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The new physiology of vision—Chapter XV. The chromatic responses of the retina

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It is of fundamental importance for an understanding of the nature of vision to know how the retina in the living state reacts when light is incident on it. Neither anatomy nor biochemistry can possibly furnish us with that knowledge. We may here recall how immensely useful the ophthalmoscope is by its enabling us directly to view the living retina and observe its state or condition. Likewise, it is evident that any technique by which the process of the excitation of the retina by light is brought within the scope of direct observation would be of the highest importance for our understanding of the visual processes. Soon after the author commenced the investigations of which the results are described in the present treatise, a technique was developed by him which had evidently great possibilities of becoming just such a tool of research as that envisaged above. Since then the technique has been improved to such an extent as to make it a precise and reliable method for the study and analysis of our visual sensations. We shall proceed to describe and explain the technique and to indicate in general terms the nature of the results which emerge.

The principle of the method: The essence of the method of observation is the use of a colour filter which freely transmits light over the entire range of the visible spectrum except over a limited and well-defined region which it completely absorbs. It is possible by the use of suitable dye-stuffs in appropriate concentrations to prepare colour filters of gelatine films on glass exhibiting the spectroscopic behaviour described. Holding such a colour filter before his eye, the observer views a brilliantly illuminated screen for a brief interval of time and then suddenly removes the filter while continuing to view the screen with his attention fixed at a particular point on it. He then observes on the screen a picture in colours which is the chromatic response of the retina to the light of the colour previously absorbed by the filter and which impinges on it when the filter is removed. Actually, as will become clearer presently, what the observer sees is a highly enlarged view of his own retina projected on the screen and displaying the response of the retina in its different areas produced by the incidence of the light of the selected wavelengths. By using a whole series of colour filters whose characteristic absorptions range from one end of the visible spectrum to the other, we are enabled to explore the behaviour of the retina over an extensive region (including especially the foveal area) under excitation by light of different wavelengths which in the aggregate cover the entire visible spectrum.

Why the phenomenon described above manifests itself is not difficult to understand. A colour filter completely absorbing a selected part of the spectrum when placed before the eye of the observer protects the retina from the incidence of light from that part of the spectrum, and if such protection continues for a sufficient period of time, it has the result of sensitising the retina for the reception of light of those wavelengths when the filter is removed. *Per contra*, light of wavelengths not absorbed by the filter being incident on the retina both when the filter is in position and after its removal, the visual sensation which it excites becomes enfeebled by the continued exposure. Accordingly, when the filter is removed, the visual response of the retina to light of the wavelengths for which its sensitivity has been enhanced is far stronger than the continuing response to the other wavelengths and manifests itself vividly to perception. The nature of the picture seen is determined by the part of the spectrum which is absorbed by the colour filters and differs enormously for the different filters employed in the study. The usefulness of the technique for the study of the functioning of the retina over its different areas is thereby vastly enhanced. As in the analogous case of the Haidinger brushes discussed in an earlier chapter, we have to take note of the essentially fugitive nature of the phenomenon. But here, again, this is no obstacle to the study of the effects. For, the image of the retina seen by the observer on removing the colour filter and which fades away is restored and can be examined again and again merely by putting back the filter in front of the eye for a little while and then removing it.

Preparation and use of the colour filters: From what has been stated above, it will be evident that the quality of the filters employed is of great importance. This includes especially the complete transparency over the visible spectrum except in a limited region where there is a complete absorption. It is therefore useful to record here how such filters are prepared. Old and unused photographic plates form excellent material for their fabrication. They are first put in a fixing bath in a dark room and kept long enough to completely remove the sensitive material. They are then washed in running tap-water for half an hour to eliminate all traces of the material of the fixing bath. The plates are then taken out and put in a tray containing distilled water and allowed to remain there for the gelatine to become quite soft. This is necessary to enable the gelatine to absorb the colouring material quickly and evenly. A small quantity of the selected water-soluble dye is put into a beaker containing distilled water and stirred well. The solution obtained is then filtered through a clean cloth to remove any undissolved particles and the clear solution poured into a developing dish of appropriate size. The plate is then immersed in the solution of the dye-stuff and by varying the time of such immersion (depending on the particular dye-stuff) the depth of colour taken up by the gelatine can be controlled. Several different shades of colour on different plates can thus be obtained. The dyed plate is then taken out and washed in water quickly and kept aside for drying. The filters thus prepared are labelled and kept arranged in closed boxes for use as and when needed.

