THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part IV. Ferroheme and Ferriheme

BY SIR C. V. RAMAN

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1. INTRODUCTION

IN the first part of this memoir, the basic facts concerning the perception of light and colour were reviewed and a mechanism of the functioning of the retina was suggested which explains them in a simple and intelligible fashion. Human vision is mediated by certain pigments present in the retina, these pigments acting as energy-receiving and energy-transferring agents; in other words, they absorb the quanta of radiational energy incident on the retina, but pass on the energy thus absorbed to the sensory mechanism, themselves returning to their original states. The second part of the memoir dealt with the problem of determining the number and nature of the visual pigments functioning in the manner indicated, the regions of the spectrum in which they respectively operate and the distribution of the pigments over the area of the retina. A method of observation was described which furnishes valuable information on these points. Considerations were also developed which pointed to xanthophyll, ferroheme and ferriheme as the three visual pigments with which we are concerned in photopic vision. In the third part of the memoir, evidence was presented which confirms and establishes that xanthophyll is the visual pigment which functions in the violet and blue regions of the spectrum.

In the present part of the memoir, we are concerned with the region of the spectrum between the wavelengths 5000 and 7000 angstroms. The facts of observation which concern us here and need interpretation are firstly, the distribution of luminosity in this region of the spectrum; secondly, the distribution of colour in it; and thirdly, a derivative property of the same, namely, the characteristics of the colour progression which find expression in the so-called hue discrimination curves determined by various observers. We shall consider these facts here in some detail and discuss their interpretation. The aim is to infer therefrom the spectroscopic behaviour

of the visual pigments and to compare the same with what is known regarding the absorption spectra of the heme pigments.

2. Luminosity, Colour and Hue Discrimination

The form of the luminous efficiency curve in the spectrum depends to a notable extent on the region of the retina used in its determination. In what follows, we shall make use of the data obtained by Walters and Wright under photopic conditions in which only the foveal region of the retina was employed. Their results are reproduced below in Fig. 1. It will be noticed that the luminous efficiency exhibits a well-defined maximum at 5600 angstroms on either side of which it descends steeply, but less rapidly so on the side of longer wavelengths than towards the shorter ones. This pronounced asymmetry of form evidently calls for explanation.

