

Spectral Properties of the Visual Receptor Elements of the Guinea Pig.

By

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The assumption that mammalian rod-eyes with a relatively small number of cones for this very reason may present the experimenter a simplified and hence analyzable picture of colour reception has already proved its value in the case of the albino rat studied with the microelectrode technique (GRANIT, 1941 a). In this animal I found after light-adaptation a narrow "red" sensitivity band with maximum in 0.600μ coupled to a "green" one. The latter was a steep and narrow curve with the maximum of the absorption curve for visual purple. The light-adapted frog's eye, on the other hand, had several narrow "modulator" bands and in addition a broad "dominator" absorption band with maximum in 0.560μ (GRANIT, 1942), that is, a band practically identical with the human photopic (day-light) luminosity curve. This region of the spectrum was empty in the rat's eye.

The same microelectrode technique has now been applied to the retina of the guinea pig which also has been found to lack a broad photopic "dominator" band and hence to be without a Purkinje shift in the strict sense of the term. But in selecting the guinea pig for the next analytical step I was fortunate in striking an animal with a remarkably prominent "blue" element. The results with this retina thus serve to justify the assumption underlying this experimental survey of the spectral properties of the receptive units in different retinae.

Method.

The guinea pig is anaesthetized with 4—5 cc 20 % urethane intraperitoneally, the skin cut away around the eye which then with a few stitches through the conjunctiva is tied to the surrounding tissue. When this has been done the cornea is cut away, the lens removed, and the animal placed under the binocular preparation microscope to be illuminated by its lamp at full strength (about 2,400 m. c.) if light-adaptation be wanted. This light was strong enough to depress the sensitivity of the eye to or below the threshold of the spectrum of my Tutton monochromator (model Hilger). Bleeding is much easier to avoid in this preparation than in the albino rat.

The microelectrode is inserted with the aid of a micromanipulator and a reactive spot found, characterized by large repetitive spikes upon illumination, and often also by spontaneous firing. The energy necessary for the absolute threshold or for a just perceptible increase of the spontaneous discharge has been determined by listening to the amplified spikes in a loudspeaker and simultaneously observing them on the screen of a cathode ray oscillograph. For this technique and the energy control of the spectrum the reader is referred to the paper by GRANIT and SVAETICHIN (1939). The whole eye is illuminated by the spectral light. As to sources of error with mammals, see author's paper on the rat's eye (1941 a).

Results.

Some General Observations.

In the guinea pig, as in the rat, it is relatively easy to obtain large isolated spikes of activity. Fig. 1a shows such a spike responding repetitively to light somewhat above the threshold for wave-length 0.540μ ; fig. 1b shows a spontaneously active element responding with an increased frequency to stimulation with wave-length 0.500μ . The variation in the size of the latter is probably due to a slight movement of the eye or the animal to judge by its gradual onset and disappearance. (The spike is an impulse discharged along the optic nerve, probably picked up near the ganglion cell, and not a b-wave (!), as has been contended. This term refers to an at least hundred times slower wave of the electroretinogram).

The spike is caused by the discharge of a single ganglion cell or else by some cells synchronized so well as to be inseparable. In this animal the typical discharge is a simple increase in frequency caused by illumination, an "on"-discharge. "On"- "off"-

