The perception of light and colour and the physiology of vision—Part II. The visual pigments

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1. Introduction

In the first part of this memoir, the view has been put forward that our perception of light and colour is made possible by the presence in the retina of certain pigments which possess the power to absorb light and to transfer the energy thus absorbed immediately to the sensory receptors, thereby enabling us to perceive the absorbed energy as light. The identification of the visual pigments which perform these functions, in other words the determination of their chemical nature and a knowledge of their spectroscopic behaviour and distribution in the retina are the foundations on which any explanation of the facts of experience regarding vision must rest. We shall address ourselves in this part of the memoir to the problem which thus confronts us.

2. Viewing the retina in action

Any observer endowed with normal colour vision can perform the experiments now to be described. These do not require elaborate equipment and enable him to see his own retina in the act of functioning and observe its response to light in different parts of spectrum. From the results of the experiments, it is possible to infer the number and the distribution in the retina as well as the spectroscopic behaviour of the visual pigments which enable us to perceive light and colour.

The technique of the observations is quite simple. The observer sits facing a brightly illuminated white screen at a convenient distance from it and views the screen through a colour filter, either monocularly or binocularly as he may find best suits him. After allowing a sufficient time for the vision to adapt itself to the effects of the colour filter, the observer fixes his vision on some particular point on the screen and suddenly removes the filter. He will then recognize on the screen a highly enlarged projection of the macular region of his own retina, including especially the fovea centralis and the foveola at its midpoint. The details of the

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picture seen and the colours it exhibits depend very much on the filter employed. The picture soon fades away but it can be restored by putting back the filter and removing it again at short intervals of time. The picture can then be examined more closely.

The appearance of an image of the observer's own retina on the screen can be explained in general terms in the following manner. The colour filter employed cuts out or enfeebles certain parts of the spectrum while transmitting the other parts of it freely. As a consequence of the removal of the filter, the spectral components which are cut out or enfeebled by it are restored and suddenly illuminate the retina. Localised sensations are then excited, determined by the luminous efficiency of these spectral components and by the response to them of the receptors located in each element of area of the retina. The sensations thus excited manifest themselves to the sensory mechanism of the eye as an image of the retina which appears projected on the screen, each element of area exhibiting its own response to the illumination suddenly falling on it.

Before we proceed to describe and comment on the results observed with individual filters, it may be useful to mention a few particulars requiring attention in the experiments. It is necessary that the illumination of the screen should be adequate and that it should be as nearly uniform as possible. The observations should therefore be made by daylight, the screen being placed facing the windows in a well-lighted room and the observer should sit facing the screen and with his back to the windows. A projection screen of the well-known kind which is plastered over with tiny glass spheres is very suitable. A medium-sized screen, say 170 cm by 125 cm, is adequate and a convenient distance from the observer to a screen of that size is 350 cm. It is possible to use colour filters of different kinds, as for example glass cells of sufficient size containing absorbing solutions or plates of coloured glass. However, for the studies now under consideration, the most convenient filters are those which can be prepared by staining gelatine films on glass (of the kind used in photography) with a water-soluble dye. By regulating the strength of the solution in which the plates are dipped and the period of immersion before they are taken out and allowed to dry, the depth of the staining can be controlled over a wide range. The filters should be held close to the eye so as to exclude all light except that passing through it.

3. Description of the effects observed

Quite spectacular effects are observed when the filter employed is a gelatine film on glass stained lightly with methyl-violet. The filter appears a purplish-blue by transmitted light. Holding it before the eye for a few seconds and then removing it, the observer sees on the screen an enormously magnified image of his own fovea as a disk of light which is green in colour, and at the centre of it a bright spot of the same hue which is the pit or depression in the fovea known as the

foveola. If the filter is held for a somewhat longer time before it is removed, the fovea is much brighter and then appears surrounded by a halo of golden-yellow hue some five or six times larger in diameter but less luminous than the fovea itself. The fovea with the foveola at its centre appears at the point on the screen at which the observer has fixed his vision before removing the filter. If he shifts his gaze, they also move, thereby showing clearly that what is seen on the screen is a projected image of the observer's own retina. (See plate 1, figure 1.)