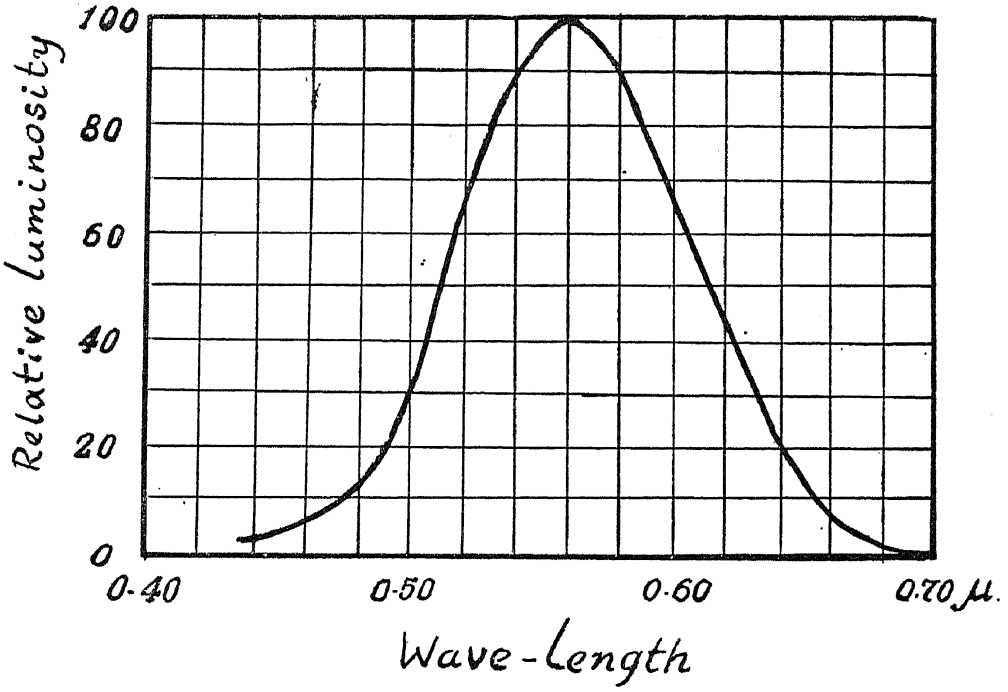
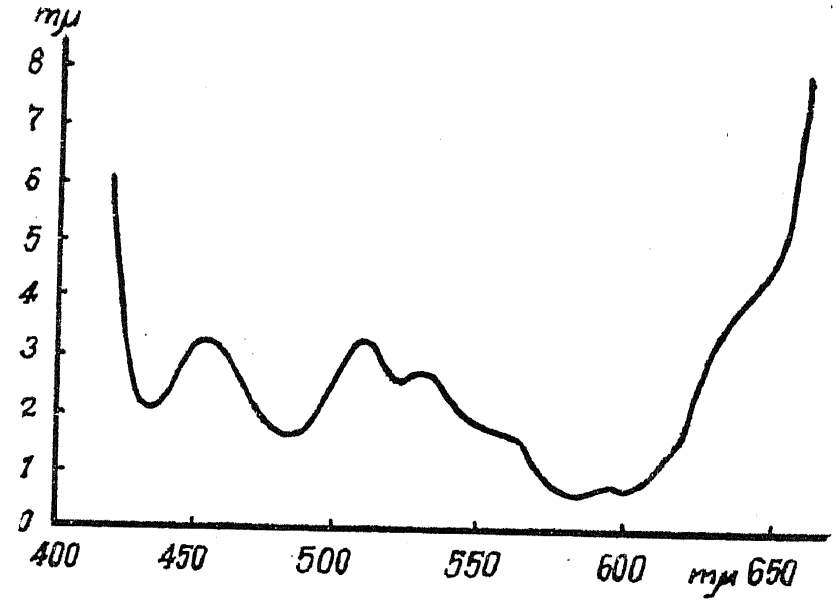


Fig. 1. Foveal luminous efficiency curve according to Walters and Wright.

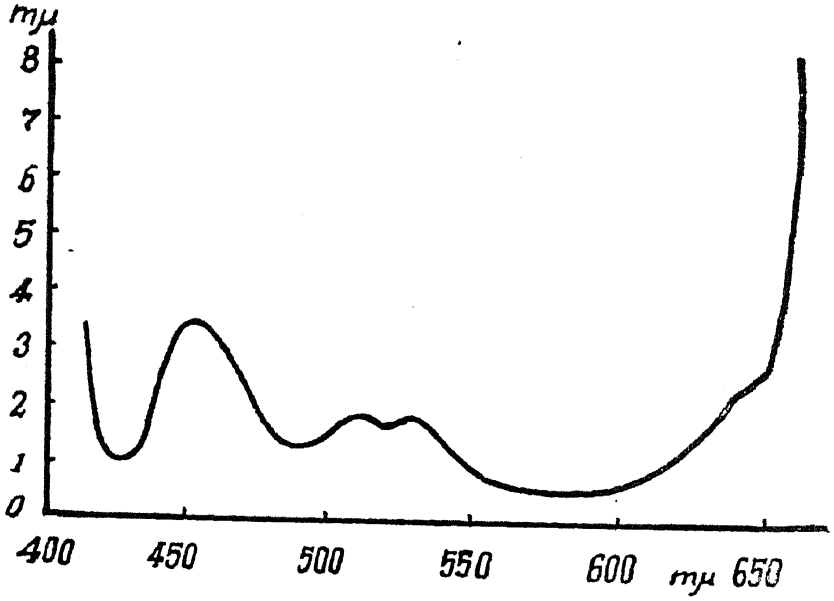
The graph of the luminous efficiency reproduced in Fig. 1 may be divided into four parts which exhibit distinctly different characters: (i) the foot of the graph from 4400 to 4950 angstroms where it exhibits a marked curvature; (ii) the steeply ascending part from 4950 to 5600 angstroms; (iii) the steeply descending part from 5600 to 6270 angstroms; (iv) the foot of the graph from 6270 to 7000 angstroms where again it exhibits a marked curvature.