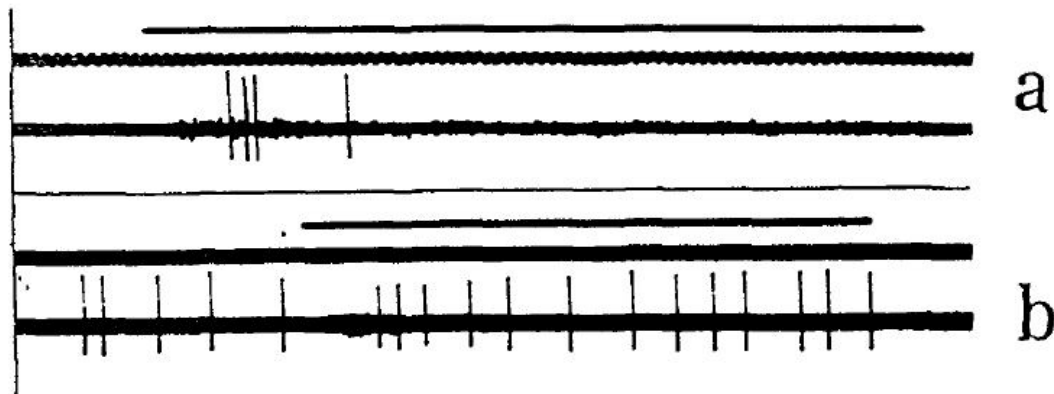


Fig. 1. a, discharge of isolated spike to stimulation with wave-length 0.540μ slightly above threshold. b, spontaneously active spike responding with increase of frequency to stimulation with wave-length 0.500μ . Light signal above each record and time in $\frac{1}{50}$ sec.

discharges are not so common and pure "off"-discharges, so often seen in the frog's eye (cf. also HARTLINE, 1938), must be rare in this retina. These observations on the type of discharge have been made in passing, and are by no means systematical. Nevertheless the large number of receptors surveyed in work of this character gives a fairly good idea of the relative distribution of different types in different types of eyes.

Average Curves for Photopic and Scotopic Retinae.

By a series of observations is meant the averaged values for the spectral sensitivity (inverse value of energy necessary for the threshold) of a given active unit or group of units. Behind each series is thus a smaller or larger number of individual measurements, dependent upon how long the unit has kept up its discharge, or, if light-adapted, how long it has been possible to follow it before it has dark-adapted, as nearly all units sooner or later do (cf. GRANIT, 1941, a, b, 1942). As in other eyes, with both rods and cones, a slow recovery for all wave-lengths precedes dark-adaptation proper, characterized by a relatively sudden onset of a fast rise of sensitivity around 0.500μ , the maximum of the absorption curve for visual purple. During the phase of slow recovery the photopic values have to be recorded.

In order to obtain curves for the average photopic and scotopic (dark-adapted) eyes it is possible to use (i) the electroretinogram which in itself is an average, or (ii) to average all series for either state of adaptation independently of the spectral distribution of sensitivity of the individual elements. I have here

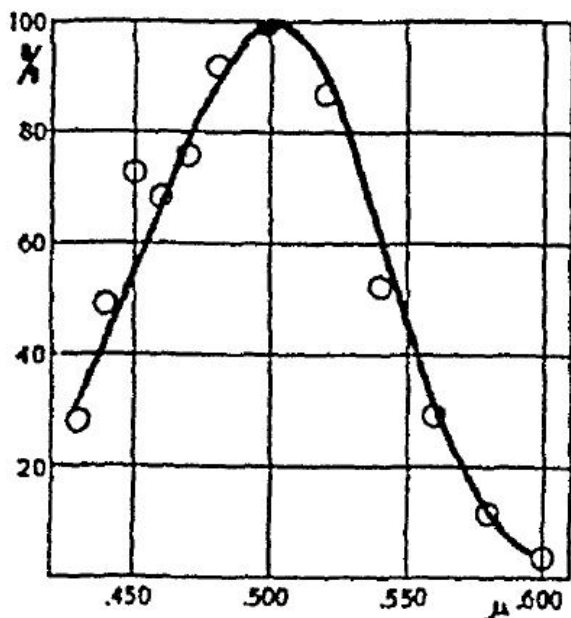


Fig. 2. Absorption curve for visual purple. The circles are averages from 20 series of measurements of spectral sensitivity of 16 scotopic guinea pigs. 137 averaged values. Spectrum of equal quantum intensity.

comprised 171, the photopic series 137 averages which now in their turn were averaged in order to show the mean spectral sensitivity of light- and dark-adapted animals.

