

**A Relation between Rod and Cone Substances,**  
Based on Scotopic and Photopic Spectra of *Cyprinus*, *Tinca*,  
*Anguilla* and *Testudo*.<sup>1</sup>

By

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Two as widely different animals as man and the frog have the same visual purple with maximal absorption around  $0.500 \mu$  and also much the same daylight or photopic "visibility" curves with maxima around  $0.560 \mu$ . For man the sensation of brightness has been the index needed in measuring these curves. For frogs, however, the number of impulses, a threshold discharge or the size of the electroretinogram has been the index used. In the former case we generally speak of a "luminosity" curve, but probably the frog perceives brightness much as man does. Nevertheless the neutral, but very awkward term "visibility" curve is commonly used for animals, and quite often also as a substitute for "luminosity" when human sensations are concerned. As is well known PARSONS has introduced the adjectives "photopic" and "scotopic" for respectively daylight- and dim light- vision, and taking up them I shall use the brief terms "photopic" and "scotopic spectra" for daylight and dim light "visibility curves".

We now know that the photopic spectrum of the frog's eye represents a composite curve (GRANIT and SVAETICHIN, 1939) consisting of narrow elementary sensitivity bands, but also, as I have been able to confirm later, of broad bands very much like the average photopic curve, but lacking its expansion into the blue end of the spectrum. Further out than in  $0.600 \mu$  I

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## 2. Photopic and Scotopic Spectra of *Cyprinus*.

Use of the string galvanometer with this fish, of which only a limited number was available, necessitated relatively strong light in order to obtain a sufficiently large deflection as index of sensitivity. This means that both photopic and scotopic elements are stimulated simultaneously, at least in the dark-adapted state. According to WUNDER (1925) this eye has roughly 38

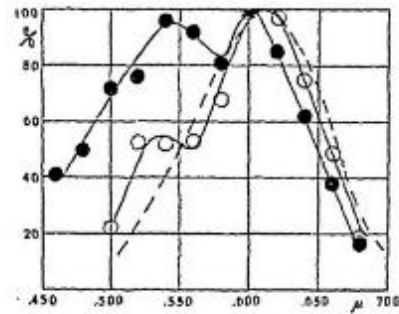


Fig. 1. Average distribution of sensitivity to supra-threshold spectral light of ● 3 scotopic and ○ 2 photopic carp. Broken line traces curve for isolated "red" element of the tortoise (GRANT, 1941, a, b). String galvanometer. Spectrum of equal quantum intensity.

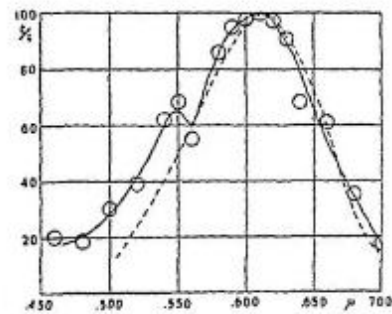


Fig. 2. Average of 230 readings from eyes of 14 tortoises (*Testudo graeca*), recorded with microelectrodes. Broken line as in fig. 1. Spectrum of equal quantum intensity.

rods per 12 cones, or a relatively high number of cones. The *a*-wave proved a better index than the *b*-wave.

Fig. 1 shows the photopic and scotopic spectra of three dark-adapted and two light-adapted carps. The scotopic curve clearly is composite and has two maxima, in 0.540 and in 0.600  $\mu$ . After light-adaptation the "green" half of the curve is greatly diminished and the remaining curve has expanded towards the long wave-lengths. Light-adaptation is probably not complete. The curve in broken lines illustrates the distribution of sensitivity of the isolated "red" receptor of *Testudo* (GRANT, 1941 a, b).

## 3. The Photopic Spectrum of *Testudo*.

In this practically pure cone-eye there is no difference between the photopic and scotopic curves. The eye only increases in sensitivity during dark-adaptation. The interesting point here

is the likeness between the photopic spectra of fish and the cone spectrum of the tortoise.

From this point of view it seemed of particular interest to average all the available data on the tortoise's eye independently of the type of curve to which they may have belonged. The curve of fig. 2 shows the result. The 230 average readings

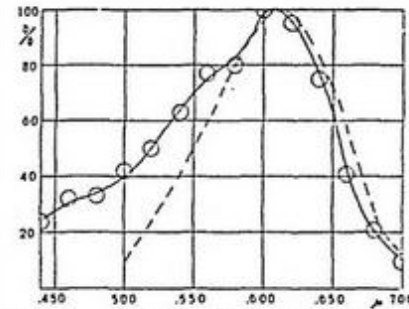


Fig. 3. Averages of 90 readings from 4 eyes of the tortoise. Broken line as in fig. 1. String galvanometer. Spectrum of equal quantum intensity.

were all obtained with microelectrodes. The curve for the isolated "red" element is also shown in the same figure. The average curve is almost identical with that of photopic carps, but its form is, of course, also determined by the relative number of readings in the different regions of the spectrum.