Spectroscopic examination shows that the filter lightly stained by methyl-violet has a strong absorption band in the orange, a weaker one in the green, and a very weak absorption in the region between them. When more heavily stained, the absorption becomes complete in all these regions and extends further into the green. A film stained heavily so that it cuts out the green completely but transmits the red and the blue of the spectrum appears of a deep purple colour. The use of such a heavily stained filter is however of no particular advantage, and on the other hand makes the phenomena rather less spectacular.

A noteworthy fact that emerges from the studies is that a filter whose absorption spectrum appears *exclusively* in the green part of the spectrum, in other words between 4900 and 5600 Å, does *not* give any observable effects of the kind referred to above. Several colour filters of this kind are available, amongst which may be mentioned very dilute solutions of potassium permanganate in water, or of iodine in carbon tetrachloride. More convenient, however, are gelatine films stained lightly by a suitable dye as, for example, rhodamine or eosine, so as to enfeeble or cut out the green region without affecting the rest of the spectrum. Holding such a filter in front of the eye for a little while and then removing it, the screen presents the same appearance as before the filter is interposed. Prolonging the period for which the filter is held before the eye prior to its removal makes no noticeable difference.

Though, as stated above, colour filters that absorb only the green part of the spectrum do not give rise to the effects under consideration, it is clear from the example of the methyl-violet filter that absorption in the green region can co-operate with absorption in adjoining regions of greater wavelengths and enable them to be observed. We may therefore conveniently divide the spectrum into three regions which we may denote as A, B and C and classify the filters and the effects they produce by reference to the regions of the spectrum in which their absorption appears. The violet-blue region of the spectrum is denoted by A, and green by B, and the yellow, orange and red may be grouped together and referred to as C.

As an illustration of the usefulness of the classification proposed in relation to the effects observed, we may here mention two cases in which very beautiful effects are observed but of a different nature. The colour filters in both cases are aqueous solutions contained in glass cells of equal thickness, 5 cm in each case. One solution is that of nickel chloride and the other of the dye-stuff lissamine-green sufficiently diluted so as to transmit plenty of light..The nickel chloride
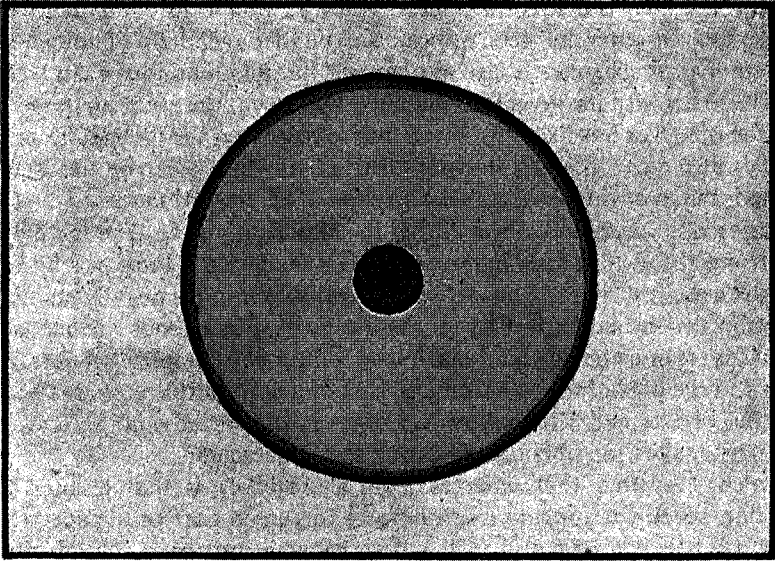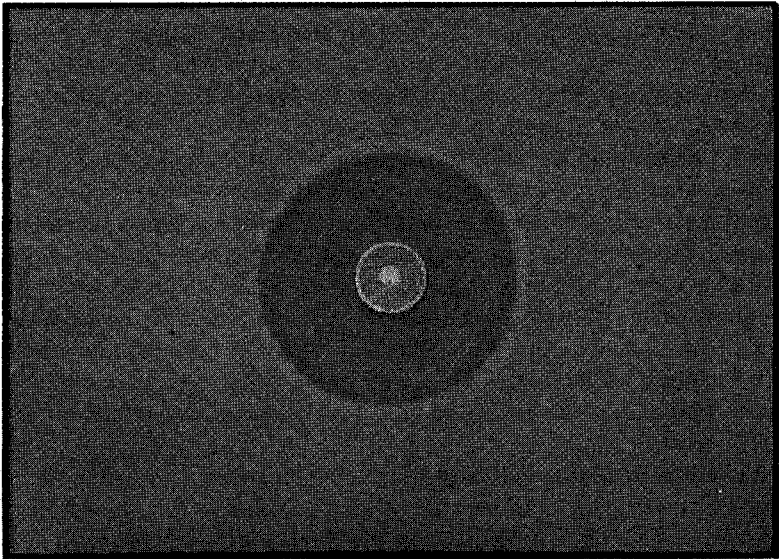
Figure 1. Methyl violet filter.

