

THE NEW PHYSIOLOGY OF VISION

Chapter XI. The Carotenoid Pigments

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THE carotenoids are a group of carbon compounds which owe their colours to a special feature in their molecular structure, *viz.*, the presence of numerous conjugated double bonds forming an elongated chain of carbon atoms joined together by such bonds. Many carotenoids are to be found occurring naturally. Thirty of these compounds of which the structure has been fully elucidated by chemical investigation are listed in the well-known treatise on the carotenoids by Karrer and Jucker (Elsevier, 1950). Their structural formulæ have there been shown in tabular form, and supplemented by another table setting out their classification as derivatives of lycopene and the three known carotenes. Of particular interest are twelve photographs in colour reproduced towards the end of the treatise. These pictures show the forms of the crystals of various carotenoids deposited from solution in appropriately chosen organic solvents.

Amongst the thirty naturally occurring carotenoid pigments of known chemical constitution listed by Karrer and Jucker, two are of particular interest in the present context, *viz.*, β -carotene and xanthophyll. Their chemical formulæ are respectively $C_{40}H_{56}$ and $C_{40}H_{56}O_2$. The chemical composition of xanthophyll is indicated by its formal description as dihydroxy- α -carotene. Both of these carotenoids are to be found widely distributed in nature. They appear invariably in the green parts of living plants as accompaniments of chlorophyll and presumably therefore play special roles in photosynthesis. Both carotenoids find their way into the human body by way of the food products consumed by the individual. They have been located in human blood serum, in fat tissues, in milk fats, in the liver and in the human placenta. β -carotene is of special importance in human physiology by virtue of its being a precursor of Vitamin-A which is essential for human health. Xanthophyll, on the other hand, does not serve as a precursor of Vitamin-A. The reason for this difference is intelligible. β -carotene has symmetric molecules which in appropriate circumstances can split into two equal fragments, each of which by the addition of a water-molecule acquires a terminal hydroxyl group and becomes a molecule of Vitamin-A. Such a splitting could scarcely be expected to occur in the case

of xanthophyll, as this substance has unsymmetrical molecules in which a hydroxyl group is already present in each of the closed rings at the ends of the molecule.

Spectroscopic Behaviour of the Carotenoids.—Towards the end of the treatise by Karrer and Jucker appear 28 figures in which the light-absorption curves of numerous carotenoids dissolved in organic solvents are reproduced. A study of these figures is highly illuminating. With some significant exceptions, the absorption by these pigments appearing in the visible region of the spectrum is restricted to its blue-violet sector, in other words, to the wavelengths between $400\text{ m}\mu$ and $500\text{ m}\mu$. Another general feature is the appearance within this range of the spectrum of alternate maxima and minima, there being usually three maxima of absorption. Following such alternations, there is a steep drop in absorption as we proceed towards longer wavelengths, and this is followed by a feeble absorption extending a little beyond $500\text{ m}\mu$ before it finally vanishes. Towards shorter wavelengths, there is also a fall which is distinctly less rapid, the absorption becoming weak at $400\text{ m}\mu$ and weaker still in the near ultra-violet. These features are illustrated in Fig. 1 for the α - and β -forms of carotene. The

