constant rate (cf. Wright, 1934, 1937). This simple relation breaks down beyond 0.450μ . It is possible that in an intact animal the readings after 6 minutes of recovery would have been higher. The readings for the first and second minutes after adaptation are nearly equal for 0.450μ . After bleaching with 0.430μ the value for 1 minute after adaptation exceeds the one taken 2 minutes later. The same holds good for 0.400μ , for which the effect of bleaching appears first after a latent period of 2 minutes, enhancement and depression in the meantime having fully balanced. However, at this wave-length only 5 experiments were available for the averaging of the results.

In order to complete this analysis we shall now compare the values for 1 minute after bleaching at different wave-lengths with the absorption curve for visual purple, plotted in broken lines in fig. 5. The latter curve is based on the work of Granit & Munsterhjelm (1937), and is derived from 801 measurements of size of potential in an equal energy low-intensity spectrum (same apparatus,

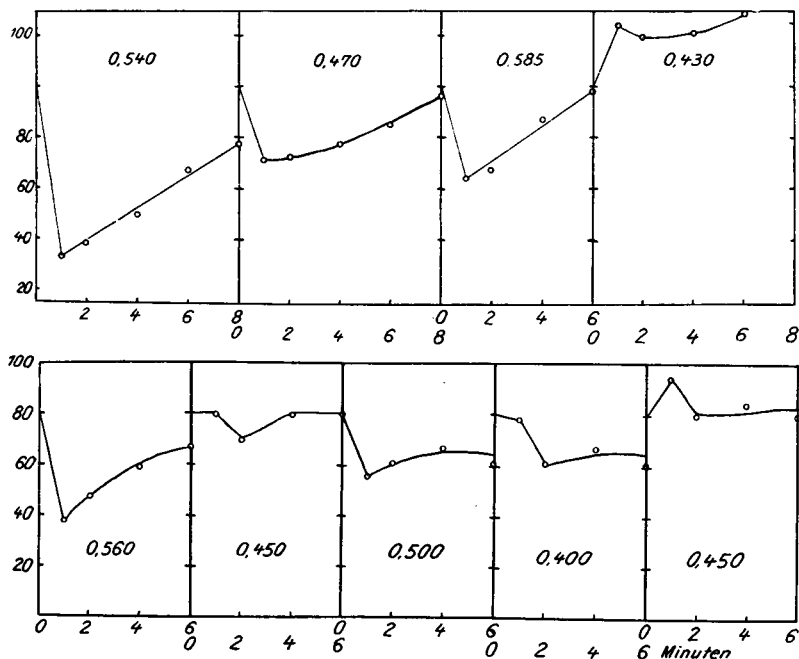


Fig. 4. Averages of all results, plotted as in fig. 3. Bleaching wave-lengths marked in the figure. Note: with wave-length 0.400μ as adapting light there is no reduction of the response 1 min. after adaptation. It follows after transient enhancement

but narrower slit-width). A correction for quantum intensity is not introduced as without it the values are directly comparable with those of fig. 4

The curve (fig. 5), drawn in full, has been derived in the following manner: assuming again visual purple to be the only substance concerned, the effect of bleaching should be proportional to its absorption curve, and thus be maximal at about 0.507μ . The best measure for the effect of bleaching would seem to be the difference between the original value of 100 for each wave-length of fig. 4 and the value for 1 minute after adaptation, in other words, the amount of reduction of the response to the test light caused by the bleaching lights. In order to correct for visual purple absorption we multiply those values by an "activity coefficient" obtained by dividing the maximal absorption coefficient for visual purple by the value for the absorption at the bleaching wave-lengths concerned. Thus, if the

absorption coefficients for visual purple for three wave-lengths are 100, 50, and 25, the corresponding "activity coefficients" are 1, 2, and 4.

As an illustration we choose the wave-lengths 0.430 and 0.585 μ . Adaptation to these wave-lengths reduces the response to the test light, given in terms of concentration of visual purple, from 100 to respectively 76 and 24 (see fig. 4). Hence the amount of reduction after adaptation and 1 minute of recovery is respectively 24 and 76. The

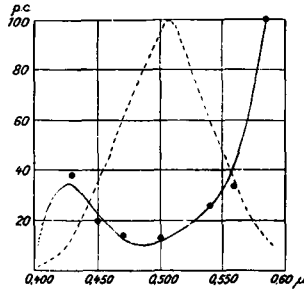


Fig. 5. Diagram showing the relative 'bleaching capacities' of different wave-lengths (curve drawn in full), calculated as explained in the text. The broken line illustrates absorption curve for visual purple as determined from measurements of size of *b*-wave of equal energy spectrum (Granit, 1937). As only 5 observations were available for wave-lengths 0.400 μ , the general direction of the curve is merely indicated in this part of the spectrum

