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The perception of light and colour and the physiology of vision—Part III. The carotenoid pigment

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1. Introduction

In the first part of this memoir, the facts of observation regarding the luminosity and colour perceived at different points in the spectrum were set out in detail. The mechanism of perception and the role played by the visual pigments in the retina which would account for the facts were then discussed. In the second part of the memoir, the number of visual pigments which function and the spectral regions in which they are effective were deduced from certain luminous effects which were described, and the considerations which enable these visual pigments to be identified were also set out.

We shall now proceed a step further and show how it is possible to connect the observed variations of luminosity and colour in the spectrum with the absorption characteristics of the visual pigments which enable us to perceive light and colour. We shall in the first instance consider the blue-violet region of the spectrum which exhibits several features of interest, including especially the fact that in that region—and no other—the unaided eye is capable of recognizing polarised light and even of determining its plane of vibration by sight. It will be shown that these and other features are explicable in terms of the structure, spectroscopic behaviour and disposition in the retina of the molecules of the carotenoid pigment xanthophyll which was identified in the second part of the memoir as the mediator of photopic vision in that part of the spectrum.

2. The carotenoid pigments

The carotenoids are so named by reason of their chemical relationship to carotene which is a plant pigment first isolated in crystal form from the roots of the cultivated carrot. They are a numerous family of compounds and are essentially plant products. Indeed, there is no evidence which would suggest that any carotenoid is produced *de novo* within the body of an animal. They enter the

body by way of the articles of food which are consumed by the animal and if assimilated, pass into the blood stream to be utilised or stored up where needed. The potential vitamin-A activity is an important function of some of them, including especially β-carotene. This is however not one of the potential uses of xanthophyll since it is not a precursor of vitamin-A. Accordingly, xanthophyll is either accumulated in certain organs or else is passed out of the body.

An interesting example of the storage of xanthophyll is furnished by the yolk of the domestic hen's egg which, as is well known, exhibits a bright yellow colour. It is a convenient source-material from which xanthophyll can be obtained for the study of its spectroscopic behaviour. Repeated treatment with warm acetone results in the extraction of the pigment and on filtration, a clear liquid exhibiting a golden-yellow colour is obtained. This contains xanthophyll and also its isomer zeaxanthin as a minor constituent. As the latter has a very similar spectroscopic behaviour, its presence is not inconvenient for the purpose in view.

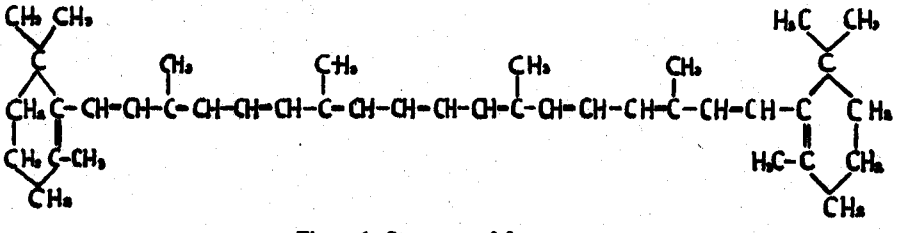

Figure 1. Structure of β-carotene.

Carotene is a hydrocarbon having the chemical formula $C_{40}H_{56}$ which appears in different isomeric forms, the most important of them being β-carotene which has the structure shown in figure 1. It will be seen that the two end-groups in the molecule have an identical structure and are joined together by a long chain in which single and double bonds alternate. It is this system of conjugated double bonds that is responsible for the absorption of light by the substance which extends into the visible region of the spectrum. The characteristics of the absorption spectrum of β-carotene in hexane solution are exhibited in the spectrophotometer curve reproduced as figure 2. It will be seen that it is negligible at wavelengths greater than 5200 Å and that it rises very steeply in the region of wavelengths smaller than 5000 Å. After passing through a succession of maxima, it drops down but much less rapidly to quite small values at the violet end of the spectrum. The absorption maxima are located at 4770, 4500 and 4250 Å, the last being visible only as a point of inflexion on the curve.