Now the number of observations in the short wave-lengths were few owing to the ease with which red-sensitive elements are obtained and the difficulty of finding other ones, so that it was deemed necessary to add a few observations on the composite response recorded with the string galvanometer. The eye of *Testudo* gives chiefly negative electroretinograms with relatively small positive phases (BERNHARD, 1941). Therefore the *a*-wave was used as index of sensitivity. The curve obtained is shown in fig. 3. The presence of blue-sensitive elements is indicated by the hump in the short wave-lengths. But I have never succeeded in isolating a blue element in this eye, be it then that they do not come isolated or are few in number or of low sensitivity relative to the energy available in my spectrum. Some caution in interpreting this result is also suggested with all red-sensitive eyes owing to the fact that the diffusely spreading light of the spectrum is reddish in character.

Only once I have found a "green" maximum coupled with relatively low red-sensitivity. This curve is shown in fig. 4. Its maximum could well be caused by a low concentration of visual violet.

The photopic spectrum of the frog's eye is composite and for this reason it is of some interest to show a number of typical curves from the eye of the tortoise. Fig. 5 illustrates that there

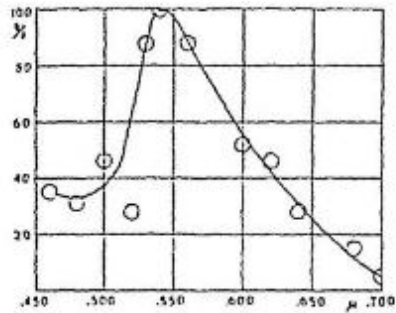


Fig. 4. Micro-electrode record of distribution of sensitivity of discharge with maximum in 0.540  $\mu$  in the retina of a tortoise. Spectrum of equal quantum intensity.

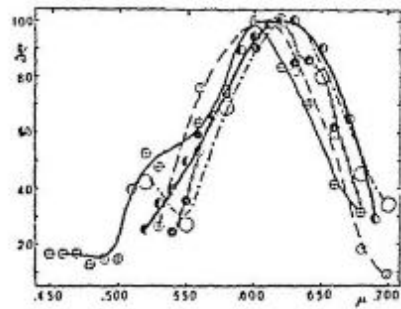


Fig. 5. Showing individual curves for the distribution of sensitivity to spectral light in tortoises. Recording with micro-electrodes. Equal energy spectrum.

is considerable variability also in this eye among the dominantly red-sensitive elements.

#### 4. The Scotopic and Photopic Spectra of *Tinca*.

Microelectrodes were used in all work with the tench. The relation of rods to cones in this fish is about 33:15 (WUNDER, 1925). The average curve for dark-adapted fish is given in fig. 6 where it is being compared with the absorption curve of the visual violet of the same animal. The latter curve is from a paper by BAYLISS, LYTHGOE and TANSLEY (1936) devoted to spectrophotometric analysis of visual purple in fishes. There is close agreement, except in the blue, where again is found a secondary rise. If dark-adaptation is insufficient or the light too strong, there is also found a hump around 0.600  $\mu$  just as in similar circumstances the frog's eye tends to give a hump around 0.560  $\mu$  (GRANT, 1937). As some light is needed to insert the micro-electrode, it is necessary to wait some time before one begins to read off the values in the different regions of the spectrum.

In fig. 7 is illustrated the average photopic curve of *Tinca*. It tends to be somewhat broader towards the red end than the curve for the isolated "red" element of *Testudo*, shown in the same figure. There is still more variability among the "red" elements of this fish than in the tortoise. The reason for this may be that more observations from the red end were available. But I am rather inclined to believe that the greater varia-

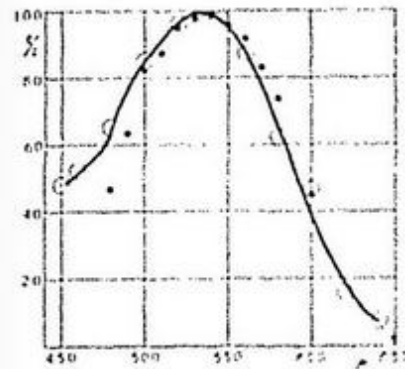


Fig. 6. Averages of 148 readings from the retina of 6 dark-adapted tenches. Micro-electrodes. Some isolated "spikes", some diffuse discharges. Black dots spectrophotometric values for visual violet, determined by BAYLISS, LYTHGOE and TANSLEY (1936). Spectrum of equal quantum intensity.