"activity coefficients" for 0.430 and 0.585 μ are respectively 8.10 and 6.67. This means that 0.430 μ is nearly but not precisely equivalent with 0.585 μ from the point of view of visual purple absorption. However, the actual effect of bleaching with this wave-length, when the correction has been carried out, is 194 as against 504 for 0.585 μ . Thus wave-length 0.585 μ is about 2.6 times more effective as an adapting light than 0.430 μ . Actually the maximum of the corrected curve is at 0.585 μ . In fig. 5 the corrected curve is plotted in per cent of this value. The effect of bleaching is minimal somewhere around 0.470 μ . It again decreases towards 0.400 μ where visual purple absorption is very small.

In order to simplify our reasoning we have worked out our results in terms of the assumption that the effect of bleaching on a test light of wave-length 0.500 μ has had to be proportional to the absorption curve for visual purple. As the eyes were dark adapted the analysis must necessarily begin with this assumption; if only in order finally to prove that the effect has little or nothing to do with visual purple.

The experiments have later been developed along photochemical lines and a brief reference to the results obtained will simplify the discussion of the present findings. Photoelectric measurements of the concentration of visual purple of digitonin extracts of retinae made immediately after bleaching with wave-lengths 0.430 and 0.585 μ have shown: (i) that the effects of bleaching with these two wave-lengths from the Tutton monochromator, adjusted as in these experiments, is small, and (ii) that the great difference between the effects of 0.430 and 0.585 μ in the electrophysiological experiments is not reproduced by the photoelectrical measurements of visual purple densities. The experiments are being continued but it is not likely that the main conclusions drawn above would have to be substantially modified.

Discussion

There are no previous experiments with which these should be directly comparable. But in two admirable papers by Wright (1934, 1937) the recovery curves after adaptation to different wave-lengths have been measured for cone vision with a sensory binocular matching method. It is interesting to note that, despite the different experimental conditions, the results agree in showing a general process of recovery proceeding at a constant rate. More interesting still is that Wright with blue adapting lights likewise found complications of the type described in this paper, above all, a relatively faster rate of recovery after adapting with blue lights. This adds weight to the suggestion, put forth by Granit and Wrede (1937) and Wright and Granit (1938) that cone vision in the short wave-lengths is mediated by a substance with "rod-like" properties.

On the basis of the material presented in this preliminary communication it is neither possible nor desirable to suggest a final explanation of the results obtained. There may be one or several factors behind them. A simple but nevertheless questionable explanation ascribes the result to a blue-sensitive substance which either is present all the time or formed in the course of bleaching of visual purple and which is capable of catalyzing regeneration of visual purple. On the chemical side there is some evidence for this. In studying the bleaching of visual purple with yellow and blue lights Chase (1937) noted that regeneration was faster after bleaching with blue light. This effect was interpreted as being due to an accessory photo-sensitive substance favouring visual purple regeneration. However, as pointed out above, our own attempts to confirm a photochemical explanation along these lines have been unsuccessful. Despite the large difference

between the bleaching effects of 0.430 and 0.585μ , the concentrations of visual purple, as measured photoelectrically after extraction of eyes (bleached precisely as in the electrophysiological experiments), were identical within the limits of error.

The electrophysiological results themselves excluded the simple explanation that immediately occurred to us. The bleaching of the visual purple might have led to cones being released and becoming capable of making a relatively greater contribution to the response to the test light (as to the cone spectrum of the frog's eye see Granit and Wrede, 1937). The greater effect of bleaching with long wave-lengths would then have been due to the cones being themselves bleached by these wave-lengths. Dark adaptation again would have suppressed the cones. However, these receptors are relatively insensitive to short wave-lengths, and the transient enhancement of the response immediately (1 minute) after adaptation with short wave-lengths was noted also with 0.400 and 0.450μ as test lights.

Considering that the most essential feature of our results is that blue light apparently stimulates a process tending to enhance the response to the test light, and that this effect is transient, any tentative explanation of our results in the first instance would have to account for the minimum around 0.470μ rather than for the maximum in the long wave-lengths of the curve of fig. 5. In our opinion the most attractive hypothesis ascribes the effect to the "transient orange", discovered by Lythgoe (1937). According to this hypothesis the transient orange which is the first product of the breakdown of visual purple is assumed to be photochemically active and the formation of it or its stability favoured by blue light. The "hump" around 0.460μ in the curve showing size of the electrical response of a dark adapted frog's eye as a function of wave-length (Granit and Munsterhjelm, 1937) may also indicate activity of transient orange. A theory based on the indicator substance "visual yellow" would seem less plausible (see Lythgoe and Goodeve, 1937). But it should be pointed out that these suggestions are put forward merely to form a working hypothesis the soundness of which should be tested by further experimentation.