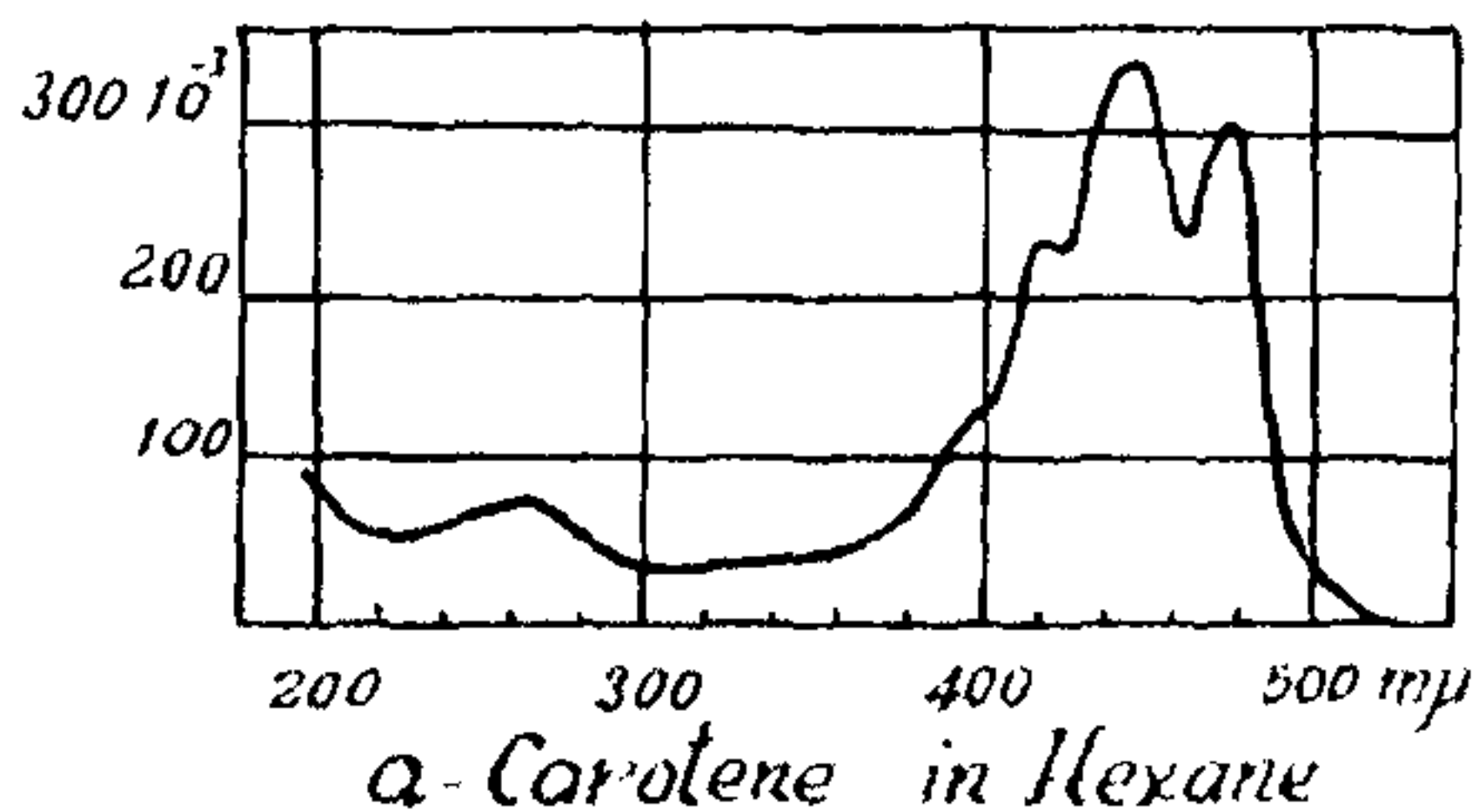