The values obtained are shown in figs. 2 and 3, corrected for an equal quantum intensity spectrum. The scotopic curve drawn (fig. 2) is actually LYTHGOE'S (1937) absorption curve for visual purple which gives a good fit. The large value in 0.450μ comes from two series with a markedly blue-sensitive element despite dark-adaptation. The photopic curve is drawn through the averaged values and LYTHGOE'S curve is indicated by the small black dots. There is a marked rise in the blue after light-adaptation but the maximum is still in the same place as in the scotopic eye.

used the latter method alone because of the little amount of potential available in the photopic eye. In averaging the series I have left out a number of casual observations in intermediate wave-lengths, as otherwise it would have been necessary to use the less reliable procedure of interpolating the corresponding values for the majority of the series in which only certain standard wave-lengths were used. For averaging were available 30 photopic series from 19 animals and 20 scotopic series from 16 animals. The photopic series

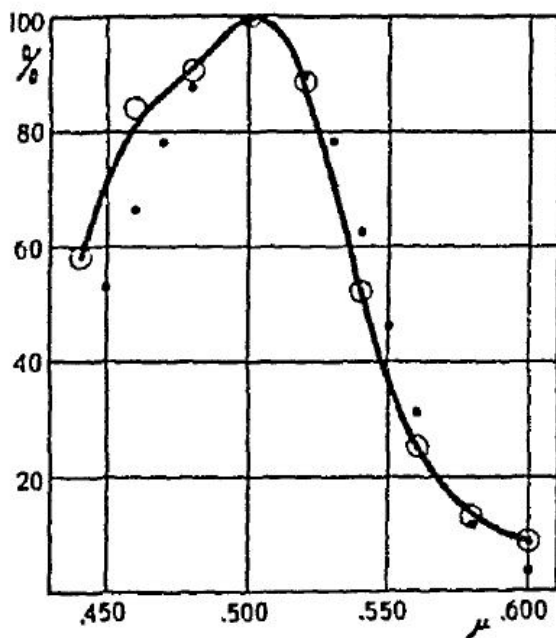


Fig. 3. Distribution of spectral sensitivity of the averages from 30 series obtained from 19 photopic animals. 171 averaged values. Black dots visual purple. Spectrum of equal quantum intensity.

Analysis of the Average Curves.

In the dark-adapted retinae of the guinea pig the spectral sensitivity curves for single spikes are identical within the errors of measurement. There is practically no indication of a systematic difference from the average curve. Not so after light-adaptation. Just as in the frog's retina (GRANIT, 1942) there is in the guinea pig a definitely higher sensitivity to blue light in some elements, particularly in an intermediate stage of recovery from light-adaptation, somewhat before dark-adaptation sets in. If all elements with maximum in the blue are grouped together, and the readings averaged, the curve of fig. 4 is obtained. This

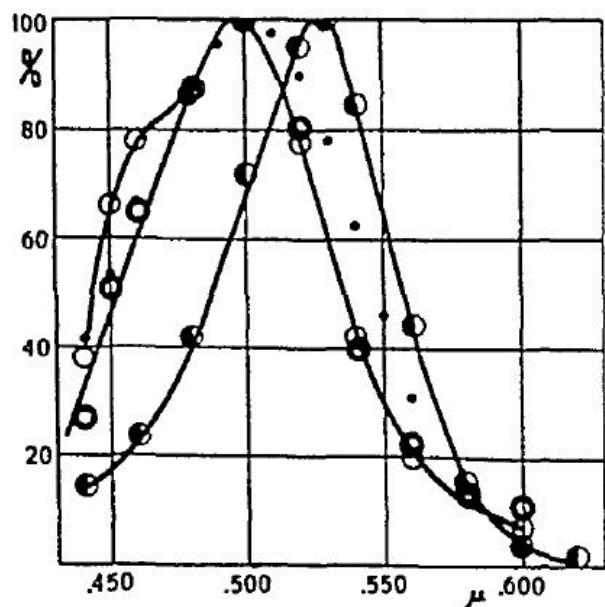


Fig. 5. *To the right:* Averages of all readings from photopic series with maximum around 0.530μ . *To the left:* Do with maximum around 0.500μ , falling into two groups either possessing or lacking hump in the blue. Black dots visual purple. All corrected for an equal quantum intensity of the spectrum.