Xanthophyll (also referred to in the chemical literature as lutein) has the formula $C_{40}H_{56}O_2$ and its structure which is shown in figure 3 exhibits its chemical nature as dihydroxy-α-carotene. The two end-groups in xanthophyll differ in their structure and this difference reflects the difference between α-carotene and β-carotene; the latter is symmetrical whereas the former is not. The

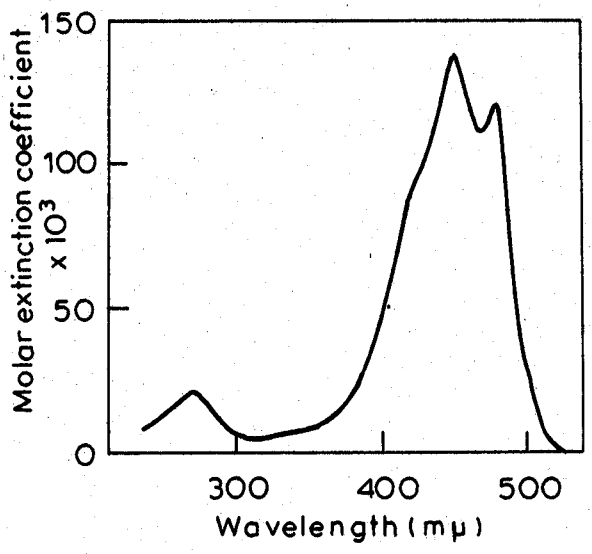

Figure 2. Absorption characteristics of β-carotene.

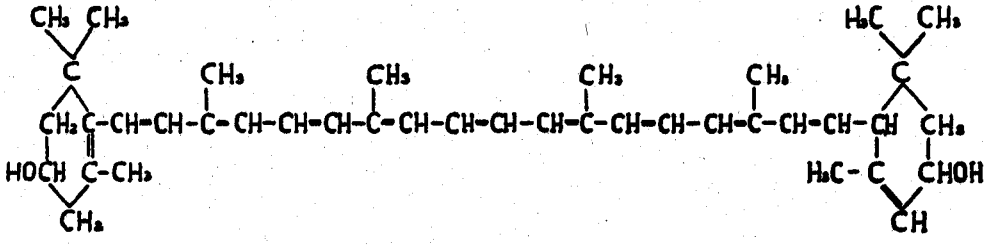

Figure 3. Structure of xanthophyll.

characteristics of the absorption spectrum of xanthophyll in ethanol solution are exhibited by the spectrophotometer curve reproduced in figure 4. It will be seen that xanthophyll resembles β-carotene in its spectroscopic behaviour; but there are significant differences. In particular, the inflexion which appears in the absorption curve of β-carotene shows up as a distinct maximum at the same point in the case of xanthophyll.

The absorption spectra of the carotenoid pigments have been explained as arising from a swinging to and fro of the electrons along the length of their molecules. The incident radiation would excite such an oscillation provided that its electric vector is parallel to the length of the molecule. Simultaneously with the electronic oscillation and as an accompaniment to it, molecular vibrations would also be excited. The energy required for exciting the latter would add up to that of the electronic excitation. Hence the absorption spectrum would spread towards

higher frequencies and smaller wavelengths. Peaks of absorption representing the successive harmonics of the most strongly excited molecular vibration frequencies may be expected to appear. It is thus possible to understand, at least in a qualitative fashion the characteristic features of the absorption curve; firstly, a steep rise at the long-wave end leading up to the first and fairly high maximum; after the first maximum, a few additional maxima and then a slow drop-down to a very weak absorption at the violet end of the spectrum.