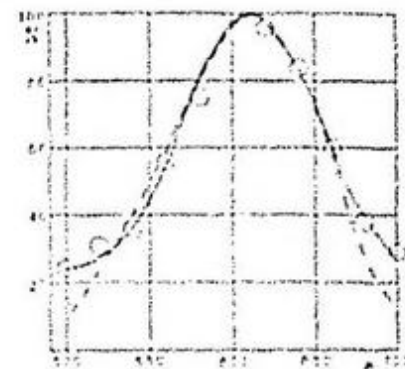


Fig. 7. Averages of 245 readings from the retina of 15 light adapted tenches. Micro-electrodes. Some isolated "spikes", some diffuse discharges. Broken line traces isolated "red" element of *Testudo* as in fig. 1. Spectrum of equal quantum intensity.

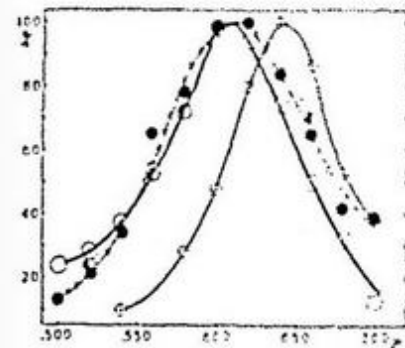


Fig. 8. Showing individual curves for the distribution of sensitivity of light-adapted tenches. Micro-electrodes. Equal energy spectrum.

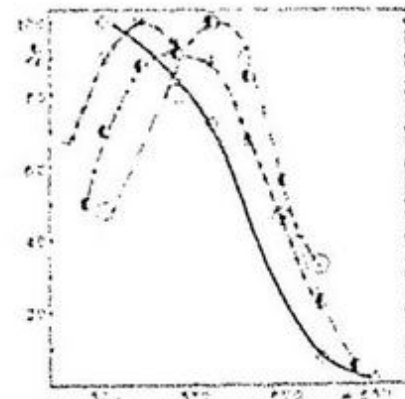


Fig. 9. Showing individual curves for the distribution of sensitivity of light-adapted red. Micro-electrodes. Equal energy spectrum.

tion is physiological. Occasionally one could find a maximum as far out as in 0.640  $\mu$  with the tortoise, but such curves were more often seen with the tench. Also there were some very narrow curves in the eye of *Tinca* just as in the frog's eye. Samples of individual photopic curves are shown in fig. 8.

### 5. The Photopic Spectrum of *Anguilla*.

The so-called freshwater eel has a visual purple of the type found in frogs and mammals (BAYLISS, LYTHGOE and TANSLEY, 1936). Its retina is dominated by rods but is also supplied with cones (BAYLISS, LYTHGOE and TANSLEY, 1936). The eyes of eel survived better than those of the other fishes and it could easily be shown that during dark-adaptation the spectrum shifted to the region of  $0.500 \mu$ . But the attention in this work was devoted to looking for the extremely red-sensitive receptors which are found everywhere in the retinae of carp, tench and tortoise. To this end the microelectrode was shifted from spot to spot in a search for such red-sensitive elements. But the search proved fruitless. In the 5 fishes available only curves of the type previously seen with the frog's eye were found. Samples are given in fig. 9. The shortening of the spectrum towards the red end is very obvious by comparison with the curves shown in the previous figures.

As a matter of fact it proved very difficult to light-adapt eel. Most curves tended to give secondary maxima between  $0.500 \mu$  and  $0.560 \mu$  and it was impossible to find any maxima outside  $0.560 \mu$ . Strong light-adaptation of the opened excised eye tended to depress the sensitivity below the threshold of the energy available in the spectrum. The reason for this is probably the relative paucity of cones (rods: cones = 143:7, WUNDER, 1925). The sensitivity to red was remarkably low also in absolute terms and not only by comparison with the maxima found. An interesting fact was the tendency of the slightly dark-adapted eel's retina to discharge for several minutes when left illuminated by the light of the binocular microscope.