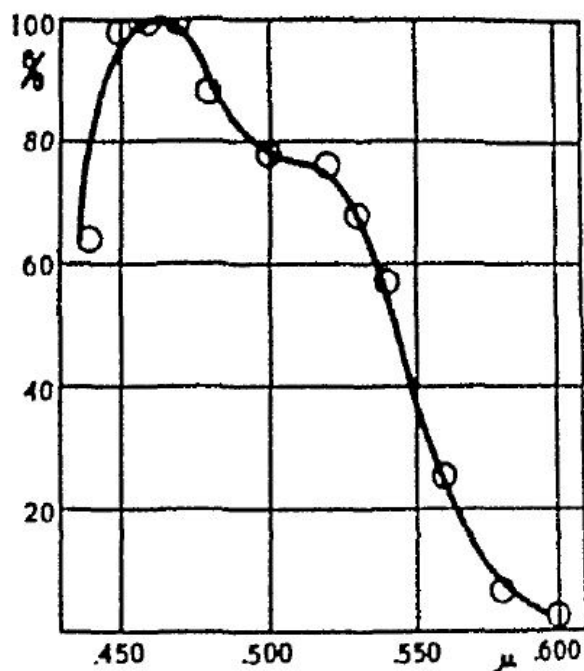


Fig. 4. Averages of all readings from photopic series with primary or secondary maximum in the blue, corrected for equal quantum intensity.

curve has a secondary hump in the green. Only once I have seen a single element with maximum in 0.450μ and as low as 40 % sensitivity in 0.500μ .

However, the most common elements have their maximum in 0.500μ . Of these there are two kinds: the one possessing, the other one lacking a hump in the blue. These are shown to the left in fig. 5. The two curves do not differ significantly in the red. When the hump is lacking it can be seen that the curve falls inside LYTHGOE'S absorption curve for visual purple and thus represents an abnormally steep and

narrow visual purple distribution. The same type of element was found in the rat's eye (GRANIT, 1941 a).

Relatively rare is the element to the right in fig. 5 with maximum in 0.530μ . I should have doubted its presence had it not been possible in one of these experiments to make 53 observations with a spike lacking spontaneous activity. Initial

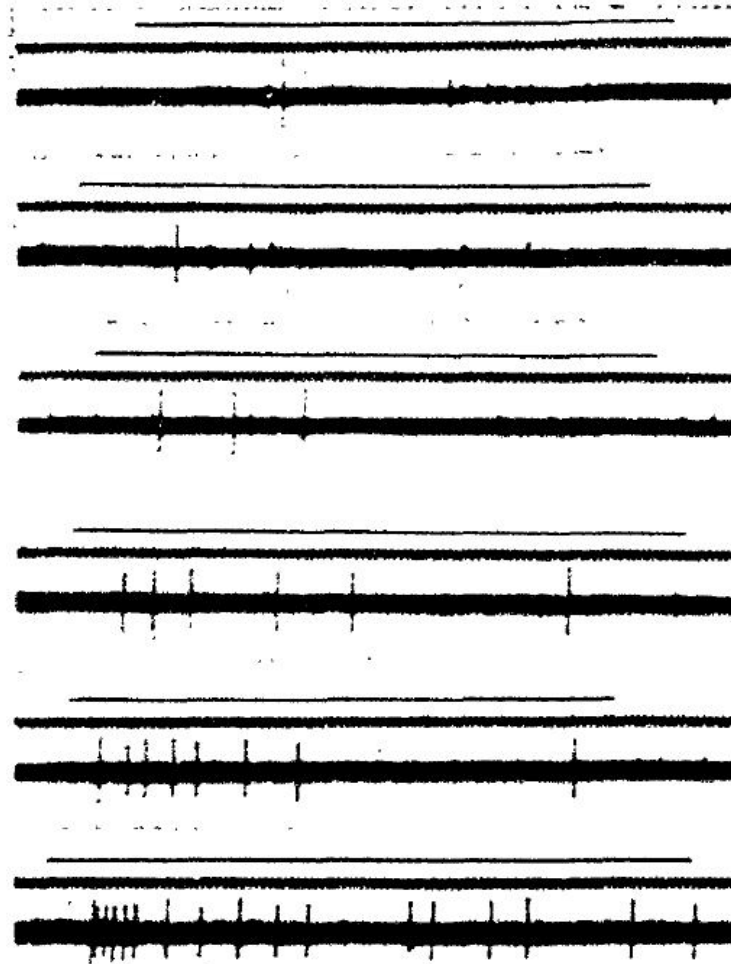


Fig. 6. Isolated unit with maximum in 0.530μ responding to different intensities, increasing from record to record downwards. The uppermost value near threshold at relative sensitivity ($1/E$): 16.0. The other inverse values of the energies used for eliciting these responses are (downwards) 14.2, 10.8, 7.2, 4.1 and 1.5.