Figure 2. Lissamine green filter.

Plate 1. Pictures of retina seen by the filter technique.

solution is completely transparent in the green, and examination by a pocket spectroscope shows that it also allows the adjoining part of the blue to pass through, but cuts out the red, orange and yellow more or less completely. It thus belongs to the class of filter which we denote as AC. The solution of lissamine-green appears blue-green by transmitted light and while resembling the nickel chloride in allowing part of the blue to come through, it cuts out the red, orange and yellow completely and also part of the green. It thus belongs to the class ABC.

Holding the nickel chloride solution before the eye for a few seconds and then removing it, a brilliant rose-red glow is seen to cover the entire screen; at the centre of the field and as a very inconspicuous feature appears a small circular area which appears of a different hue and brightness from the rest of the field. On the other hand, when the lissamine-green solution is used for the observations, a highly magnified image of the fovea, bright yellow in colour and more luminous than the rest of the field, and the foveola at its centre are the most conspicuous features which the observer sees on the screen. A reddish glow covers the entire screen, but around the foveal image appears a halo of indefinite hue. (See plate 1, figure 2).

The filters of the species designated as A and as AB transmit the red, orange and yellow regions of the spectrum perfectly but cut off the shorter wavelengths to varying extents. As their cut-off in the spectrum moves from the extreme violet into the green and then further, the colour of the transmitted light progressively alters from the palest yellow to the deepest orange. All filters of the kind mentioned exhibit effects having certain features in common, though differing in detail. For example, using a filter of pale yellow hue having a cut-off at 4500 Å, its sudden removal from before the eye after an adequate interval results in the appearance of a violet-tinted glow which covers the entire screen. Neither the fovea nor the foveola nor any halo surrounding them can be seen. But at the centre of the field in the direct line of the observer's vision, there is a hint of the glow being somewhat dimmer than elsewhere on the screen. With another filter of a deep yellow colour which has a cut-off at 5100 Å, similar effects are noticed, but the glow which appears covering the screen is blue in colour and much brighter than that observed with the pale yellow filter. Orange filters give effects which are similar to those seen with the deep yellow filter but are not by any means more conspicuous; on the other hand, the effects are rather less conspicuous since the glow does not display any vivid colour and also ceases more quickly to be observable. Thus, an extension of the region of absorption by the filter from section A of the spectrum into section B does not make any real difference to the results, apart from making them less easy of observation.