3. Xanthophyll and the spectral colour sequence

The colour of the spectrum to any observer with normal colour vision is green at 520 mμ, blue at 480 mμ and violet at 420 mμ. Referring to figure 4, it will be seen that the absorptive power of xanthophyll is zero at 520 mμ, has reached its first maximum at 480 mμ and that at 420 mμ it has passed its third maximum and is on its downward course. If xanthophyll is the visual pigment which functions in the wavelength range between 4000 and 5000 Å, the change-over from green to blue should appear in the vicinity of the steeply rising part of the absorption curve at about 490 mμ. This, indeed, is what is actually observed; the change of colour with wavelength is shown by the investigations already reviewed in the first part of this memoir to be very rapid in the vicinity of that wavelength. Similarly, one

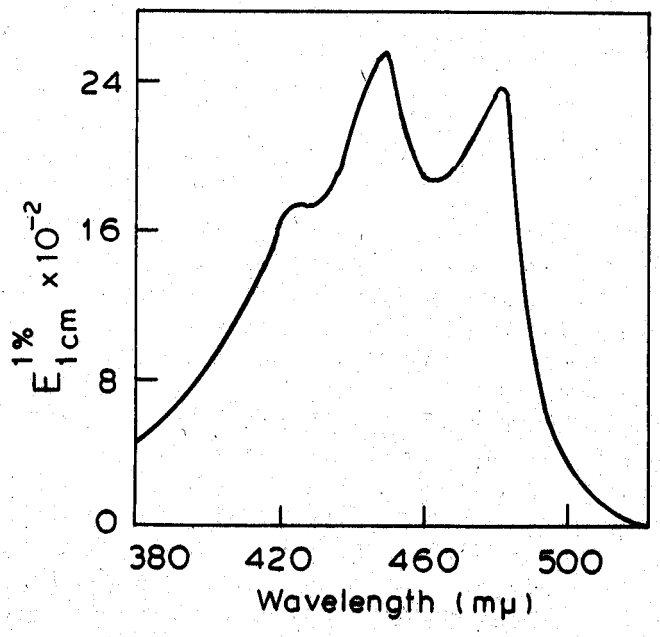

Figure 4. Absorption characteristics of xanthophyll.

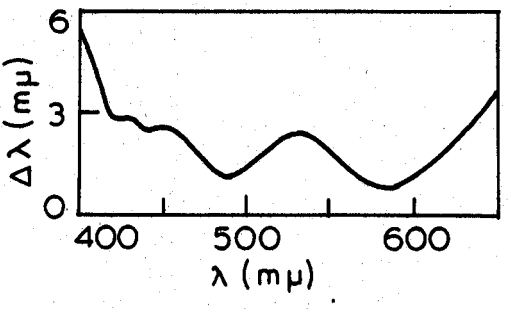

Figure 5. Hue discrimination curve in the spectrum.

would expect the change from blue to violet to occur in the vicinity of the steeply falling part of the absorption curve around 440 mμ. At this wavelength, again, it has been observed that the colour changes rapidly with alteration of wavelength. In other words, the absorption-curve of the visual pigment and the colour sequence in the spectrum exhibit a close correlation, as indeed is to be expected since it is the visual pigment which by its absorptive properties enables the radiation to be perceived.

Figure 5 reproduces the hue discrimination curve in the spectrum which appears on page 229 in the late Dr P J Bouma's posthumously published book entitled *Physical Aspects of Colour*. It is stated that the curve represents the average of the results of a dozen different investigators whose published papers are listed in the book. (Some of these references are not available for consultation at Bangalore.) The graph shows a small dip at 420 mμ besides the more conspicuous ones at 490 mμ and 440 mμ, mentioned above. The dip at 420 mμ appears in the same position as the steep fall in the absorption curve of xanthophyll which commences after it has reached and passed its third maximum.