silence under the electrode greatly enhances the accuracy of the experiment. In fig. 6 is given a number of photographs of the response of this particular unit together with the inverse values of energy necessary for eliciting the responses illustrated. In fig. 7 is found the spectral distribution of sensitivity of this unit. Elements of this type, narrow bands with maxima around $0.530-0.540 \mu$, have also been found in the frog's eye (GRANIT, 1942).

The dominant impression from the actual experimental situation is that invariably there is low sensitivity towards the red, the curves in this region falling inside the absorption curve for visual purple, but unpredictable great variations in the blue from case to case, and that the maxima in general are gathered around 0.500μ . This impression is based also on a great number of less systematic experiments than the ones leading to more or less complete series.

Nevertheless I am not absolutely certain that a "red" element can be wholly excluded. There are some rare cases in which the values around 0.600μ have tended to be at or just above 10 % of the maximum which is a little too much even for visual purple (about 3 %).

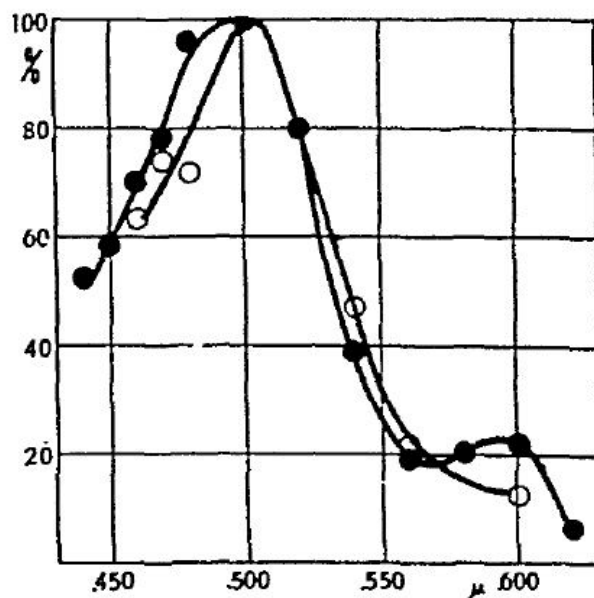


Fig. 8. Spectral distribution of sensitivity of a group of spikes with indication of secondary rise in 0.600μ . Open circles refer to readings taken 1—20 min. after cessation of light-adaptation, filled circles same from 20—50 min. afterwards. Equal energy spectrum.

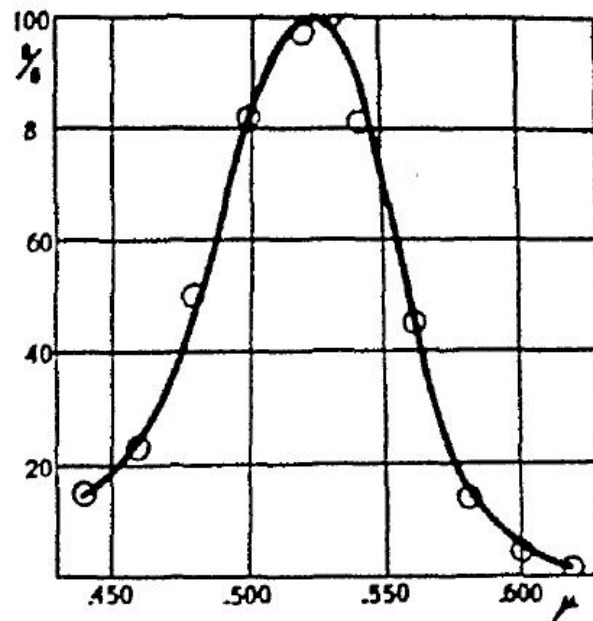


Fig. 7. The spectral distribution of sensitivity of the active unit of fig. 6. 53 averaged values corrected for equal quantum intensity.