On the basis of the average positions of the colour boundaries in the spectrum as placed by observers with normal vision, we may divide the

visible spectrum into four sectors thus: Blue-violet, $\lambda < 495\ m\mu$; Green, $495\ m\mu < \lambda < 566\ m\mu$; Orange-yellow, $566\ m\mu < \lambda < 627\ m\mu$; and Red, $627\ m\mu < \lambda$. It will be noticed that these sectors represent also the divisions of the luminous efficiency curve indicated above, except that the green-yellow boundary is placed at 566 $m\mu$ instead of 560 $m\mu$ where the maximum luminous efficiency appears.



(*a*)



(*b*)

FIG. 2. Hue discrimination in the spectrum (after Haase).

Measurements of the smallest change of wavelength needed for a perceptible change of colour have been made and reported by numerous observers. Their results agree in respect of the major features but show some differences in detail. This is not surprising, since the characteristics of human vision are by no means the same for all observers, and the techniques and physical conditions of observation are also not identical in all the investigations. Two slightly different forms of these hue discrimination curves have already been reproduced in earlier parts of this memoir. Figure 2 (*a*) and Fig. 2 (*b*) above represent the results reported by Haase for two different levels of illumination, Fig. 2 (*b*) representing the results for the higher level of the two which was ten times greater in intensity than the other. Figure 3 below reproduces the results of E.P.T. Tyndall for the wavelength range between 450 mμ and 650 mμ. The individual observations made by him at various times are marked in the graph by crosses and circles and show a remarkable consistency except in the vicinity of the humps at 530 mμ and 630 mμ respectively.

Tyndall's observations place the wavelength of minimum limen at 575 mμ where it has the value of 0·5 mμ; at greater wavelengths it increases, at first quite slowly and then more and more rapidly, reaching large values at the red end of the spectrum. The results reported by Haase and shown in Figs. 2 (*a*) and 2 (*b*) exhibit generally similar features in the same region. At the wavelengths around 530 mμ where Tyndall's data exhibit a considerable scatter, Haase's results show two minor humps instead of the single hump at 535 mμ reported by others.

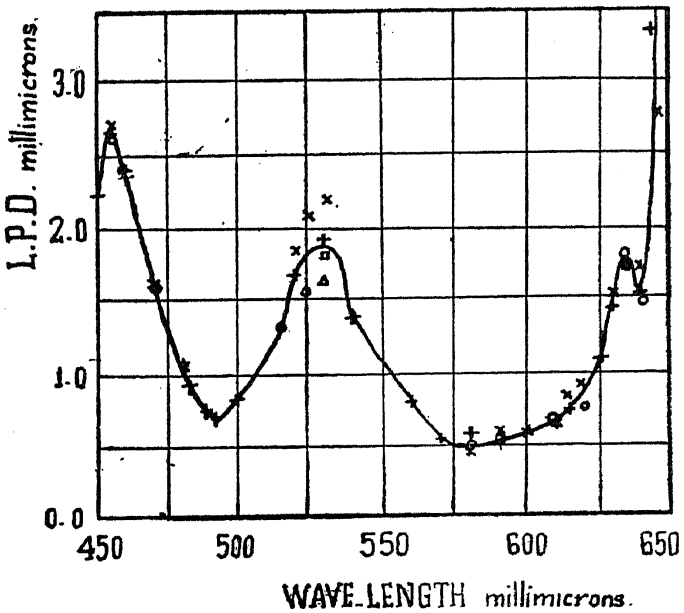


FIG. 3. Hue discrimination curve (after E. P. T. Tyndall).