Gelatine filters of the classes C and AC may be prepared by staining them *very lightly* with the dyes methylene-blue and lissamine-green respectively. Holding such filters before the eye and then removing them, the entire area of the screen exhibits a glow of a colour which differs for the two species of the filter, orange in one case, and rose-red in the other. But no image of the fovea or foveola appears on the screen. Only filters of the classes BC and ABC exhibit the later

phenomenon. They may be prepared in great variety by staining gelatine films to any desired depth by blue or green, dyes (methylene blue and lissamine-green for example). Most blue glasses belong to the class BC and most green glasses to the class ABC. A photographic filter exhibiting a delicate green colour was however found to belong to the class AC. It cuts out the violet rays in the spectrum and visibly enfeebles the red, but has no noticeable absorption in any other part of the spectrum. Holding it before the eye and then removing it, the observer sees a beautiful purple glow covering the entire screen, while an inconspicuous feature appears at the centre of the field of vision in the shape of a small circular area where the glow is feebler than elsewhere.

   Observations have been made with numerous filters of the classes BC and ABC. With every one of them, the foveal disk and the foveola at the centre are conspicuous features. It is also possible in many cases to observe a coloured halo five or six times larger in diameter than the foveal disk but of much lower intensity superposed on the general field of illumination on the screen following the removal of the filter.

4. The three visual pigments

We now proceed to consider the significance of the facts of observation reported above. We have seen that the entire spectrum may be divided into three sections which we have denoted as A, B and C respectively, and that the colour filters whose absorption appears exclusively in one or another of these three behave quite differently. It is a reasonable interpretation of the facts that the retina contains three pigments which are effective as absorbers of light and mediators of vision respectively in these three sections. We shall refer to them hereafter as pigments A, B and C respectively. Pigment A functions in the blue-violet region of the spectrum, pigment B in the green and pigment C in the orange, red and yellow. The facts of observation described earlier, however, compel us to qualify this statement and to recognize that the absorption spectrum of pigment C overlaps that of pigment B in the green to an appreciable extent. It is also necessary to assume that when the incident light causes pigment C to function, this results in the simultaneous functioning of pigment B in the same region of the retina, provided the wavelength of the incident light is within the region of overlap of the absorption spectra of the two pigments.

   The effects observed on the viewing screen following the removal of the filter enable us to draw some useful inferences regarding the distribution in the retina of the visual pigments which give rise to them. The position is clearest with regard to pigment A which is the mediator of vision in the blue-violet region of the spectrum. The blue or violet glow covering the entire area of the screen seen with filters of class A clearly indicates that the visual pigment of that class is distributed over an extent of the retina many times larger in area than the fovea itself.

The appearance on the viewing screen of a highly enlarged and luminous image of the fovea and of the halo encircling it clearly demands the co-operation of the two pigments B and C, since these effects are only noticed when filters of the classes BC and ABC are employed. The colours displayed by the fovea and the halo in various cases also point to the same conclusion. We are thus led to infer that pigment B is present in its maximum density in the region of the fovea and has a considerable though smaller density in an area surrounding the fovea and having five or six times its diameter. The observations further indicate that pigment C is present in association with pigment B in the areas referred to and that it is also to be found distributed over the area of the macula well beyond the region in which the coloured haloes surrounding the fovea are observed.
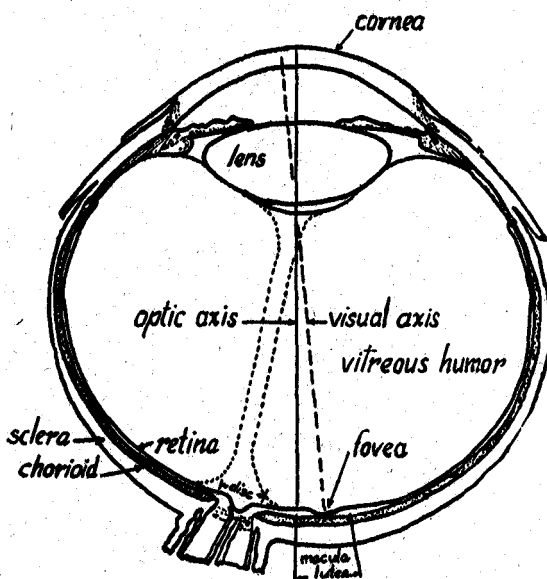

Figure 1. Horizontal section of the right human eye.