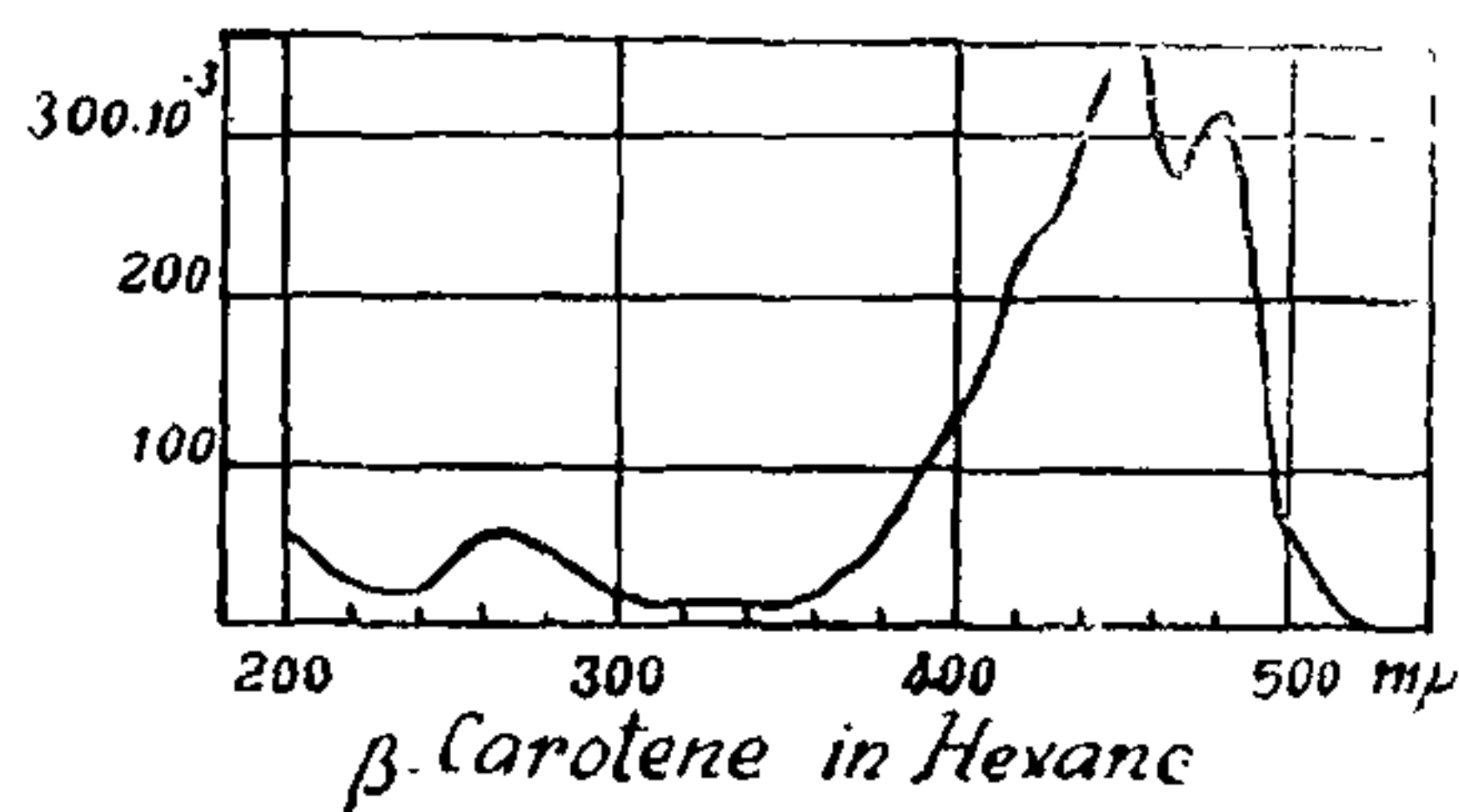