3. THE COLOUR SEQUENCE IN THE SPECTRUM

The luminous efficiency curve reproduced in Fig. 1 above represents a summation of the effects of the three visual pigments functioning in their respective regions of the spectrum. It gives no indication of any special features related to those appearing in the hue discrimination curve at various points in the spectrum. We have therefore to assume that any special features appearing in the absorption curves of the individual pigments which determine their respective luminous efficiencies have been smoothed out and rendered unobservable by reason of such summation. However, since the econd visual pigment which functions in the green sector of the spectrum plays the major role in human vision, we are justified in assuming that the pronounced maximum at 560 mμ exhibited by the luminous efficiency curve arises principally by reason of a very conspicuous maximum at or near the same wavelength in the absorption spectrum of that pigment. On the other hand, in the blue-violet region of the spectrum, the first visual pigment which functions in that region would principally be responsible for the observed luminous efficiency. Likewise, the third visual pigment would be responsible for the observed luminosity in the region of longer wavelengths and its extension in that direction would account for the asymmetric form of the luminous efficiency curve.

The experiments and observations described in the second part of this memoir showed very clearly that there is a considerable overlap in the absorption spectra of the second and third visual pigments. That there is such an overlap and that it has a most important effect on the visual sensations excited by light appearing in the regions of such overlap is the clue to an understanding of the facts set forth above regarding the distribution of colour in the region of the spectrum now under consideration. The luminous efficiency curve itself indicates that there is a large drop in the absorptive power of the second visual pigment at wavelengths greater than 560 mμ and we may safely assume that this drop continues over the whole range in which that curve goes steeply down, in other words, up to about 627 mμ. The colour change from green to yellow, from yellow to orange and then from orange to red appears precisely in this region. It is a justifiable inference that these changes are a consequence of a rapid falling off in the luminous effect due to the second visual pigment and its progressive replacement by the luminous effect of the third visual pigment which, though inherently much weaker than that of the second pigment, nevertheless effectively determines the observed visual sensations in the regions of the spectrum where it is relatively more important. Continuing this line of

argument, we infer that when, at about 627 mμ, the colour of the spectrum passes over from orange to red, the second visual pigment has ceased to be effective, in other words, that its absorption strength is practically zero. On the other hand, the luminous efficiency curve itself indicates that the visual effect due to the third pigment persists up to the extreme red end of the spectrum, slowly and progressively decreasing with increasing wavelength.

4. Hue Discrimination in the Spectrum

The mechanism of the perception of light and colour by the eye which was indicated in the first part of the memoir was based on the quantum theory of radiation. It recognizes that the physical basis for the differences in colour perceived by the eye in the different parts of the spectrum is the fact that the magnitudes of the light-quanta differ from one end of the visible spectrum to the other, being least at the red end and greatest at the violet. To account for the remarkably high power of discrimination of colour actually exhibited by our eyes, it was postulated that the visual pigments in the retina which are the mediators of vision absorb the energy quanta incident on them and immediately pass on the energy thus absorbed to the sensory mechanism and return to their original energy states. Accepting this hypothesis, the following two questions arise which require an answer: What is the factor which limits the power of the eye to discriminate differences in colour in different parts of the spectrum? Why is it as great as it is in some parts, and why is it less in others? We shall now endeavour to find answers to these questions.

As is well known, the absorption of light by molecules embedded in solid or liquid media is manifested in the form of diffuse spectral bands exhibiting only remnants of the structure shown by their spectra in the state of vapour. As a typical example, we may mention the absorption spectrum of toluene in hexane solution observed in the near ultra-violet. Some nineteen bands are indeed discernible in the region between 270 mμ and 240 mμ, but only the first few of them are sharp and intense, and as we proceed further into the ultra-violet, they become weaker and more diffuse, and the individual bands can only with difficulty be distinguished apart from each other.