We may reasonably look for some evidence in support of the foregoing findings in the appearance of the retina as seen in the ophthalmoscope. The fundus of the human eye is visible in that instrument by reason of the diffusion of the light incident on the retina by the retina itself and by the materials behind it. The opinion expressed in the ophthalmological treatises (and copied therefrom into other books) is that the appearance of the retina is due to the diffusion of light by the choroid coat which lies behind the retina. Between the retina and the choroid coat, however, lies the pigment epithelium, the function of which is to absorb unwanted light traversing it in either direction. The efficiency of the pigment epithelium as an absorbing screen has therefore a very great influence on the

nature of the picture seen in the ophthalmoscope. This is indeed evident on a comparison between the appearance of the retina in the four cases of albinotic individuals, persons of fair complexion, and persons of dark and very dark complexions respectively. The blood-vessels in the choroid are clearly seen through the retinae of albinotic individuals. They are not seen in the other cases, what is actually visible being the surface of the retina upon which can be distinguished the optic disk, the retinal blood-vessels and the macula. The hue exhibited by the retinal surface is orange-red in persons of fair complexion, while in the case of negroes it is brick-red.
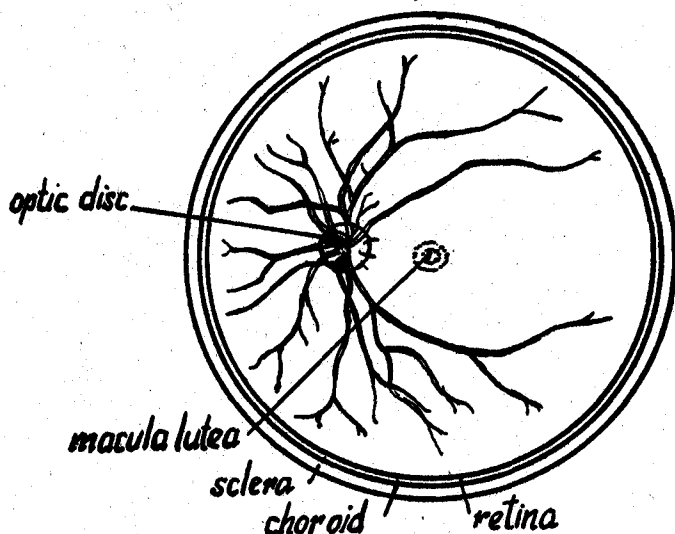

Figure 2. Fundus of the human eye.

What we are here specially interested in is the macular region which is physiologically the most important part of the fundus. This region is devoid of any visible blood-vessels, though it is encompassed on all sides by the twigs reaching down to it from the retinal blood-supply system. A specially significant fact is that in all cases, the macular region is of a distinctly darker tint than the rest of the fundus. Further, the fovea itself exhibits a blood-red hue in all cases. These are conspicuous features in the coloured illustrations of the fundus appearing in the ophthalmological treatises. The presence of pigments which between them cover the entire spectrum would explain the deeper colouring of the macular region. The presence in and around the fovea of a pigment which exercises a powerful absorption in the green region of the spectrum would account for that area exhibiting a blood-red hue. Thus, what is actually seen of the fundus through the ophthalmoscope does not contradict the conclusions reached through the present studies, but on the other hand gives them the clearest possible support.
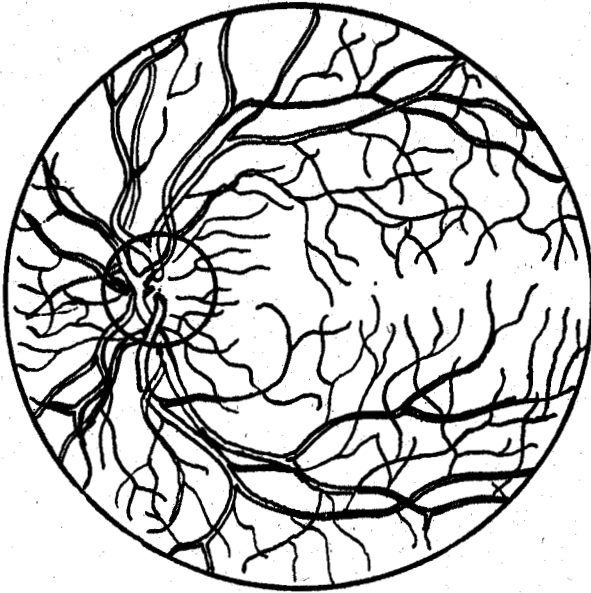
Figure 3. Blood-vessels in the retina.