From amongst the numerous dye-stuffs available, several were selected after a preliminary examination of the transmission of light by their aqueous solutions. In all, some 30 different dyes were chosen and some 150 colour filters were prepared therefrom. Four, or five, or six different depths of colour were fixed on plates for each dye, as the comparative study of the effects observed with such a sequence of filters was found to be useful and instructive. To examine the spectral transmission by the filters, the most convenient procedure is for the observer to view the first-order diffraction spectrum of the glowing filament in a tubular lamp through a replica grating and the filter held together before his eye, and to notice the effect on the spectrum of removing the filter. The comparison with each other of the filters of different depths of colour made with the same dye can be quickly effected in this fashion.

For an observer to study the results of using the colour filters in the manner explained above, a screen of the kind used for projection work containing a great many small glass spheres embedded in plastic is found to be particularly suitable. Placed facing the windows in a well-lighted room, such a screen is quite brilliant and this indeed is necessary for any impressive phenomena to be observed. With a screen 175 × 120 cm in area, 350 cm is a convenient distance from the screen for the observer to station himself. The area of the screen under observation is then of sufficient width to include an enlarged picture of an extensive region of the retina. That what the observer notices when the filter is removed is a picture of his own retina becomes evident when it is remarked that the foveal disk is the central feature seen in every case. This is located at and around the point on the screen at which the observer's attention is fixed at the instant of withdrawing the filter from before his eye.

We shall proceed to describe one after another the effects observed with the filters prepared with various individual dyes and their relation to the spectral characteristics of the filter. The integrated picture of the retina which emerges from these studies will form the subject of a later chapter.

Filters of crystal violet: Colour filters prepared with this dye exhibit quite spectacular effects. Extremely conspicuous is the brilliant disk of green colour appearing at the centre of the field around the point of fixation of vision at the instant of removal of the filter. Its position as well as the actual size of the disk show it to be a highly enlarged image of the fovea of the observer's own retina. At the centre of the disk a bright spot can be seen which is evidently the foveola, in other words, the bottom of the foveal depression in the retina. The foveal disk also exhibits a rim which is distinctly brighter than the region inside. The foveal disk also exhibits a rim which is distinctly brighter than the region inside. There are also indications of a radial structure visible within the area of the disk. Outside the foveal disk and concentric with it, the observer notices an extensive area of circular shape of which the diameter is some five times greater than that of the foveal disk, but which is much less luminous than the latter. This area exhibits a greenish-yellow colour, much less saturated in hue than the green of the foveal disk. The outer margin of this area appears fairly well defined. Beyond this circular area and surrounding it is a region exhibiting an orange-yellow colour.

A sketch of the effects described above is reproduced as figure 1. The differences in colour and luminosity between the foveal disk and the surrounding areas cannot, of course, be properly exhibited by shading in a black and white sketch. Even the details of the structures seen visually within the foveal area cannot thus be exhibited. Nevertheless, the figure may help to convey some idea of the effects observed, supplementing a purely verbal description.

All the five filters prepared with crystal violet exhibit a blue colour by transmitted light, but the depths of their colours are very different. The effects described above are shown only by those filters which had been dyed to a sufficient depth of colour to make the absorption by crystal violet really effective. Only in three out of the five filters which were prepared was this actually the case. Spectroscopic examination shows that the absorption by the dye manifests itself as two distinct bands, one in the green and the other in the orange, the former ranging from 540 to 570 mμ, and the latter from 590 to 620 mμ. In the most heavily-dyed filter, these bands have spread out and their overlap results in a complete cut-off of the region of wavelengths between 530 and 640 mμ, while the rest of the spectrum is transmitted without any noticeable absorption. In the two less heavily-dyed filters the two bands can be seen to be distinct from each other, but nevertheless the absorption of the yellow of the spectrum in the wavelength range between 570 and 590 mμ is quite strong. The absorption of the yellow is however quite weak in the fourth filter of the series and scarcely noticeable in the fifth.

A comparative study of the effects observed with all the five filters is highly instructive when considered in relation to their respective spectroscopic behaviours. Even with the most lightly-dyed filter, it is possible for the observer to notice on the illuminated screen a picture of his own retina. But for this to be possible, it is necessary to hold the filter before his eye for a longer period before removing it than in the case of the more highly-coloured filters. In the picture then seen, the orange-yellow field in the outer region is the most conspicuous feature, while the circular area and the foveal disk which it surrounds too are scarcely noticeable. But these features appear more distinctly with the next filter in the series, while with the three other filters, they become progressively more and more conspicuous.