#### Relation between the Visual Purples and the Cone Substances.

KÖTTGEN and ABELSDORFF (1896), GRUNDFEST (1932) and BAYLISS, LYTHGOE and TANSLEY (1936) all came to the conclusion that the maximum of the difference spectrum of visual violet was located between  $0.535$ — $0.540 \mu$ , but the last mentioned authors also describe a number of other forms of visual purple with maxima intermediate between the two previously known substances. WALD (1939) objects to the use of difference

spectra and seems to hold that there are only two substances, the visual purple (*rhodopsin*) and the visual violet, which he calls *porphyropsin*. The latter substance is found in freshwater fishes. According to his view intermediate maxima must be given by mixtures of rhodopsin and porphyropsin. This question cannot yet be regarded as experimentally decided though WALD's suggestion seems probable. But it is of no immediate interest here as the eyes of the tench (BAYLISS ET AL.) and the carp (SAITO, 1938, WALD, 1939) only contain visual violet. The maximum of this substance, if not measured as a difference spectrum, is between  $0.520$  and  $0.530 \mu$  (SAITO, 1938, WALD, 1939). The measurements presented above for the tench show the values for  $0.520$  and  $0.540 \mu$  to be respectively 96 and 100 %. The difference is small and absorbing filters may influence electro-physiological measurements and also spectrophotometric ones, until solutions of visual violet can be guaranteed to be chemically pure.

Too much emphasis should not be laid on the precise distance in the spectrum between, on the one hand, visual purple and the photopic spectrum, and, on the other hand, visual violet and the photopic spectra found in fishes possessing porphyropsin. The photopic spectra are dominated by components with maxima which may be some  $0.050 \mu$  apart, components which may represent narrow or broad sensitivity bands. Though in the eyes of fish having visual violet they average out to a maximum between  $0.600$  and  $0.620 \mu$ , individual narrow curves in the frog's eye also may reach a maximum as far out as in  $0.600 \mu$ . But then individual sensitivity bands in the eye of the tench may go still some  $0.050 \mu$  further out into the red. And this is not a peculiarity of fish cones as such since the photopic eye of the eel containing the ordinary visual purple is even less sensitive, on an average, to red than that of the frog.

The principal conclusion must therefore be that in eyes containing visual violet with absorption shifted further out towards the red end by comparison with the type  $0.500 \mu$  visual purple, the photopic spectra of the dominating cone substances are also shifted further out in the same direction. This result is difficult to understand, particularly in the light of the experiments with *Anguilla*, unless it be assumed that the cone substances are chemically related to the rod substances. This assumption is as old as the knowledge of visual purple but it is now based on facts which otherwise are difficult to explain except as sheer coincidences! It is quite possible that such substances are being formed in the

rods during illumination. Such changes I found it necessary to assume in order to explain results with the rat's eye (GRANIT, 1941 c) but these substances must also be *preformed* in the cones. The discovery in *Testudo* of a cone-system with similar properties as those found in photopic eyes containing visual violet shows that *preformed* cone-substances cannot be distinguished from those found after light-adaptation in fish.

Visual violet and visual purple behave similarly in solution but the spectra of bleached products are, for the latter substance, uniformly displaced 0.020—0.030  $\mu$  towards the red (WALD, 1939). Thus, for instance, the yellow carotinoid retinine<sub>1</sub> extracted by WALD after decomposition of visual purple has its counterpart in a deep yellow retinine<sub>2</sub> obtained from similarly treated solutions of visual violet. Of great interest is the fact that the vitamin A<sub>1</sub> in the eyes of frogs and fishes possessing visual purple is replaced with vitamin A<sub>2</sub> in eyes having visual violet (WALD, 1939).

We may regard visual purple as a chromoprotein (WALD, 1935—36; KRAUSE and SIDWELL, 1938) with a protein carrier in which every molecule "carries" about 10 chromophores (BRODA, GOODEVE and LYTGOE, 1940). It can easily be imagined that a small shift in the structure of the linkage between chromophores and carrier suffices to change the absorption spectra and stabilize the structure into something less sensitive to light than the original form. WEIGERT (see summary by WEIGERT and MORTON, 1940) has made interesting models of visual purple dissolved in gelatine reproducing characteristics of colour reception. And the "green" substance of the rat's eye has been shown to have the absorption spectrum of a "narrow" visual purple curve (GRANIT, 1941 c).

v. STUENITZ (1937, 1940) has studied "cone substances" from the eyes of frog, fish and tortoise and in all cases found maxima around 0.560  $\mu$ . Actually the cone substance of the tortoise, as we have seen, has its maximum shifted about 0.050  $\mu$  towards the red from that of the frog. This difference is not reproduced by v. STUENITZ's methods and his results for the tortoise thus do not show anything like the order of agreement found between my results and the measurements of visual purple absorption spectra.<sup>1</sup> For this reason his work fails to convince.