Figure 4. Perifoveal capillaries in the retina.

A further remark may be made here. Ophthalmologists not infrequently in their examination of the fundus make use of light from which the red rays have been excluded by a suitable filter. It is stated that the background of the retina then appears of a yellowish-green colour, the macula standing out as a lemon-yellow area, and that the blood-vessels running through the retina appear almost black with sharply defined outlines. That the macula is distinguishable by its colour from the rest of the retina in these circumstances has been explained as due to presence in it of appreciable quantities of the yellow pigment which gives to the macular region its anatomical name of *macula lutea*. This yellow pigment may reasonably be identified with our pigment A, the absorption of which appears exclusively in the blue-violet region of the spectrum.

5. Identification of the visual pigments

We now proceed to consider the problem of determining the chemical nature of the pigments present in the retina which are the mediators of photopic vision. We have designated them above as (A), (B) and (C) and formed a general idea of the spectral ranges within which they are operative. They should be capable of existing in association with the materials which form the living substance of the retina. Their absorption spectra should appear in the regions indicated and it is necessary that when they absorb light radiations, they should also be capable of passing on the energy thus absorbed immediately to the receptors in the retina. Finally also, since we are concerned with photopic vision and therefore with high levels of illumination, it is necessary that the pigments should possess in a reasonable measure the power to resist disruption or decomposition by the action of light.

The advances in organic chemistry made of recent years have resulted in the elucidation of the structure of numerous naturally occurring colouring matters and created a great body of knowledge connecting chemical constitution with optical behaviour. A well-established result which emerges from these researches is the relation between the manifestation of colour by a substance and the presence in its molecules of unbroken chains of alternate single and double bonds. It is found that the longer the chain of such bonds is, the further into the region of the visible does the absorption spectrum of the substance extend. Many naturally occurring pigments also fall into two classes, one in which the chain of alternate single and double bonds is an extended straight line, and the other in which it forms a closed ring within the molecule. The group of substances known as the carotenoids belong to the first class, while in the second class we find various substances playing highly important biological roles, including especially heme and chlorophyll. It is interesting and significant that the green leaves of growing plants contain colouring matters belonging to both of the classes referred to. Two

of them, viz., β-carotene and xanthophyll belong to the first class and two others, viz., chlorophyll-a and chlorophyll-b to the second.

As is well-known, the pigments present in green leaves form the pathway by which organised nature finds access to solar energy. They enable the energy of solar radiation to be utilized for the synthesis from carbon dioxide and water of carbohydrates and other constituents of living matter. The mechanism of this process—known as the photosynthesis by plants—has been the topic of numerous investigations. It is recognized that chlorophyll with the strong absorption of light which it exhibits at the extreme red end of the spectrum plays a leading role in photosynthesis and the view formerly prevailed that the other pigments present did not participate in the process. More recent studies indicate that the latter conclusion needs modification and that the carotenoid pigments also do play a part in photosynthesis. Cogent evidence is also forthcoming that the pigments in green leaves, including especially chlorophyll, act as energy-receiving and transferring agents and thus enable photosynthesis to take place. Whether they actually participate in the chemical changes which result in the formation of new compounds and if so in what manner are questions which at the present time remain in the speculative stage.

