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The visual pigments and their location in the retina

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The faculty of perceiving light and colour which is one of our most precious posessions and plays an immensely important role in our lives is made possible by the marvellously organised structure of the retina and its connections with the cerebral centres. It is not surprising, therefore, that the details of that structure have been the subject of innumerable researches in the past. It is appropriate that we commence the present communication by briefly recalling those features of the retinal structure which have a bearing on the subject which will be dealt with here.

The retina may be described as an outlying part of the central nervous system to which it is connected by a tract of nerve fibres, namely the optic nerve. The nervous structure is encased within two coats which serve the purposes of protection and nutrition. Externally, we have the fibrous tunic which is white and opaque, namely, the sclera. Between this and the retina is a layer of which the function is primarily nutrient. This is known as the choroid and is a tissue almost entirely composed of blood vessels. Behind this again lies the retina which functions as the organ for the reception of visual impressions.

The retina itself is a multi-layered structure. The two innermost layers adjoining the choroid coat are respectively the pigment epithelium and the layer of rods and cones. These latter are recognized as the visual receptors. The two outermost layers of the retina are the so-called inner limiting membrane and the layer containing the optic nerve fibres. Between these two sets of layers appears an elaborate organisation of connective cells, pictures of which will be found in the anatomical treatises.

The area of the retina can be usefully divided into two parts, a central region measuring five to six mm in diameter and the peripheral part which is a much larger area surrounding it. An important part of the central retina is the area known as the *fovea*. This is a shallow rounded pit of which the diameter is about 1·5 mm. At the bottom of this pit is the area known as the foveola which is about 0·3 mm across. The depression of the fovea below the general level is due to the practical disappearance of the inner layers of the retina, compensated somewhat by the increased thickness of the layers containing the rods and cones. Outside the fovea and in the central retina, two further regions have been recognized and distinguished from each other on morphological grounds, namely the *parafovea*

which is a belt 0·5 mm wide all round the fovea, and a second belt known as the *perifovea* which is about 1·5 mm across.

It is well known that the *fovea* plays a highly significant role in human vision. It is the region of the retina on which the image of any object falls towards which we direct our vision. When the fundus of the living eye is viewed through an ophthalmoscope, the fovea can be glimpsed at the centre of the region of the retina known as the macular area. The fovea is seen somewhat more conspicuously with the ophthalmoscope when the fundus is viewed in red-free light, it being then visible as a spot of yellowish hue surrounded by a greenish-yellow field. This effect arises from the presence of a yellow pigment in the macular area which permeates diffusely the retinal tissues from the outer nuclear layers inwards.

The present communication is concerned with the visual perception of colour and the part played by the retina in such perception. It is useful here to recall the basic facts of the subject. The visible spectrum is comprised in the wavelength range between 400 and 700 mμ, the perceived colour altering continuously from one end of the range to the other. It may be demarcated into six regions, designated as violet, blue, green, yellow, orange and red respectively; the limits between them are indicated in figure 1 by broken vertical lines whose positions have been taken as 436, 495, 566 and 627 mμ respectively. The luminous efficiency of visible radiation reaches very low values at either extremity of the range. Intermediately, as shown in figure 1, it reaches a maximum at 560 mμ, in other