FIG. 1. Light Absorption Curves

two corresponding dihydroxy derivatives known as xanthophyll and zeaxanthin exhibit absorption curves closely resembling those of the respective carotenes. It should be mentioned that the precise positions of the maxima of absorption between $500\text{ m}\mu$ and $400\text{ m}\mu$ are noticeably influenced by the choice of solvent for the observations. The actual positions of the maxima observed in hexane solution are for β -carotene $477\text{ m}\mu$, $450\text{ m}\mu$ and $425\text{ m}\mu$. The corresponding figures for xanthophyll in alcohol solution are $476\text{ m}\mu$, $447\text{ m}\mu$ and $420\text{ m}\mu$.

The relationship between the colour and the constitution of carotenoids have been extensively studied and empirical relations have been deduced. These will be found set out fully and discussed in the treatise of Karrer and Jucker. The absorption of light by the carotenoids in the visible region of the spectrum has been ascribed to electronic oscillations along the chain of conjugated double bonds. It can be predicted on this basis that the wavelengths and intensities of the maxima of absorption would increase with the number of conjugated ethylenic bonds in the molecule.

Of particular significance are the exceptions to the general rules regarding the form of the absorption curves which have been stated above. It will suffice to mention here three such cases, viz., rhodoxanthin ($\text{C}_{40}\text{H}_{50}\text{O}_2$), astaxanthin ($\text{C}_{40}\text{H}_{48}\text{O}_4$) and astacene ($\text{C}_{40}\text{H}_{48}\text{O}_4$). Their absorptions extend into the visible spectrum well beyond the usual limit of $500\text{ m}\mu$. Such extension is accompanied by noteworthy changes in the form of the absorption curve, the alternation of maxima and minima between $500\text{ m}\mu$ and $400\text{ m}\mu$ becoming less pronounced or even completely disappearing. A single wide-band maximum is then observed in this region. These features are illustrated in Fig. 2. All three carotenoids whose absorption curves appear in the figure are derivatives of β -carotene. The changes noticed in their absorption curves are the result of the introduction of oxygen atoms, each replacing two hydrogen atoms, in the closed rings which terminate the molecule. Astacene, for example, in which the wide-band maximum of absorption appears at $500\text{ m}\mu$ may be described as tetraketo- β -carotene. That the spectroscopic behaviour of β -carotene is profoundly altered by this change in the chemical nature of the end groups in the molecule is not surprising.

Role of the Carotenoids in Vision.—Visual pigments function by reason of their presence in the retina as well as their ability to absorb

light in particular regions of the spectrum and to transfer the energy thus absorbed through the optic nerves to the cerebral centres of perception. By reason of the powerful absorption of light exhibited in the wavelength range between $400\text{ m}\mu$ and $500\text{ m}\mu$, carotenoid pigments are qualified to function as receptors of vision in this range. Studies on the visual perception of polarised light in the blue-violet sector of the spectrum and its relation to the