It would seem, therefore, that the pigments in green leaves and the pigments in our retinae play somewhat similar roles in their respective fields of activity. Hence, there are good grounds for assuming that the pigments concerned are either the same or else are very similar. Four pigments are needed for vision, three for photopic and one for scotopic vision. Two of them may well be carotenoids, one for each type of vision. There is no reason, however, for assuming that all four pigments are carotenoids. On the other hand, it is most improbable that this would be the case. Pigment B plays a role in human vision of special importance as it covers the part of the spectrum having the highest luminous efficiency. One would naturally expect that such a pigment would be a product of biological activity in the human organism and not a substance carried into the body through the medium of food products. Further, it is difficult to believe that any carotenoid would possess all the properties needed for pigment B, viz., an absorption spectrum lying in the region between 5000 and 6000 Å, a high efficiency as an energy-receiving and transferring agent and the chemical stability needed to resist disruption or decomposition under intense illumination. We are thus led to infer that pigment B belongs to the class of organic compounds which derive their colour from the presence in their molecular structure of a ring of alternating single and double bonds. That pigment C also belongs to that class is *prima facie* very probable.

Chlorophyll-a and chlorophyll-b are pyrrole pigments which are chemically very similar to each other. They owe their colour to a combination of four pyrrole nuclei joined together in a complex molecule, a special feature of importance being that a magnesium atom appears linked to the nitrogen atoms of the four pyrrole groups in the molecule. When the magnesium atom is removed by the

action of acids, the solution turns brownish and ceases to exhibit the colour characteristic of chlorophyll, thus demonstrating the importance of the metallic atom in relation to its spectroscopic behaviour. When we seek for a pigment which could perform in the retina a role analogous to that performed by chlorophyll-*a* in the green leaf, the obvious and indeed the only choice is the metallo-porphyrin compound known as ferroheme (or simply as heme). This is also a pyrrole pigment, being a compound of iron in the divalent state with one of the porphyrin group of compounds. The porphyrins contain a closed ring of eighteen bonds which are alternately single and double in their structure, and the iron atom occupies in heme a position in relation to the four nitrogens in the molecule which is the same as that of the magnesium atom in the chlorophyll structure. There is thus good reason for assuming that heme possesses the properties needed to enable it to function very efficiently as a visual pigment in the retina.
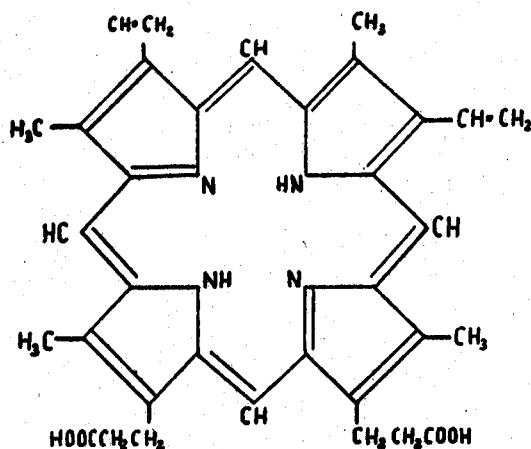

Figure 5. Molecular structure of protoporphyrin.

It is appropriate here to mention that heme in combination with different proteins plays a role of outstanding importance in the life of both plants and animals. The compound of heme with globin known as hemoglobin is present in the red-blood cells of vertebrates and by its ability to combine reversibly with oxygen without undergoing oxidation performs the unique and indispensable role of a carrier for the storage and transport of oxygen in the organism. Another heme-protein known as myoglobin is the respiratory pigment occurring in muscle cells of both vertebrates and invertebrates. The cytochromes are the most widely distributed of all the heme proteins and occur in the cells of nearly all aerobic organisms, both plant and animal. Catalase and the peroxidases are other

heme proteins which function as enzymes. This brief statement indicates the ubiquitous nature and extraordinary versatility of the heme proteins in performing biological roles of importance.