The absorption of light by the molecules of the visual pigments embedded in the retina and the transfer of the absorbed energy to the sensory mechanism would necessarily be influenced by various factors, including especially the thermal agitation in the medium. The absorption of radiation involves the electronic energy levels of the molecules of the pigment and the vibrational levels coupled with them. Thermal agitation, on the other hand,

appears as the energy of translatory movements. In solid and liquid media, the molecules may be regarded as being continuously in a state of collision, and hence exchanges of translatory energy *may* occur simultaneously with the exchanges of electronic and vibrational energy. Whether this happens at all and the extent to which it occurs may be expected to depend on the circumstances of each particular case. To obtain a rough idea of the effect of such exchanges, we proceed on the basis of the highly simplified picture of the process indicated by the following equation

$$h\nu - h\nu^* \approx \pm kT$$

in which h, k, and T are respectively Planck's constant, Boltzmann's constant and the absolute temperature of the retina, while $h\nu$ is the energy of the light incident on it and duly absorbed and $h\nu^*$ is the energy actually transferred to the sensory mechanism. The plus and minus signs refer to the cases in which the energy transferred is respectively diminished and increased by the presence of thermal agitation.
Taking

$$h = 6{\cdot}62 \times 10^{-27} \text{ erg.sec.}$$
$$k = 1{\cdot}38 \times 10^{-16} \text{ erg.deg.}^{-1}$$
$$T = 310°$$

$(\nu - \nu^*)$ when expressed as wave-numbers comes out as $\pm$ 215. In other words, the precision with which the eye can recognize a variation in the magnitude of the light quantum as a variation in colour in the spectrum would be diminished to the extent of 430 wave-numbers. When expressed as a wavelength spread in millimicrons, it comes out as directly proportional to the square of the wave-length in the spectrum, in other words, some four times greater at the red end of the spectrum than at the violet end. The calculated figures are shown in Table I below.

TABLE I

Effect of thermal agitation on hue discrimination

Spectral region in mμ	750	700	650	600	550	500	450	400
Spectral spread in mμ	24	20	18	16	12	10	8	6

Comparing the figures shown in Table I with the actual facts of observation as reported by various investigators, it is immediately obvious that except at the extreme red and extreme violet ends of the spectrum, the eye actually exhibits a power to detect changes of colour with change of wavelength far in excess of that indicated by the calculations appearing in Table I. Especially in certain parts of the spectrum, *viz.*, around 440 mμ, 490 mμ and 590 mμ, one finds a remarkable and obviously highly significant sensitivity to colour change with wavelength which closely approaches the theoretical perfection indicated by the principles of the quantum theory when the effect of thermal agitation is ignored. The inference is obvious, *viz.*, that the manner of calculation adopted greatly overestimates the influence of thermal agitation on hue discrimination. The transference of radiational energy to the sensory mechanism which takes place at the retina through the medium of the absorbing pigments present in it is presumably a very rapid process, and it is by no means inevitable that it would be influenced by the thermal agitation in the medium in the manner and to the extent contemplated in the calculations.

We are thus compelled by the facts to approach the subject of the varying power of hue discrimination in the spectrum from a different standpoint. We may reasonably expect it to be closely related to specific features in the absorption spectra of the visual pigments functioning in the respective regions of the visible spectrum; these features are not necessarily observable in the luminous efficiency curve, being masked by reason of the superposition of the effects of all three pigments in it. In the hue discrimination curve, on the other hand, they may well be expected to manifest themselves. In other words, the form of the hue discrimination curve is an indicator of the features of the absorption spectra of the individual pigments and is thus of prime importance in relation to the physiology of vision.

Already, in the third part of this memoir, it has been shown that the form of the hue discrimination curve in the blue-violet sector of the spectrum stands in the closest relationship to the special features in the absorption spectrum of the visual pigment functioning in that sector, *viz.*, xanthophyll. The minima of the limen of wavelength change for a perceptible alteration in colour appearing around 490 mμ and 440 mμ were, in fact, explained as due to the steep ascent and descent appearing in the absorption spectrum of xanthophyll respectively at those wavelengths. It is evident that we have likewise to seek for the explanation of the very remarkable minimum of limen noticeable in the wavelength region around 590 mμ on a similar basis.