Since, despite 5 minutes of illumination with an adapting light of short wave-length, the response to the test light is sometimes but little depressed or may even be increased, it deserves to be emphasized that mechanisms of precisely this character would be needed in order to explain certain fundamental phenomena of colour vision and colour contrast. It is curious that only the general depressing effect of light adaptation, seen also in our experiments, and not the favourable effect

of greenish-blue lights has been noted before, and still more curious that the selective effect should appear in a dark adapted eye. A priori it would not have seemed worth while to undertake these experiments at all. Their main significance would seem to lie in the fact that as soon as a selective effect of wave-length has been found, a possible mechanism for colour differentiation in the retina has likewise been discovered, as by definition differentiation of colour is a selective effect of wave-length.

Summary.

The size of the electrical response to wave-length 0.500μ (the *b*-wave) has been measured in the originally dark adapted excised frog's eye before and after bleaching the eye for 5 minutes with some other wave-length in an equal energy spectrum.

Adaptation to long wave-lengths between 0.600 and 0.500μ causes a relatively greater depression of the response to the test light than adaptation to short wave-lengths between 0.500 and 0.400μ . With long wave-lengths recovery is linear for the first 4 minutes if given in terms of concentration of visual purple, as calculated from the size of the response to the test light. Bleaching with short wave-lengths makes recovery follow complicated curves, indicating that during adaptation with these wave-lengths a process tending to increase the size of the response to the test light likewise is activated.

When an eye has been adapted and allowed to recover a number of times, the first test response 1 minute after adaptation may be greater than the response following 1 or 2 minutes afterwards, sometimes even greater than the response given by the test light *before* adaptation. These effects are likewise seen with adapting lights between 0.450 and 0.400μ . They have only occasionally been seen with long wave-lengths.

An attempt has been made to compute the spectral distribution curve of the bleaching effect. It coincides with neither rod nor cone spectrum of the frog's eye determined with the same apparatus, nor can it be explained as a sum of the effects to be expected from these two spectra superimposed.

It is pointed out that the selective effect of wave-length on the retina, quite independently of what else it may signify, represents a mechanism that can be utilized for colour differentiation which by definition is selective sensitivity to wave-length and that it operates in the general direction of colour contrast.

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References.

- Chaffee, E. L. and Hampson, A., *J. Amer. Opt. Soc.* 1924. **9**. 1.
 Chase, A. M., *Science (N. Y.)*. 1937. **85**. 434.
 Dartnall, H. J. A. and Goodeve, C. F., *Nature*. 1937. **139**. 409.
 Graham, C. H. and Riggs, L. A., *J. gen. Psychol.* 1935. **12**. 279.
 Granit, Ragnar, *Nature*. 1937. **140**. 972.
 Granit, R. and Munsterhjelm, A., *J. Physiol.* 1937. **88**. 436.
 Granit, R. and Wrede, C. M., *J. Physiol.* 1937. **89**. 239.
 Kahn, R. H. and Löwenstein, A., *Arch. Opth. (Graefe)*. 1924. **114**. 304.
 Köttgen, E. and Abelsdorff, G., *Z. Psychol. Physiol. Sinnesorg.* 1896. **12**.
 161.
 Lythgoe, R. J., *J. Physiol.* 1937. **89**. 331.
 Lythgoe, R. J. and Goodeve, C. F., *Trans. Opth. Soc.* 1937. **57**. 88.
 Therman, P. O., Neurophysiology of retina in the light of chemical methods
 of modifying its excitability. *Acta Soc. Scient. Fenn. Nova Series. B. 2*.
 Helsingfors 1938.
 Trendelenburg, W., *Z. Psychol. Physiol. Sinnesorg.* 1904. **37**. 1.
 Waller, A. D., *The signs of life*. London, 1903.
 Wright, W. D., *Proc. Roy. Soc. B.* 1934. **115**. 49.
 Wright, W. D., *Proc. Roy. Soc. B.* 1937. **122**. 220.
 Wright, W. D. and Granit, R., On the correlation of some sensory and phy-
 siological phenomena. *Brit. J. Opth. Monogr. Suppl.* In print. London
 1938.