We shall later discuss the spectroscopic behaviour of heme in detail. It will suffice here to mention that all the heme proteins exhibit an intense absorption of light in the green region of the spectrum. Hemoglobin, for example, exhibits even in very dilute solutions a powerful absorption in the region of wavelengths between 5000 and 6000 Å, its maximum appearing around 5550 Å. That this absorption is a characteristic property of the porphyrin groups of compounds when a divalent metal atom occupies the central position in the molecule is shown by the fact that a powerful absorption with its maximum located at or near the same wavelength is also exhibited by several different porphyrins in combination with diverse metallic atoms which are divalent.
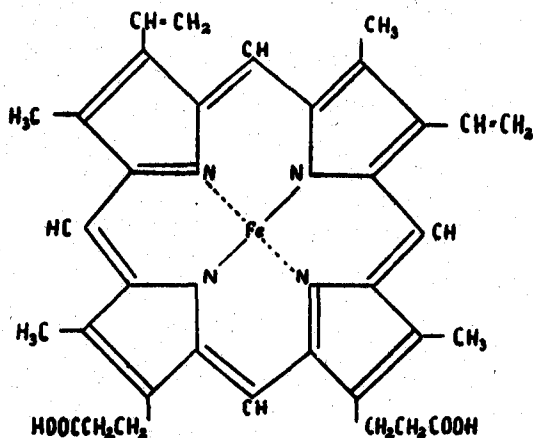

Figure 6. The molecular structure of heme.

The considerations set forth above accordingly lead us to identify ferroheme as our pigment B. We thereby add to the list of the many biological functions performed by ferroheme that of being the principal mediator of vision in the photopic range. Indeed, the absorption of light by ferroheme is so strong that no great quantity of this pigment would be needed in the retina to enable it to function effectively as the mediator of vision. That it is indeed the principal visual pigment finds strong support in the fact that the position of its absorption maximum is not very different from the wavelength of maximum visual sensitivity in the spectrum. The generally accepted value for the latter is 5550 Å, but it may actually be a little greater for persons with normal colour vision.

That the pigment A which functions in the region of wavelengths between 4000 and 5000 Å is a carotenoid is indicated by the fact that the absorption spectra of

carotenoids appear just in that region. As already mentioned earlier, the two carotenoids that are invariably found in the green leaves of growing plants are β-carotene and xanthophyll. These colouring matters are also present in the plasma of human blood and in human milk in varying amounts, evidently as the result of the consumption of articles of food containing them. There is however a noteworthy difference between the two pigments, viz., β-carotene is a precursor of vitamin-A, whereas xanthophyll is not. In other words, xanthophyll does not undergo fission and become transformed to vitamin-A in the human body. We may, therefore, with confidence assume that it is xanthophyll, and not β-carotene which is the pigment that finds its way into the human retina to function as a receptor in photopic vision.

The identification of pigment C has next to be considered. We have already noticed that while the absorption spectrum of this pigment appears principally in the yellow, orange and red regions of the spectrum, it also overlaps to some extent the region in which pigment B is operative, namely, the green of the spectrum. The reasons which make it probable that pigment C belongs to the same group of pyrrole compounds as pigment B have already been indicated. That the two pigments co-operate with other in producing the luminous effects described earlier on these pages is also significant. We are thus led to recognize that pigment C is closely related to pigment B. The suggestion that it is only an oxidised form of that pigment, in other words that it is ferriheme naturally presents itself. The spectroscopic and other evidence which supports and confirms this identification will be presented in a subsequent part of the present memoir.