<sup>1</sup> "In Bezug auf ihre Lichtabsorption verhalten sich die Ätherextrakte aus Fisch- und Froschnetzhäuten genau gleich den von den Schildkröten erhaltenen." (v. STUENITZ, 1937).

Evidence for a common origin of rod and cone substances has also been supplied by JEANS and ZENTMIRE (1934) who in the belief that they measured dark-adaptation of the rods studied the early phase of dark-adaptation with a sensory method and found this phase to be sensitive to lack of vitamin A in the food. To those acquainted with the subject of vision it was clear that cone-adaptation was being studied. With improved technique HECHT and MANDELBAUM (1940) confirmed their results and drew the obvious conclusion which JEANS and ZENTMIRE had missed.

### Colour Reception.

A discussion of colour reception is better postponed to theoretical papers dealing with all the results assembled with different types of eyes. The results presented in this paper show that colour reception in fishes and tortoises is organized on the principles previously described for frogs (GRANIT and SVAETICHIN, 1939) and rats (GRANIT, 1941 c). It is also well known that fishes can be trained to react on the basis of a high degree of colour discrimination. (See *c. g.* a review by v. FRISCH, 1925, and later papers mentioned in a book by v. STUENITZ, 1940).

In this connexion I only want to point out the limitations of the micro-electrode technique which chooses the common types of elements at the expense of rare ones. From this point of view it is interesting to compare the average curve from the eye of the tortoise, taken with string galvanometer and large electrodes (fig. 3), with the curves obtained with micro-electrodes (fig. 5). There is a hump in the green and one in the blue region, but with the micro-electrode I have only once had a definite "green" maximum and never a blue one, though some of the "red" curves have had a hump in the blue region. With my very much larger material of curves from the frog's eye I have about 3—4 % of definitely and predominantly "blue" elements. If with the very red-sensitive fishes and tortoises the expansion and secondary humps in the blue and violet region merely were due to irradiated reddish light in the spectrum it would be difficult to understand the high degree of selectivity with which sensitiveness to short wave-lengths occurs from experiment to experiment. Considering that fishes are red-sensitive it is remarkable how far out into the violet they are capable of reacting to light. This is also the experience of those who have studied

colour discrimination with training methods (see *c. g.* SCHIEMENZ, 1924; WOLFF, 1925).

The explanation of the fact that dominantly blue- or violet-sensitive elements have not been found with the micro-electrodes must be that these elements are relatively few or else that they are coupled to the other ones and thus not independent. The curves indicate that these elements would be sufficiently sensitive, at least in the eyes of the tench, to be above the threshold of the apparatus.

In the tortoise it is clear that the broad average curve recorded with string-galvanometer hides other elements than the "red" one which is so easy to isolate.

### Summary.

The distribution of sensitivity to spectral light of light-adapted ("photopic spectra") and dark-adapted ("scotopic spectra") fishes and the tortoise (*Testudo graeca*) have been determined for isolated or a restricted number of elements with micro-electrodes, leading off from the retina to amplifier, cathode-ray and loudspeaker. In some cases the average distribution of sensitivity has been directly recorded with string-galvanometer.

Fishes with "visual violet" absorbing maximally around 0.530–0.540  $\mu$  have scotopic spectra determined by the absorption curve of this substance; fish (eel) with "visual purple" absorbing maximally around 0.500  $\mu$  have a scotopic spectrum determined by the latter substance. The sensitivity to short wave-lengths, however, is greater than is to be expected from spectrophotometric measurements of visual purples.

After light-adaptation fishes with visual violet give an average photopic spectrum with maximum between 0.600–0.620  $\mu$  whereas the photopic curves of the eel (visual purple) do not have maxima further out than in 0.560  $\mu$ .

The fact that the photopic curves for different species are shifted towards the red end in proportion to the scotopic curves of the same species is regarded as evidence for chemical inter-relationship between rod and cone substances.

The practically pure cone-eye of the tortoise is found to have its system of colour reception organized on the same principles as that of photopic fish with eyes containing visual violet.

The average curves are composite curves, consisting of ele-

mentary curves, some of which are broad, others narrow, and having maxima which may be some 0.050  $\mu$  apart in the spectrum. This should form a basis for colour discrimination.

In addition it is necessary to postulate at least one separate substance sensitive to short wave-lengths.

Comparison of the average curve for the tortoise, as recorded with string-galvanometer, with that of the isolated "red" element shows that the latter alone cannot be responsible for the high values and the "hump" in the green and the blue regions of the spectrum.

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