Earlier, it has been remarked that the drop of the luminous efficiency at wavelengths greater than that of its maximum at 560 mμ should be

ascribed to a steep drop in the absorptive power of the second visual pigment in that region. Indeed, the latter drop should evidently be even steeper than that of the luminous efficiency curve. For, in the latter curve, the effect of the third visual pigment is superposed and since the latter evidently diminishes with increasing wavelength in this region, the steepness in the drop of the luminous efficiency curve would be diminished by reason of such superposition. We are thus entirely justified in inferring that in the region of wavelengths greater than 560 mμ, the second visual pigment exhibits a very steep drop in absorption with the result that it is practically negligible at wavelengths greater than 625 mμ. Accordingly, in the region of wavelengths between 560 mμ and 625 mμ, we should expect the limen for colour discrimination to exhibit a large diminution and that it should reach its lowest value in the region of wavelengths somewhere between those limits where the absorption curve of the second visual pigment exhibits its steepest fall, *viz.*, around 590 mμ. This is what is actually observed to be the case. The two D lines of sodium appear in this vicinity, and it is a well-established experimental result that when these two lines are equalised in their intensity, they exhibit an observable difference in colour.

5. THE ABSORPTION SPECTRA OF THE HEME PIGMENTS

The considerations of a general nature which lead us to the identification of the two visual pigments functioning in the green and red sectors of the spectrum as ferroheme and ferriheme respectively have already been set out in the second part of this memoir. We shall, in what follows, review the known facts regarding the absorption of light by the heme pigments with a view to compare them with the behaviour of the retinal pigments as indicated by the facts of visual experience.

The iron-protoporphyrin complex known as heme appears associated with different proteins in biological material. The extraordinary versatility which heme exhibits in performing a variety of physiological functions is ascribable to its association with the appropriate proteins in the different circumstances. Extensive studies have appeared in the literature regarding the absorption spectra which it exhibits in various cases. Indeed, the identification of the different heme proteins in biochemical research is largely based on their spectroscopic behaviour. The absorption of light by the heme proteins owes its origin in the first instance to the special structure characteristic of the porphyrin group of compounds, *viz.*, the tetrapyrollic group containing a closed ring of eighteen bonds which are alternately single and double. The character of the absorption is however modified when a metal atom enters the porphyrin structure and finds its place at the centre

of the ring. Further modifications in the spectroscopic behaviour appear when the metal-porphyrin structure associates itself with other nitrogenous materials, including especially different proteins.

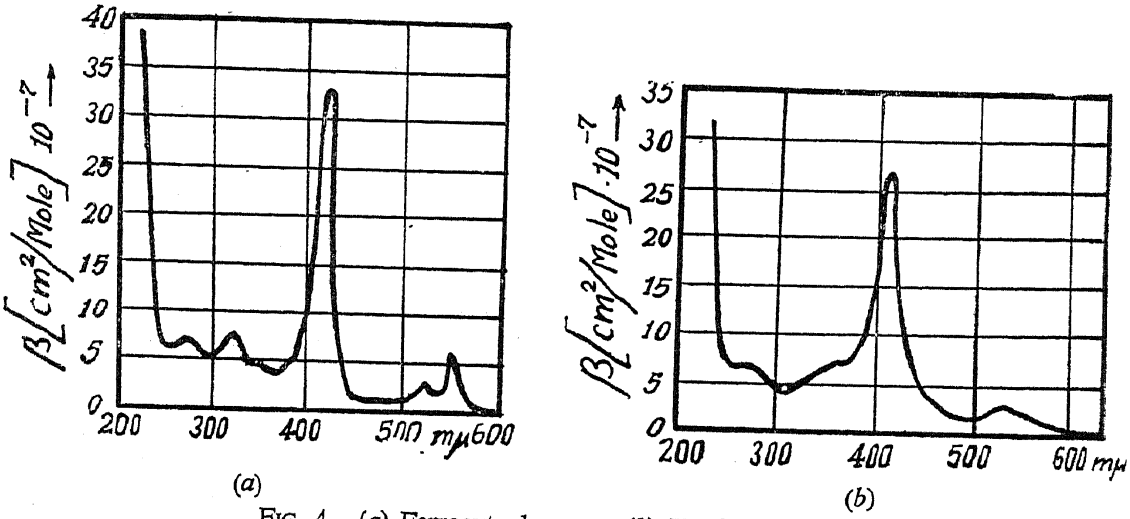
The porphyrins when dissolved in organic solvents exhibit a typical four-banded absorption in the visible spectrum with some indications of further details. The strength of the bands increases towards shorter wavelengths and in addition there is a still stronger band at about 400 mμ known as the Soret band, having been first observed by that author in the absorption spectrum of hemoglobin. The four bands of protoporphyrin, for example, in ether-acetic acid solution have their maxima at 632, 576, 537 and 502 mμ respectively, while indications of subsidiary bands at 605 and 585 mμ have also been noticed. It is worthy of remark that at the temperature of liquid air, the bands sharpen and are partly split up and shifted to shorter wavelengths.