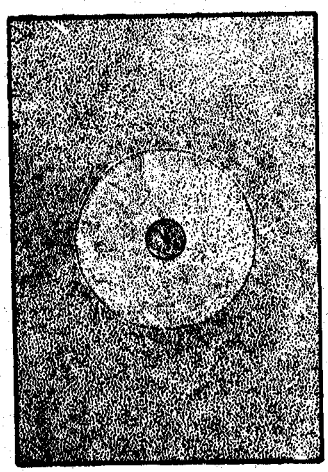

Figure 1. Foveal disk and surrounding area.

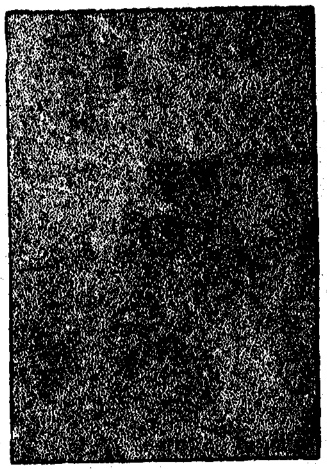

Figure 2. Foveal disk and surrounding area.

From what has been stated above, it is apparent that the absorption of light by the filters in the yellow region of the spectrum, viz., between 570 and 590 mμ, plays a highly important rôle in giving rise to the observed effects. Indeed, only when such absorption is present do we observe the very striking manifestation of a brilliantly luminous disk in the foveal area. That the disk appears of a green colour and not just yellow is an indication that the absorption by the filter of the spectral region between 540 and 570 mμ also then takes part in exciting the foveal region of the retina. The co-operation of the entire region of the spectrum between 540 and 590 mμ is clearly needed for observing the brilliant foveal disk as well as the much larger circular area which appears surrounding it. On the other hand, the absorption of the part of the spectrum between 590 and 640 mμ gives rise to the orange-yellow hue observed in the outer parts in the field. Its effect on the region of the fovea and the area immediately surrounding the fovea is submerged in the much larger contributions arising from the spectral region between 540 and 590 mμ.

Filters of methyl violet: This well-known dye-stuff is closely related to crystal violet in its chemical constitution and its spectroscopic behaviour also resembles that of crystal violet. It is, however, not very easy with it to prepare a set of colour filters of the same high quality as with crystal violet. This may be due to impurities present in the commercially available material. Nevertheless, the filters actually prepared with it which have the necessary depth of colour exhibit effects similar to those observed with crystal violet and approaching them in their spectacular character. The most striking results are those observed with filters which show a complete extinction in the spectral range between 540 and 630 mμ and perfect transmission in other parts of the spectrum. Thus, they support the same conclusions as those based on the observations made with crystal violet.

Cyanin filters: A set of six filters were prepared with this well-known dye-stuff, their colours by transmitted light ranging from a deep blue to a light blue. The absorption spectra of the filters showed a regular progression, the deepest filter exhibiting a practically complete extinction of the yellow, orange and red regions in the spectrum, while the lightest filter showed a well-defined absorption band in the wavelength range from 630 to 670 mμ. The visual effects produced and observed with these filters also alter in a progressive fashion. With the filter which exhibits a cut-off extending from the yellow towards greater wavelengths, the observer notices a disk of yellow light with a bright spot at the centre and a bright rim around its margin appearing in the foveal region. Surrounding this and exhibiting a yellow colour, a circular area also manifests itself which has a diameter some three times greater than that of the foveal disk. Outside this again, there is a field of light extending to the outer limits of the screen and exhibiting an orange hue.

Observations with the other five filters show that the yellow foveal disk and the surrounding yellow region become less and less prominent in the series relatively to the outer parts of the field. With the two lightest filters, they can be observed only with some difficulty. On the other hand, the outermost areas continue to be visible and to exhibit colour. This colour shows a perceptible change from an orange to a reddish hue in the sequence.

From these observations, we are led to infer that the foveal region and the brighter area immediately surrounding it are made conspicuous by reason of light in the spectral range between 570 and 590 mμ being incident on the retina. On the other hand, the orange and the red sectors of the spectrum are responsible for the luminosity appearing in the outer parts of the field.