Besides the features referred to above, the blue-violet region of the spectrum exhibits other observable characteristics which should be mentioned and commented on here. The luminous efficiency which at 500 mμ is very much less than the maximum appearing in the greenish-yellow part of the spectrum shows a further rapid fall as we proceed towards shorter wavelengths. This feature is represented in figure 6, the observations being those made by Jainski using a flicker method and a retinal illumination high enough to ensure that the results refer to photopic conditions. His observations do not take us beyond 460 mμ. But the simplest visual observations suffice to show that the luminosity of the spectrum becomes very small as we approach further towards its violet end. The other characteristic of the spectrum to which attention may be drawn appears in figure 5 reproduced above. The minimum of wavelength difference perceptible as a colour change exhibits a progressive increase as we approach the violet end of the spectrum, being some five times greater at 400 mμ than it is at 500 mμ. This

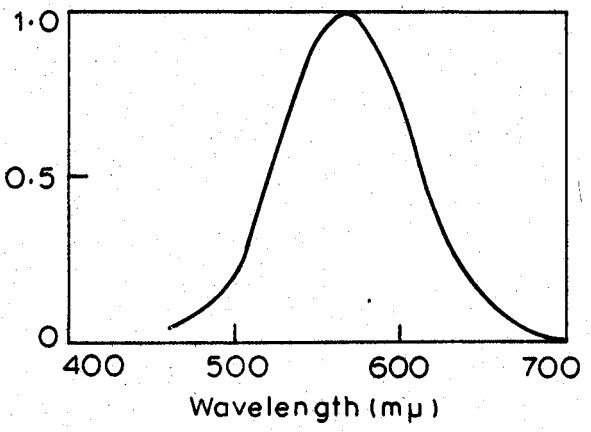

Figure 6. Photopic luminous efficiency curve.

increase accompanies and is superposed upon the undulations in the value of the minimum which appear in the same range of the spectrum. These features of visual experience are clearly related to the absorption characteristics of xanthophyll; the fall of luminous efficiency goes hand in hand with the decrease in absorptive power and the decreasing power of hue discrimination with the diminishing slope of the absorption curve, as we pass from the blue to the violet and approach the end of the visible spectrum.

It appears worthwhile also to mention here that very simple visual observations with a pocket spectroscope and a moderately strong solution of xanthophyll prepared in the manner already stated suffice to establish the principal features in its absorption spectrum. With an absorption cell of small thickness, say 1 cm, one observes an enfeebled transmission in the blue and violet and a succession of absorption maxima in that region. This region exhibits a distinct edge at about 490 mμ beyond which there is free transmission of light. A bluish-green section is visible which precedes the green and the rest of the spectrum. With a greater absorption path, say 3 cm, there is a complete cut-off of the violet and blue regions of the spectrum and a distinct enfeeblement of the bluish-green region. With a large absorption path, say 10 cm, the bluish-green section is also completely cut off, and we observe an absorption edge located at 510 mμ which separates the fully absorbed from the freely transmitted parts of the spectrum.

4. Effects observed with polarised light

We have already had occasion to notice two very striking properties of xanthophyll, viz., the elongated form of its molecules and the restriction placed on

their power to absorb light and therefore also on the power of xanthophyll to function as a visual pigment, viz., that the radiation falling on the molecules should have its electric vibration—or at least a component of it—parallel to the length of the molecules. These properties would have no observable consequences if the molecules of xanthophyll were orientated at random in the retina. This, however, is not the case in the foveal region which exhibits a depression or pit with the foveola at its centre and with sloping sides having a maximum slope angle of about 20°. As a consequence, the nerve fibres in the retina are normal to its surface only at the centre of the pit or depression. Elsewhere they slope away from the normal and as a result, the region of the fovea presents a radially disposed fibrous structure. The xanthophyll molecules are themselves highly elongated bodies and as they are part of the retina, they would naturally set themselves parallel to the elements of the fibrous structure in which they are located. In other words, the visual pigment would present to the incident light a radially disposed array of molecules with their long axes pointing towards the centre of the fovea. This radial disposition would be least evident at the centre of the fovea, would be most conspicuous in the region around the centre where the nerve fibres slope outwards and would cease again to be noticeable outside the foveal region.