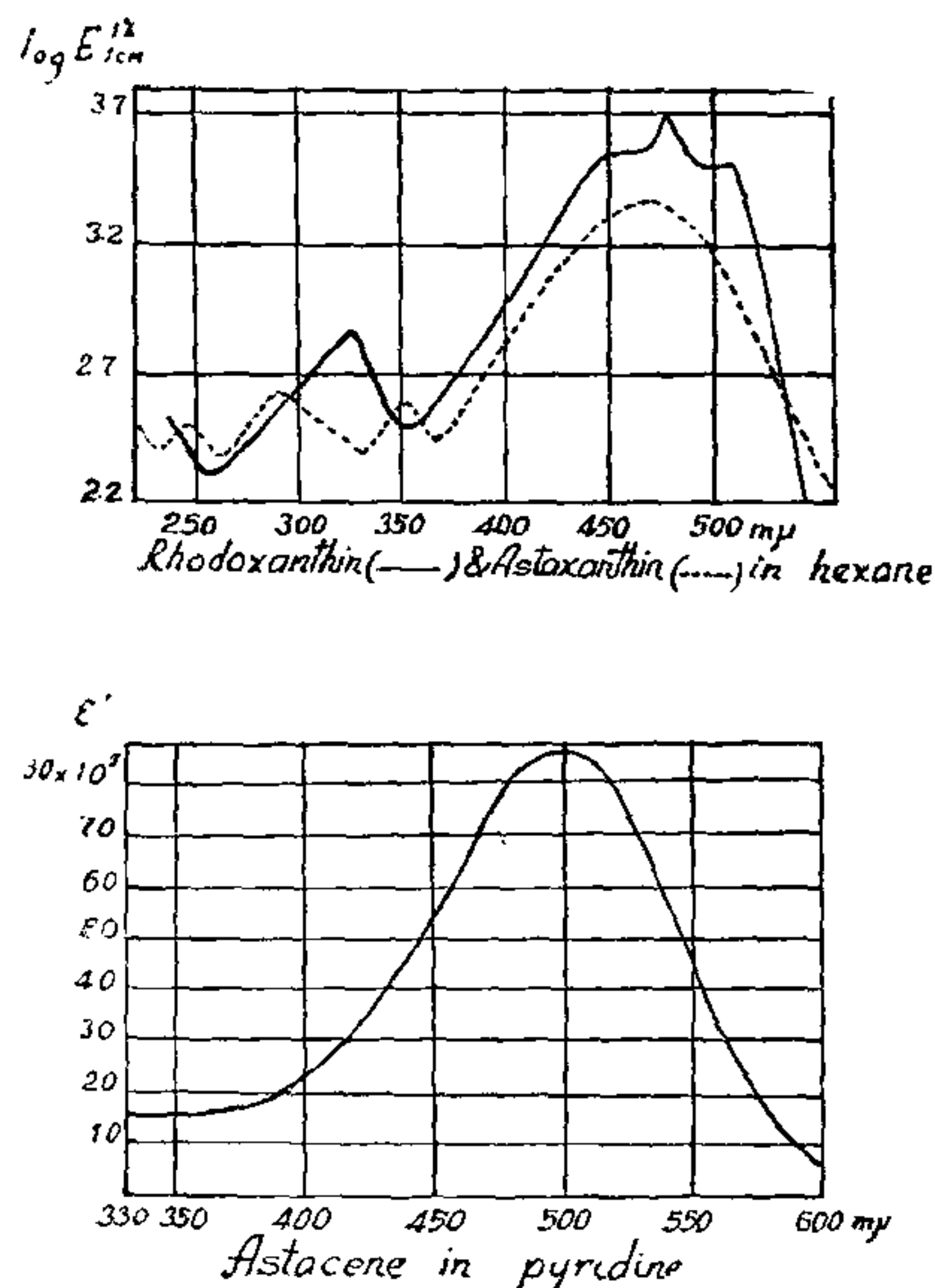


FIG. 2. Light Absorption Curves

structure of the fovea have been described in two earlier chapters. They pointed to the conclusion that a carotenoid is indeed the visual pigment which enables us to perceive light and colour in that range of the spectrum. We shall revert to the same theme in the present chapter and describe further observations which confirm the stated finding and enable us to identify the pigment as xanthophyll.

The nature of the visual pigment functioning at low levels of illumination is another problem of great interest. It is a characteristic of vision at low levels of brightness that we do not perceive the red end of the spectrum and in consequence, dim-light vision is practically confined to the spectral range between $400\text{ m}\mu$ and $600\text{ m}\mu$. It has been shown in an earlier chapter that the ability to perceive feeble light

with these spectral characteristics is not an exclusive feature of "rod vision", since it is exhibited just as perfectly by the cones in the retina. The ability to perceive dim light is indeed a general and fundamental aspect of human vision. From the spectral characteristics of dim-light vision, we may proceed to infer the features which we may expect to find exhibited by the light-absorption curve of the visual pigment which enables us to perceive dim light. This, in its turn, should assist us in identifying the pigment. Visual observations of the spectrum of dim light show that its maximum brightness is located at about $500\text{ m}\mu$. What has been stated above and is illustrated by the absorption curves reproduced in Fig. 2 indicates that the visual pigment functioning in dim light is a derivative of β -carotene in which the two groups at the ends of the molecule have both been modified suitably so as to give an absorption curve of the same general shape as that of astacene shown in Fig. 2. We shall consider this matter more fully as we proceed.