But absorption caused by intraretinal vessels or bleeding in the eye could perhaps explain these cases (cf. GRANIT, 1941 a). One such case is shown in fig. 8. The rat's eye has a well defined maximum in this region (GRANIT, 1941 a). But in its eye the "red" receptor quite often is the only one left immediately after light-adaptation whereas in the eye of the guinea pig wavelength 0.600μ generally is below the threshold of the monochromator until some recovery from light-adaptation has taken place.

Conclusion.

In animals with good colour vision such as fishes, the tortoise and the frog (GRANIT, 1941, a, b, 1942) there has always been found a broad "dominator" photopic band carrying the Purkinje shift. But in the rat, and now in this animal, all the sensitivity bands have been of the narrow "modulator" type. These are also found in animals with good colour vision from which I have concluded that they must serve to modulate the spectral distribution of the dominator. The latter must be mainly responsible for photopic white (brightness). Brightness, after all, is the fundamental sensation of the eye. So far I have seen a dominator band in only one of the three mammals hitherto examined, *i. e.* in the cat (GRANIT, 1942 unpublished).

The guinea pig thus has three narrow modulators, one no doubt identical with the narrow band in the rat's eye representing some simple modification of visual purple. The "green" modulator with maximum in 0.530μ and the "blue" band are probably identical with corresponding bands in the frog's eye.

The experiments have shown that the "blue" and the visual purple green (0.500μ) are difficult to isolate in the photopic eye of the guinea pig. This may be simply a matter of convergence of receptors of different type towards the same ganglion cell from which recording takes place. Other physiological consequences of convergence have recently been analyzed by HARTLINE (1940) in the frog's optic nerve. But for some reason or other the "blue" band was easier to separate from visual purple in the frog's eye where it also was of the narrow modulator type with maximum between $0.450-0.470 \mu$. As a first approximation I shall therefore assume that the "blue" band of the guinea pig's retina is of a similar kind and of a width intermediate between the two "green" bands of this eye. Its maximum should perhaps be placed nearer to 0.450μ on account of the fact that it never goes to the right of 0.460μ , despite an additional green component tending to expand it in this direction.

Synthesis of the Average Photopic Curve.

The three elements found must combine to give the average photopic curve of fig. 3 for this animal. The form and position of two of the elementary curves will be taken to be given directly

by the experiments, whereas the third, the "blue" one, is drawn in accordance with the principles set forth above. The question is now in what proportion these elementary modulator curves should be combined to give the average curve for the light-adapted guinea pig's eye.

This problem may, of course, be solved by a method of trial and error, but since I actually solved it with the aid of a simple assumption this assumption may just as well be mentioned in this paper. Having no particular reason to hold one of the elements to be more sensitive to light inside its own range than another I took them to be of equal sensitivity and assumed

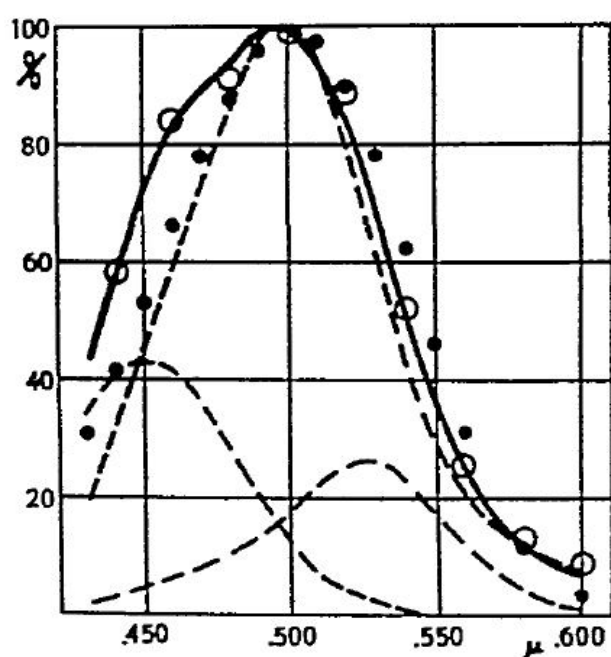


Fig. 9. Curves in broken lines represent the three elementary narrow "modulator" bands in a proportion adding up to curve drawn in full. The circles are from fig. 3 and thus refer to averages of all light-adapted guinea pigs. Black dots visual purple. See text. Spectrum of equal quantum intensity.

the height of the individual curves to be proportional to the number of elements of this particular type. An approximative value for the relative number of each colour element could be obtained experimentally from the number of incidence of each type of maximum in the 30 photopic series. Let the incidence of the most common element with maximum around 0.500 μ be 100; then the "blue" element has occurred 42.8 times and the 0.530-element 26.4 times. The curves shown by the broken lines of fig. 9 illustrate the three elementary modulators in this proportion.