If the observer views an extended source of unpolarised light, the special features of the foveal region referred to would not result in anything noticeable. With polarised light, however, the situation would be different. The molecules of xanthophyll which are parallel to the direction of optical vibration would function as absorbers and give rise to the sensation of a bright brush of light along that direction. The molecules perpendicular to the direction of vibration would be unable to absorb the light and hence a dark brush would appear along that direction. In other words, the observer would see an image of his fovea projected

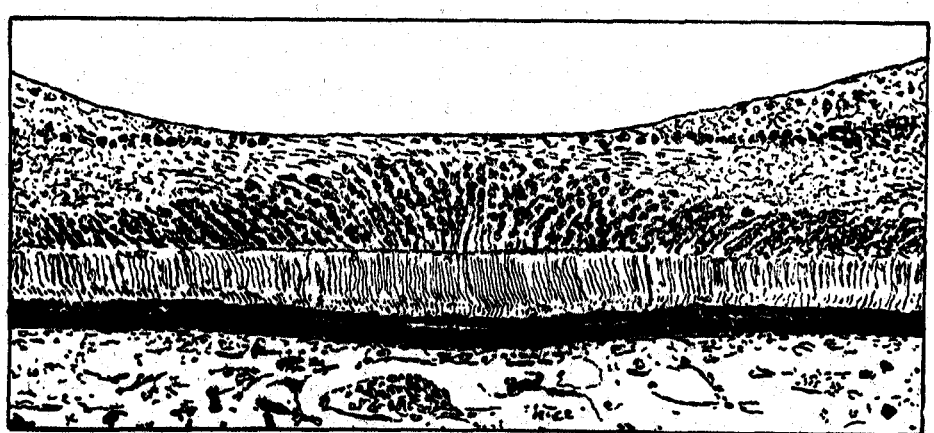

Figure 7. Structure of the foveal region.

in space which presents the aspect of a cross, the arm parallel to the direction of vibration in the incident light being bright and the arm perpendicular to it being dark.

Since the absorption of xanthophyll covers only the violet and blue regions of the spectrum but does not extend to greater wavelengths, it follows that the foveal cross seen in polarised light would be at its best if the incident light lies within those spectral regions. Light of greater wavelengths would not exhibit the phenomenon and hence its presence would only add to the general illumination of the field and thereby make the foveal cross a much less conspicuous phenomenon than it really is. This remark is of special importance in view of the relatively low luminosity of the blue and violet regions in comparison with the rest of the spectrum.

Since xanthophyll is a photopic visual pigment, it follows that the foveal cross requires for its observation that the illumination of the field with polarised light is sufficiently strong to ensure photopic conditions for the spectral region under consideration. When the illumination is reduced and we pass over to scotopic conditions, the effect should weaken and ultimately cease to be observable.

It should also be remarked that the phenomenon of the foveal cross should be equally well or even better seen binocularly as monocularly. For, what is actually observed is a picture of the foveal region projected on the field of view and when both eyes are used, their foveal regions are in register, in other words, both are seen at the same point in space.

It also deserves to be emphasised that the foveal cross seen in polarised light and the picture of the fovea seen with colour filters of various sorts described in the second part of this memoir are closely related phenomena. In both cases we are concerned with a localised excitation of the visual receptors and the possibility of directly perceiving the results of such excitation. The closeness of the analogy will become plainer when we presently take up a description of the methods of observation and the results obtained.

5. Observation of the effects

The phenomenon originally discovered by Haidinger viz., a faint brush which enables the eye to recognize polarised light and ascertain its plane of vibration is ordinarily both inconspicuous and fugitive in character. It however becomes a striking and conspicuous effect in the following circumstances; the field viewed by the observer should be of adequate intensity, e.g., a cumulus cloud lit by sunlight or a brilliantly illuminated white screen; the observer should hold before his eyes (in addition to a polaroid) a colour filter which is transparent to the blue and violet rays and completely cuts out the rest of the spectrum; the polaroid should be rotated, or else oscillated continuously instead of remaining in a fixed orientation. With these arrangements, one observes a dark brush and crossing it

transversely, a bright brush; the dark brush is very dark and the bright brush is brighter than the surrounding field. The two brushes together form the figure of a cross which fills an area which is readily recognizable as a projection in space of the fovea centralis of the observer's own retina. As the polariser is turned round or oscillated, the cross turns round or oscillates synchronously with it around a point which is the projection in space of the foveola of the observer's retina. If the polaroid and colour filter are both large enough, the whole phenomenon can be viewed binocularly.