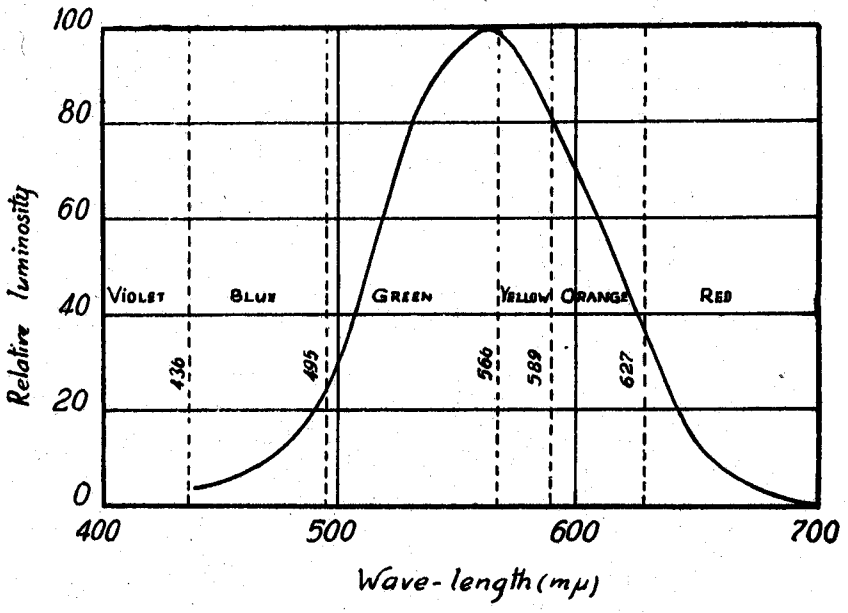

Figure 1. Luminous efficiency of the visible spectrum.

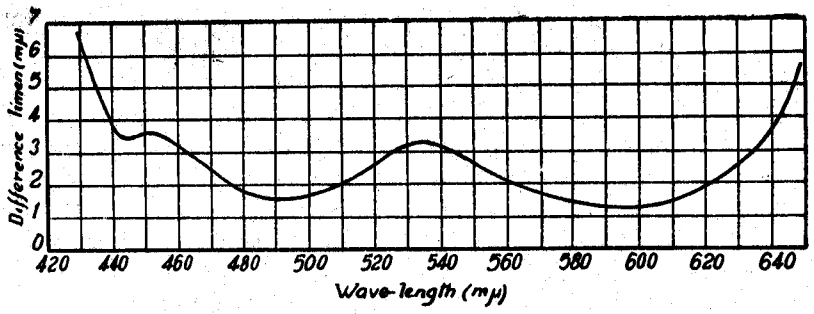

Figure 2. Hue discrimination in the spectrum.

words not far from the boundary between the green and yellow sectors of the spectrum. The most rapid increase of the luminous efficiency as we move towards greater wavelengths appears at about 520 mμ and the most rapid fall at about 590 mμ.

Figure 2 shows the hue discrimination curve, in other words, the smallest change in wavelength which manifests itself in vision as a perceptible change of colour in various regions of the spectrum. It will be noticed that except near the ends of the spectrum, a change of 4 mμ is more than sufficient to produce an observable change of hue. Indeed, over the greater part of the spectrum, the power of colour discrimination is much greater. Dips in the curve appear at 444, 492 and 595 mμ, these wavelengths being nearly the same as those at which the observed colour changes from violet to blue, from blue to green and from yellow to orange respectively.

The present communication records the results obtained and the conclusions reached from a study of the functioning of the retina in its central region in terms of colour sensitivity. The method of investigation is that devised by the author and described in earlier publications by him, but it has now been much improved by reason of the attention paid to important details of the technique. The method of observation makes use of a set of colour filters so chosen or prepared that they are more or less completely opaque to a limited region of the visible spectrum but transmit other parts of the spectrum freely without any sensible absorption. Holding such a filter in front of his eye, the observer views a brightly illuminated white screen, fixing his vision at some particular point on the screen and after a short interval of time, varying from a few seconds to a few minutes according to the circumstances of the case, suddenly removes the filter from before his eye. He then observes on the screen an enlarged picture of his own retina, the nature of which varies with the filter used. From the nature of the picture seen, the spectral sensitivity of the retina and its variations over its central region, and especially the dependence of such sensitivity on the choice of the spectral region may be inferred.

How the effects observed in this manner with the aid of the colour filters arise is

an important question regarding which some remarks of a preliminary nature may be made here. It is evident that if the colour filter cuts off a limited part of the spectrum, in the light which reaches the observer's eye through the filter that part of the spectrum would be missing, and hence it would also be missing in the light falling on the retina. The interval of time during which the observer views the screen through the filter is much too short for any retinal fatigue to be produced by the parts of the spectrum transmitted by the filter. *But it may suffice to enhance the sensitivity of the retina to the parts of the spectrum cut off by the filter.* The extent of such enhancement may be expected to depend on the circumstances of the case, viz., the part of the spectrum screened off by the filter, the state of adaptation of the retina to light before the filter is put in, the illumination of the screen which is viewed and finally the duration of time for which the filter is held in front of the eye before it is removed.