Colour and Luminosity in the Spectrum.—Much knowledge regarding the visual perceptions of luminosity and colour emerges from very simple observations made with a long straight metallic filament stretched inside a tubular lamp and carrying an electric current as the source of light, and a replica-diffraction grating held before the eye as the dispersing apparatus. Altering the current through the filament with the aid of a rheostat, the light emission can be raised step by step from a dull red glow to the intense white light emitted by the filament at the highest temperature which it can carry. The results of the observations thus made will be described and discussed in a succeeding chapter. Here, we shall confine ourself to those features which have a bearing on the present topic, *viz.*, the nature of the visual pigments which function in the blue-violet sector of the spectrum.

In the continuous spectrum of a moderately luminous source of white light, an observer can readily trace a progression of colour and luminosity as we pass along the spectrum. A feature which is immediately obvious is that the colour alters as we proceed quite slowly in some regions of the spectrum and quite rapidly in others. The transition from the blue to the green of the spectrum is one of the regions in which the changes are particularly rapid. $490\text{ m}\mu$ is the wavelength at which the colour changes most rapidly and this can be fixed quite accurately by simple visual observations made with a

wavelength spectrometer. Why such a rapid change occurs at this point in the spectrum is readily understood by reference to the absorption curves of the carotenes reproduced as Fig. 1 above and even better from that of xanthophyll exhibited in Fig. 3.

It will be seen that the strength of the absorption goes down steeply from a large value at $480\text{ m}\mu$ to a relatively small value at $500\text{ m}\mu$, the steepest fall being at $490\text{ m}\mu$. Hence, if xanthophyll is the visual pigment which is principally functioning in the spectral range between $400\text{ m}\mu$ and $500\text{ m}\mu$, the chromatic sensation excited by it would become weaker and tend to disappear as we proceed from $480\text{ m}\mu$ to $500\text{ m}\mu$, while the chromatic sensation excited by the visual pigment functioning between $500\text{ m}\mu$ and $550\text{ m}\mu$ would *pari passu* gain in strength. The rapid progression in colour and our ability to locate it precisely at $490\text{ m}\mu$ are thus accounted for in a very satisfactory manner.