Filters of disulphine blue V.S: Five filters were prepared with this commercially available dye-stuff. The first two gave a complete extinction of the yellow region of the spectrum and of all greater wavelengths. The absorption in the yellow was very weak with the third filter and non-existent with the fourth and the fifth. These three filters exhibited a powerful absorption in the orange and red sectors of the spectrum, a dark band in the wavelength range between 630 and 670 mμ being a conspicuous feature.

With the two filters which gave a perfect extinction of the yellow, observations showed the foveal region as a bright yellow disk with a luminous spot at the centre and a bright rim around the margin. Surrounding this was a circular region which was less luminous and had a diameter some three times greater than that of the foveal disk. The rest of the field exhibited an orange-red glow. Only the latter phenomenon was exhibited by the three weaker filters, barely a trace of the foveal disk and of the surrounding region being distinguishable from the rest of the field. Thus, the observations point to conclusions similar to those indicated by the observations with the cyanin filters.

Colour filters of magenta: A set of three filters were prepared with this well-known dye-stuff. All three showed a strong absorption in the wavelength range from 550 to 580 mμ, accompanied by a weaker and more diffuse absorption in the wavelength range between 500 and 550 mμ, while the rest of the spectrum showed no observable diminution of intensity in its passage through the filter. In effect, the most heavily-dyed filter cut off the whole of the green in the spectrum, while the other two filters were less effective in this respect.

All the three filters behave similarly when held by the observer before his eye and then quickly removed while he continues to view the illuminated screen with his attention fixed at a particular point in it. The only difference noticeable as between them is that the less strongly-dyed filters have to be held before the eye for a longer interval of time before being removed. Following the removal of the filter, the entire area of the screen exhibits a greenish-yellow glow which vanishes after a few seconds. But it may be instantly restored by putting back the filter and then removing it again. In effect, the observer sees on the screen a projection of his own retina as illuminated by light in the wavelength range between 550 and 580 mμ. This is made evident by the appearance at the centre of the field of a disk which does not exhibit the greenish-yellow glow seen over the rest of the screen and which is differentiated from the surrounding area by its relative feebleness and its pale blue colour.

From the foregoing, it emerges that the effects observed with the magenta filters are strikingly different from those exhibited by the other filters and described in the preceeding paragraphs. These differences are clearly attributable to the regions of the spectrum exciting the response of the retina being different. It may be remarked that in the present case, we are concerned exclusively with the response of the retina to light appearing in the green sector of the spectrum.

Colour filters of rhodamine B: Four filters were prepared with this well-known dye by varying the time of immersion of the gelatine film in the bath of its solution. The depth of the colour exhibited by them in transmitted light showed the progressive change to be expected in the circumstances. Even the most lightly-dyed filter shows a complete extinction of light in the wavelength range between 540 and 580 mμ. In the more heavily-dyed filters, this absorption band widens asymmetrically towards shorter wavelengths, resulting in a complete extinction of the green region in the spectrum. A spread of the absorption towards longer wavelengths in the more heavily-dyed filters is also noticeable, but the yellow and orange of the spectrum are not totally extinguished. Thus the effect of the rhodamine filters is principally to block out the green of the spectrum. The phenomena observed with them are essentially similar to those noticed with the magenta filters. It is therefore unnecessary to describe them here again in detail.

Yellow colour filters: Various dyes when incorporated into gelatine films result in filters exhibiting a yellow colour by transmitted light. Auramine-yellow may be mentioned as a good example of such a dye-stuff. Spectroscopic examination shows that when the absorption is confined to the extreme violet end of the spectrum, the colour of the transmitted light is a pale yellow. As it progresses further and covers more of the spectrum, the colour deepens. Finally it becomes a rich golden-yellow when all wavelengths less than 500 mμ have been cut off. A further advance into the green of the spectrum beyond 500 mμ results in the transmitted light exhibiting orange-yellow hues.

Observations made with all such filters exhibit certain common features. Following the removal of the filter from before the observer's eye, a glow of colour appears over the illuminated screen, its brilliance depending notably on the depth of colour of the filter as also on the period of time for which the filter is held before the eye prior to its removal. The colour of the glow ranges from violet to blue or a bluish-white, depending on the extent of the spectral cut-off by the filter employed. At the centre of the field around the point of fixation of his vision, the observer notices a circular area—evidently the projected image of the fovea of his own retina—where the glow referred to above is absent and a pale yellow hue is perceived instead (figure 2 attempts to represent this effect by a black and white sketch.) A dark spot is sometimes also seen at the centre of the foveal disk, with some indications of structure within the area.