In the earlier studies by the author, the colour filters employed were gelatine films on glass stained to the desired extent. While there is much to be said in favour of such filters, it has been found desirable in critical studies to use instead, aqueous solutions of various dye-stuffs contained in glass cells, 10 by 10 cm^2 in area and 2·5 cm in depth, which is then the effective absorption path. The advantage of using such cells is that the filter may be quickly prepared by dissolving a little of the dye-stuff in distilled water, and then by diluting the solution to the desired extent. The spectrum of the light transmitted by such a filter may be observed at different dilutions and the state of dilution may be adjusted suitably. This procedure is very helpful, since strong solutions of the dye-stuffs used absorb extensive regions of the spectrum, but when sufficiently dilute the region of cut-off is greatly restricted and may indeed then be confined to the specific absorption bands characteristic of the dyestuff.

Amongst the dye-stuffs which had been employed in the present investigation may be mentioned the following, the names being those under which they are commercially available: (1) Acridine orange, (2) Eosine, (3) Rhodamine, (4) Coomassie brilliant blue, (5) Methyl violet and (6) Lissamine green. Of particular importance are the observations made with solutions of these dye-stuffs of such dilution that the absorption is strong or nearly total in a limited region of the spectrum, while the rest of the spectrum is freely transmitted. Using this technique, the entire spectrum may be surveyed in detail. It emerges that it divides itself according to the observed results into five distinct regions: (1) 400–495 mμ, (2) 495–540 mμ, (3) 540–560 mμ, (4) 560–590 mμ, and (5) 590–700 mμ. Absorption only in the first of these regions may be obtained with appropriately diluted solutions of acridine orange. Absorption appearing only in the second region may be obtained with very dilute solutions of eosine; an absorption appearing only in the third and fourth regions with dilute solutions of Coomassie brilliant blue or methyl violet and in the fifth region alone with very dilute solutions of lissamine green.

The experimental results may be briefly summarised as follows. Working in the

first region which comprises the blue-violet parts of the spectrum, the observer notices on the screen following the removal of the filter, a blue glow covering the entire area of the screen. In the second region, namely 495–540 mμ no effect of any kind is noticeable, since on the removal of the filter, the observer sees the white screen as before. In the fifth region, namely 590–700 mμ, following the removal of the filter the observer sees a rose-red glow covering the entire screen. Using filters whose absorption is effective in the third and fourth regions, in other words between 540 and 560 mμ and between 560 and 590 mμ, very striking effects which reveal the structure of the retina are observed. The actual picture of the retina seen by the observer with such filters exhibits colours depending on the part of the spectrum which is cut off or weakened by the filter. But its general configuration is sufficiently well illustrated by the drawing in black and white reproduced as figure 3.

Four distinct areas appear in figure 3, whose correspondence with the different parts of the central retina may be checked by actual measurement of the angular dimensions of these features as seen on the observing screen. Around the point on the screen at which the observer's vision is fixed appears the foveal disk (enormously magnified) with the central pit or foveola and the umbo or navel very distinctly noticeable therein. Surrounding the fovea is seen a third region which appears encircled by a distinct halo along its margin. Outside this appears a fourth region without any distinct outer limits defining its extension. In general,