The importance of using a colour filter which transmits the blue and violet regions of the spectrum and excludes the rest becomes clear when the observer substitutes for it a complementary filter, viz., a yellow glass plate which cuts off the blue and violet regions and transmits freely the rest of the spectrum. *Not a trace of the foveal cross can then be seen.* It follows that the cross owes its origin to a visual pigment whose absorption appears in the violet and blue regions but does not extend to the rest of the spectrum. A more detailed examination may be made in different ways. One can, for example, view through the polaroid a field illuminated by a monochromator and alter the wavelength progressively. Alternatively, we may use the monochromatic radiations of the mercury arc; the brushes are fairly well seen with λ 4538 and also, but not so well, with the λ 4046 radiations. A simple method by which the entire spectrum may be scanned is to view the first order spectrum of a linear source of white light produced by a glass diffraction grating held along with a polaroid in front of the observer's eye. The foveal cross can then be seen, if the observer directs his vision to the blue or violet region of the spectrum. But they are not visible in other parts of the spectrum, the transition from invisibility to visibility occurring rather abruptly around 4900 Å. The cross is clearest in the wavelength region between 4800 and 4300 Å. It is less distinct at still shorter wavelengths but nevertheless continues to be visible to the violet end of the spectrum.

A technique of observation different from that described above yields highly interesting results. With the colour filter held before his eye, the observer suddenly interposes a polariser in front of it. The cross then comes into view and slowly fades away. After a little while, the observer suddenly removes the polaroid. The cross is then seen once again but rotated through a right angle, the dark arm appearing in the place of the bright arm and *vice-versa*. On putting the polariser back, the cross regains its original configuration; and when the polariser is taken out once again, the cross turns round once again. It is thus clear that a sudden removal of the polaroid produces an effect of the same nature as that of turning it round through a right angle. This effect is clearly analogous to the phenomena observed with colour filters described in the second part of this memoir. The sudden removal of the polaroid results in the component vibration cut off by it being restored and allowed to fall upon the retina. As a consequence, the foveal cross is seen again but turned round through a right angle.

A convenient technique for studying the effects due to polarised light at

different levels of illumination is to use a powerful and completely enclosed source of light, e.g., a mercury arc or a tungsten lamp and to isolate the effective part of its spectral radiation by a colour filter which covers an aperture placed close to the source. The light which issues from the aperture can be received on a translucent diffusing screen and the light emerging through the latter is viewed by the observer through a polaroid from an appropriate distance. If the arrangement is set up in a long darkened chamber, the brightness of the field under observation can be varied over a large range by the simple device of moving the diffusing screen from a position close to the aperture from which the light issues to another sufficiently far away. Observations made in this manner show that the foveal cross is visible only when the illumination which falls on the diffusing screen is strong enough to allow ordinary print to be read. If the illumination be diminished further so that print ceases to be readable, the cross becomes indistinct. When the illumination is so feeble that a printed page appears to the eye as a mere blur, the cross ceases altogether to be visible. The disappearance of the foveal cross thus goes hand in hand with the disappearance of the visual acuity which is a characteristic of photopic vision. It is also observed that the field of view as seen through the polaroid shows a progressive change in colour from a brilliant blue to a pale blue as the illumination is diminished to the point at which the foveal cross ceases to be noticeable. This is a further indication that we have then moved out from the photopic to the scotopic level of illumination in the blue and violet regions of the spectrum.