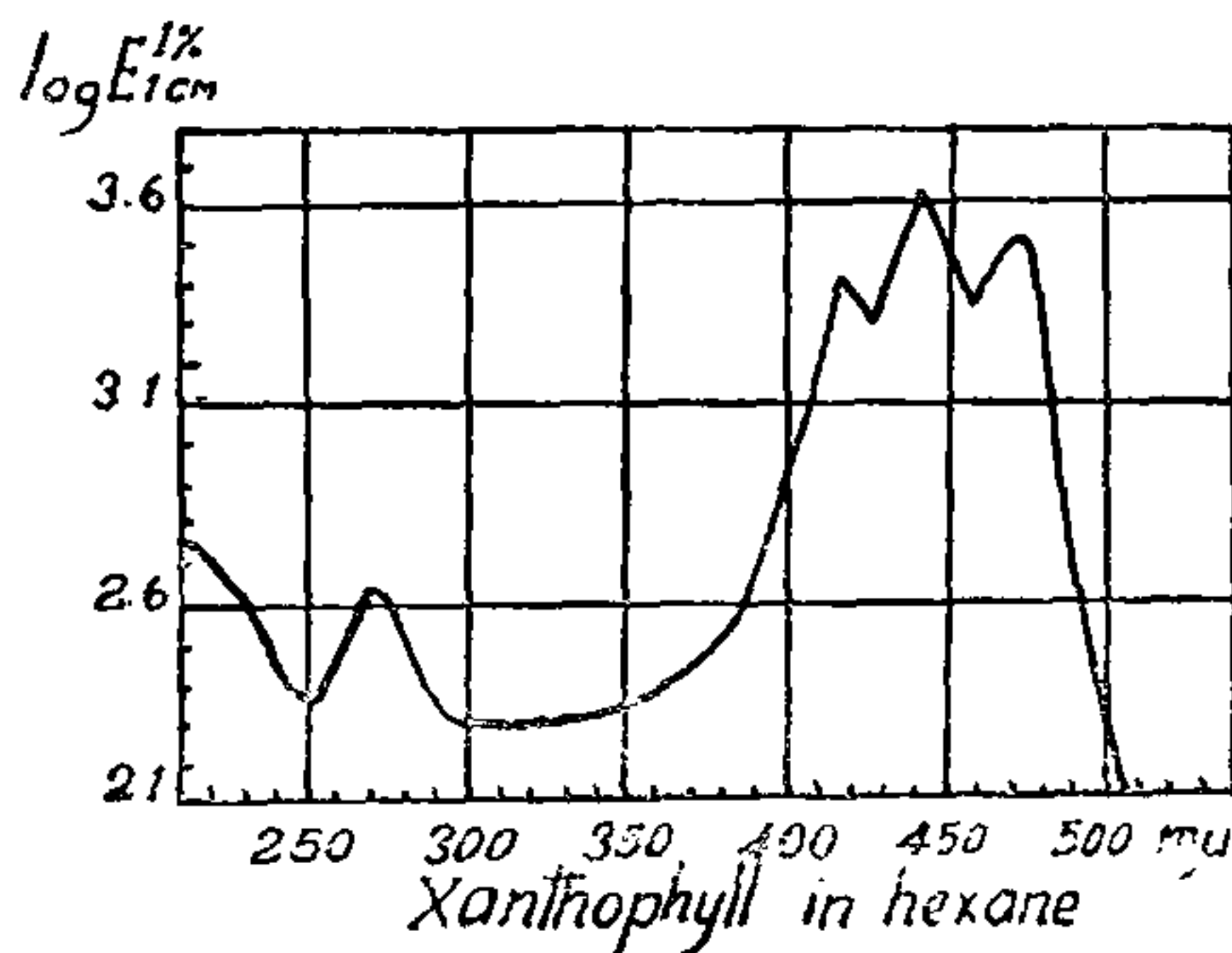


FIG. 3. Light Absorption Curve

It is worthy of remark that the absorption by xanthophyll does not actually disappear at $500\text{ m}\mu$ but continues to be sensible at $510\text{ m}\mu$ beyond which it ceases to be significant. This is clear from the absorption curves and it may also readily be verified by visual observation of the light-transmission through a solution of xanthophyll (mixed with a little zeaxanthin) obtained by the extraction of the yellow pigment of egg-yolk with hot acetone. With a sufficient absorption path, the cut-off of the spectrum appears at $510\text{ m}\mu$ accompanied by a sensible weakening upto $520\text{ m}\mu$, beyond which there is perfect transparency. It follows from these circumstances that the contribution of xanthophyll to the colour perceived in the spectrum should extend well beyond $490\text{ m}\mu$ where the change from blue to green is most

rapid. Indeed, visual observation shows that the green of the spectrum has a distinct "blue edging" extending upto about $510\text{ m}\mu$.

Further confirmation that xanthophyll is the visual pigment functioning between $400\text{ m}\mu$ to $500\text{ m}\mu$ is forthcoming when a continuous spectrum of moderate intensity is surveyed through the eye-piece of a wavelength spectrometer. We notice three points in the spectrum at which impressive changes in its character are noticeable. The first is at $490\text{ m}\mu$ as has already been mentioned and discussed. The second is at $465\text{ m}\mu$ and the third is at $435\text{ m}\mu$. At $465\text{ m}\mu$, there is a marked change of colour and at $435\text{ m}\mu$ there is a marked change of intensity. The three zones in the spectrum thus marked off are also those into which the spectrum is divided by the three peaks of absorption depicted in Fig. 3. The observed differences in colour and intensity between the three zones are explicable in terms of the large differences in the absorptive power of xanthophyll in those regions of the spectrum, when the other circumstances of the case are also taken into account.