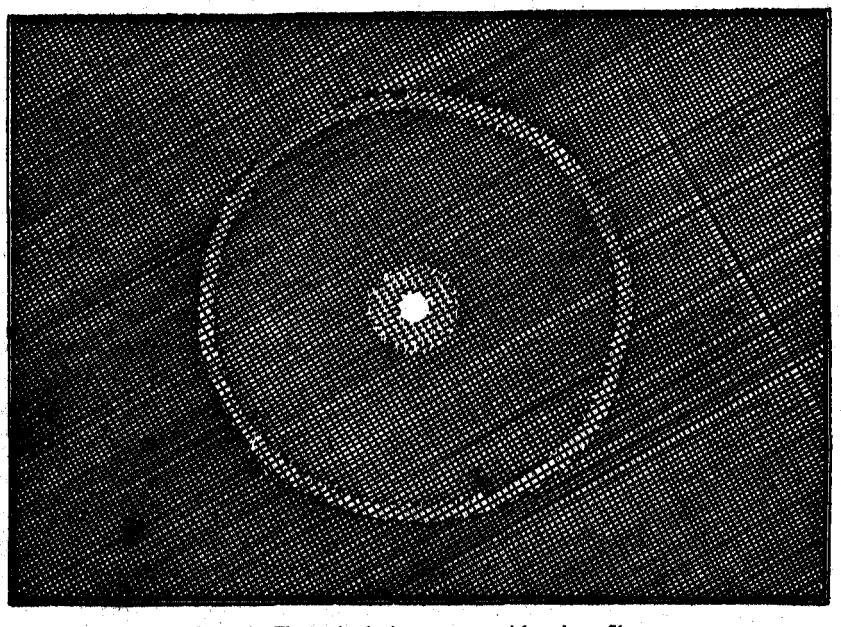

Figure 3. The retinal picture seen with colour filters.

by far the most luminous part of the picture is the fovea and this also exhibits vivid colour. The parafoveal and perifoveal regions are less luminous and distinctly less colourful.

Observations made with solutions of Coomassie blue, methyl violet and lissamine green in different states of dilution make it evident that the retinal picture seen with the filter technique is different in the two cases where the absorption by the filter appears respectively in the third and fourth spectral ranges, viz., 540–560 mμ and 560–590 mμ. In the third range, the fovea appears as a disc of a green hue and the perifovea also appears of the same colour. When the absorption is in the fourth range, the fovea is seen as of a bright yellow colour and the perifovea is likewise of that hue though less brilliant.

We may now proceed to consider what the observations described above signify in relation to the role played by the retina in the perception of colour. The effects observed are of a transitory nature in every case, but they differ enormously in the different parts of the spectrum. These differences are evidently connected with the variations in the luminous efficiency of radiation in the different parts of the spectrum. In the blue-violet and in the red regions of the spectrum, the effect observed following the removal of the filter is a glow covering the entire screen. It is noteworthy that for such an effect to be observed, it is necessary to hold the filter for an appreciably longer period in front of the eye. It may therefore be reasonably explained as due to the sensitisation of the retina for the wavelengths absorbed by the filter by its screening effect. On the other hand, the luminous efficiency of the spectrum is fairly high in the region between 495 and 540 mμ and it is not surprising, therefore, that no effect at all is observed when the filter which has an absorption in this region is put in front of the eye and then removed.

We may now proceed to consider the explanation of the effects pictured in figure 3. They appear only when the colour filters used have absorptions in the wavelength range lying between 540 and 590 mμ. This spectral range is precisely that where the luminous efficiency of the spectrum is highest. It follows that the origin of these effects is quite different from those of the effects observed in the blue-violet and red regions of the spectrum which have very low luminous efficiencies. That the character of the effects is very different is also not surprising.

A reasonable explanation for the effects pictured in figure 3 appears to be that the visual pigment functioning in the spectral region between 540 and 590 mμ is not identical with those functioning in the blue-violet, green or red sectors of the spectrum and that it is distributed in a highly non-uniform manner over the central part of the retina, being concentrated in the foveal area and in the regions immediately surrounding it. Following the removal of the filter, the regions of the retina containing the pigment under reference are lit up and flash into view. Such an effect can, of course, only be transitory. But it is worthy of note that it is restored in full strength when the filter is quickly put back and then again suddenly removed. The same procedure may be repeated as often as desired,

thereby enabling the details of the retinal picture to be carefully studied. We are justified by these facts in inferring that what is actually perceived is a picture of the distribution of the visual pigment over the area of the retina under examination exhibiting the part of the spectrum incident on it and in which it functions as a receptor.

It thus emerges from the present investigation that the visual pigment which functions in the yellow sector of the spectrum and is responsible for the very high luminous efficiency and the very high power of colour discrimination indicated by figures 1 and 2 for that part of the spectrum is quite distinct from the pigments which function in the red and green sectors of the spectrum and is not a mere superposition of these two pigments functioning jointly. The identification of that pigment presents a problem which will not be discussed here. But a useful hint is furnished by the observations which indicate that the pigment has *two* maxima of absorption, one between 540 and 560 mμ, another between 560 and 590 mμ, the latter being much the more pronounced of the two. Incidentally, it may also be remarked that the concept of three visual pigments or three fundamental sensations which forms the core of the Young–Helmholtz theory of vision is contradicted by the results of the present study and is therefore unsustainable.