The spectroscopic behaviour of the porphyrins in the form of complex salts formed by their combination with different metals has been extensively investigated, using the synthetically prepared substances in solution in different organic solvents. Generally speaking, it is found that the four-banded spectrum of the porphyrins is replaced by a two-banded spectrum, the position of the two maxima as well as their relative intensity varying with the metal which has entered the structure of the porphyrin. Ferrocoproporphyrin, for example, shows a strong absorption band at 550 mμ and a weak one at 520 mμ. The great difference between the spectroscopic behaviour of the complexes formed by the combination with iron in the ferrous and ferric states may be illustrated by the case of mesoporphyrin. Whereas the ferrous compound with it exhibits, in a buffered acetic acid solution, a band covering the region 555 mμ to 565 mμ in the green, the ferric compound shows two bands, one in the green between 530 mμ and 542 mμ and another in the red between 630 mμ and 640 mμ.

The association of the iron-porphyrin complexes with other nitrogenous substances to form what are known as hem*o*chromes and hem*i*-chromes respectively has a notable influence on the character of their absorption spectra. The hem*o*chrome structure is characterised by the appearance of a two-banded absorption spectrum of which the first or α-band is very sharp and also much more intense than the second or β-band of shorter wavelength. The positions of these two bands are observably influenced both by the nitrogenous base and by the porphyrin. In the hem*o*chrome formed by protoporphyrin with pyridine for example, the α-band has its maximum at 558 mμ, while the β-band appears at 525 mμ.

On the other hand, the hemochrome formed by the combination of ferrous iron with mesoporphyrin and pyridine has its absorption maxima at 547 mμ and 518 mμ.

A reference should also be made here to the absorption spectra of the cytochromes, of which several have been reported and which are distinguished from each other by their spectroscopic behaviour. The best-known of them is cytochrome *c* which has been isolated and spectroscopically investigated in the pure state. It shows absorption maxima at 550 mμ and 521 mμ, the former or α-band being the more intense of the two. Figure 4 below reproduces the absorption characteristics of cytochrome *c* as determined by Theorell.



(*a*) (*b*)

FIG. 4. (*a*) Ferrocytochrome. (*b*) Ferricytochrome. Absorption spectra of cytochrome *c* after (Theorell).

Figure 4 (*a*) represents that of the reduced form, *viz.*, ferrocytochrome *c* and Fig. 4 (*b*) that of the oxidised form, *viz.*, ferricytochrome *c*. It will be noticed that while the absorption by the ferrous pigment exhibits a strong, sharp band located at 550 mμ and a weaker one at 521 mμ and is practically confined to the green region of the spectrum, the ferric compound has an absorption extending into the red with a broad diffuse maximum in the green around 530 mμ. This difference between the ferrous and ferric forms is further illustrated in Fig. 5 (due to Horecker and Kornberg) which shows that the absorption by the latter goes right up to the extreme red end of the spectrum.