These modulator bands combine to the curve drawn in full which, as the figure shows, gave a good fit with the values from fig. 3. I have then neglected the possibility of a very rare "red" modulator which would be so low as to be of little significance. The modulators would permit some discrimination in the green, blue and violet regions of the spectrum and the brightness perception (luminosity curve) would in both states of adaptation roughly reproduce the absorption curve for visual purple, corrected for losses in the cornea which here has been removed.

Whether the animal utilizes this mechanism of colour discrimination, laid down in the retina, is of less interest from the present point of view. I have not found any reliable experiments designed to answer this question.

Is the "Blue" Substance Lactoflavin?

Lactoflavin was first found in the retina by H. v. EULER and his collaborators (v. EULER and ADLER, 1933). Elsewhere I have reviewed the literature on this subject (GRANIT, 1938). Eyes of fishes are particularly rich in lactoflavin but it is chiefly, if not exclusively, found in the pigment layer. As it absorbs maximally around 0.450μ in the visible spectrum it *could* be the "blue" substance of the eyes of guinea pigs and frogs, or else it may serve to extend the blue side of the spectrum by fluorescence, as has also been suggested by v. EULER and ADLER.

Since sensitivity to blue is well marked in the guinea pig and low in the albino rat (GRANIT, 1941 a) it seemed worth while to compare the lactoflavin content of these two types of mammalian eyes. Prof. H. v. EULER and Dr ADLER kindly undertook to make these measurements. I am greatly indebted to them for this generous aid and for permission to publish their figures.

Their method has been described elsewhere (ADLER, v. EULER, GUENTHER and PLASS, 1939). The experimental material consisted of 100 retinæ of albino rats and 68 retinæ of guinea pigs. The rat's retina was found to contain 0.005—0.01, the guinea pig's retina 0.01—0.015 γ of lactoflavin. The quantities found are thus very small and the difference, though in the right direction, does not either suggest that the reception of blue in the guinea pig is mediated by lactoflavin.

The only alternative explanation, at present worth considering, is the possibility that the "blue" substance is a photoproduct of bleached visual purple, discovered by LYTHGOE (1937) and called by him "transient orange". LYTHGOE and QUILLIAM (1938) have preliminarily placed its maximum in 0.470μ but at least one of their curves indicates a maximum in 0.460μ .

Summary.

Microelectrode, amplifier and cathode ray have been used for the recording of "spikes" of activity from isolated elements of

the retina of the guinea pig in response to monochromatic light of known energy distribution.

The average distribution of sensitivity to spectral light of photopic (light-adapted) and scotopic (dark-adapted) eyes suggests that visual purple is the main substance mediating sensations in this eye. These curves are given in figs. 2 and 3.

Individual units of scotopic retinae follow the absorption curve for visual purple very closely.

In the photopic state most units are activated by a narrow sensitivity band with the maximum of visual purple; some units respond to a narrow band with maximum in 0.530μ . But there are also elements with maximum in the blue region around 0.460μ . As a rule these units cannot be isolated from those with maximum in 0.500μ .

But assuming the "blue" elements to represent narrow bands of the type given by the "green" ones (maxima in 0.500 and 0.530μ) the average photopic curve, which possesses a hump in the blue region of the spectrum, can be combined by adding up the three elementary curves in the right proportion. Thus it has become possible to give a complete description of the spectral properties of the photopic elements in the guinea pig's eye.

Isolated elements are easily obtained in this eye and their reaction types have been described.

The possible relation of the "blue" substance to lactoflavin and "transient orange" has been discussed. Of all retinae hitherto studied this one has the most prominent sensitivity to blue light.

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