6. THE OBSERVABLE CONSEQUENCES

If white light is spread out into a spectrum of small dispersion by a diffraction grating, the latter appears to the eye to consist of three bands

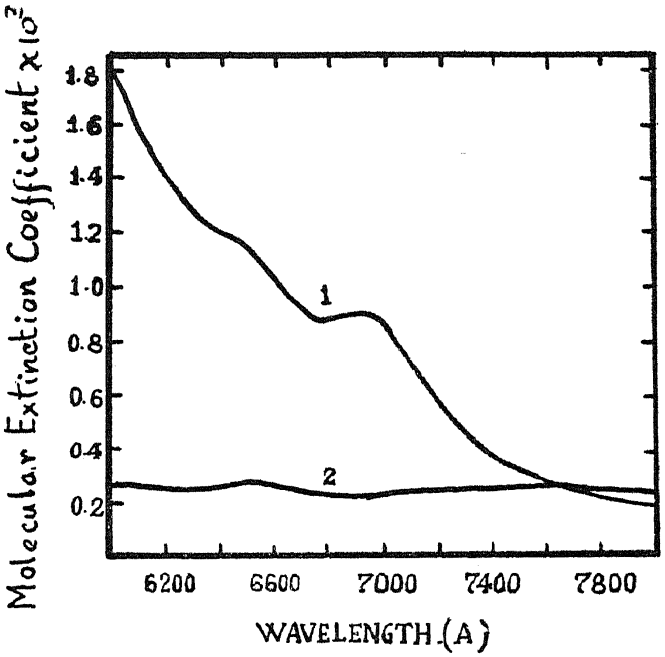


FIG. 5. Absorption spectrum of cytochrome *c* in the extreme red.
(1) Oxidised form; (2) Reduced form.

of colour of approximately equal width: a blue-violet band which is highly colourful but of low luminosity and covers the region from 400 mμ to 500 mμ; a green band from 500 mμ to 600 mμ and a red band from 600 mμ to 700 mμ. The two latter bands are also colourful, the red perhaps more so than the green, but the red is less luminous, especially near the extremity of the spectrum. The yellow-orange section of the spectrum appears merely as an edging between the green and the red. The most luminous part of the spectrum is in the green, not far from its yellow edge. The rapid changes in colour from blue to green at about 490 mμ, and from yellow to orange at about 590 mμ are also obvious to inspection. One is tempted to associate the three principal bands of colour with three visual pigments functioning in three distinct regions of the spectrum. But the appearance of the yellow and orange as a transition between green and red is a clear indication that there is an overlap of the absorption spectra of the second and third visual pigments.

The various related features of the spectrum mentioned above are readily intelligible in the light of the spectroscopic behaviour of the heme pigments discussed in the foregoing pages. The pigments formed by the combination of ferrous iron with protoporphyrin absorb light in the region of wavelengths between 500 mμ and 600 mμ. In all cases, the maximum of this absorption appears at or near 560 mμ which is also the wavelength

of maximum luminous efficiency in the spectrum. Then again, the absorption drops down quickly with increasing wave-length between 560 mμ and 600 mμ. This feature explains the low values for the limen of hue discrimination observed in that region. The absorption by the ferric forms of the pigment, on the other hand, appears both in the green and red sectors of the spectrum; it is, however, weaker and more diffuse than that of the ferrous forms. This situation explains the appearance of yellow and orange as transition colours between the green and the red in the region where the absorption by the second pigment is weak. Likewise, the diffuseness of the absorption by the ferric pigment and its progressive weakening with increasing wave-length explain the diminishing luminosity and the increasing limen of hue discrimination as we approach the red end of the spectrum.

Some remarks should be made here regarding the Soret band of absorption. Had this been effective as a mediator in human vision, the violet end instead of being the feeblest part of the spectrum, would have been the most luminous. The inference is obvious, that the Soret band is not active as a transmitter of the energy absorbed which, presumably, is dissipated in some other fashion.

The Soret band is a general feature in the absorption spectra of the whole porphyrin group of compounds, even when a metal atom is not present at the centre of the tetrapyrollic group. There is therefore no reason to believe that it would act in the same way as the absorption bands in the visible region appearing by reason of the presence of the metal atom. It may be remarked in this connection that chlorophyll participates in the photosynthetic activity of green leaves by reason of its characteristic absorption band in the red; the Soret band, so far as is known, remains entirely inactive.