Perception of Polarisation.—Still another confirmation that xanthophyll is the visual pigment functioning in the $400\text{ m}\mu$ to $500\text{ m}\mu$ range of spectrum is furnished by the effects noticed when this region of the spectrum is surveyed from point to point by an observer holding a polaroid in front of his eye and swinging it to and fro in its own plane through 90° . A well-dispersed continuous spectrum exhibiting an adequate intensity over its entire range is essential for such observations. When these requirements are secured, the following features come to light:

- (a) The phenomenon of the brushes described in an earlier chapter continues to be noticeable, though much enfeebled, in the region of wavelengths between $500\text{ m}\mu$ and $520\text{ m}\mu$. It disappears completely at wavelengths greater than $520\text{ m}\mu$.
- (b) The brushes can be seen over the entire range of the spectrum from $500\text{ m}\mu$ to the extreme violet end.
- (c) Their clearness depends much on the luminosity of the spectrum in the region under observation. There are also indications that it exhibits variations, being greatest in certain regions and distinctly less in others.

Presence of Xanthophyll in the Retina.—The observational evidence set forth above justifies the inference that xanthophyll is present in the living retina and that it functions as a visual

pigment. But it is not superfluous to add that its presence is also attested by independent evidence. Xanthophyll may be identified and indeed has been identified in the past as the material responsible for the yellow pigmentation of the macular region of the retina. In a later chapter, we shall also present direct observational evidence for the presence diffused over an extensive area of the retina of a pigment which absorbs light in the blue-violet sector of the spectrum and enables us to perceive light and colour in that sector.

Some remarks regarding the question whether β -carotene is or can be the visual pigment functioning in bright light may be made here. There are weighty reasons for excluding that possibility. Blue-blindness is a very rare condition and this indicates that the visual pigment necessary for the perception of the blue in the spectrum is present in abundant measure with little possibility of its running short. Xanthophyll is not a vitamin precursor and not being needed for other purposes can find its way into the retina through the blood stream to the extent needed and be replenished whenever necessary. If β -carotene were present in the retina along with xanthophyll, it would function as a visual pigment in much the same way. The differences between the form of their absorption spectra might perhaps lead to detectable differences in their functioning. But this possibility scarcely needs consideration, since β -carotene has other physiological functions to perform which make it most unlikely that it is present in unmodified form in the retina to the same extent as xanthophyll.

Perception of Dim Light.—It is indisputable that there is present in the retina a material that enables us to perceive dim light in the spectral range between $400\text{ m}\mu$ and $600\text{ m}\mu$ and which has its maximum luminous efficiency at or near $500\text{ m}\mu$. But the idea which has so far prevailed that this material is a constituent part of the structure of the "rods" and that it functions only in "rod-vision" is definitely false. As has been shown in earlier chapters, dim light can be perceived also by the cones in the retina, including especially those in the foveal region where there are only cones and no rods. It follows that the visual pigment is spread and distributed through the substance of the retina in such manner as to permit of rods and cones alike functioning in dim light. Studies which base themselves on the extraction of material from the rod-structures by chemical or mechanical methods are therefore not really

relevant to the problem of determining the nature of the visual pigment.

The physiology of vision is concerned with the functioning of the retina in the living state. It follows that we have to rely principally on the actual facts of visual experience and to base their interpretation on other facts and on well-established principles. The basis for all considerations regarding the nature of the visual pigment is, *firstly* that its spectral sensitivity extends over the entire visible spectrum upto 600 m μ , but that it does not extend further towards the red end and, *secondly* that the maximum of its luminous efficiency appears at about 500 m μ . These facts by themselves make it practically certain that the pigment is a carotenoid. Indeed, if we look through the 30 light-absorption curves reproduced at the end of the treatise by Karrer and Jucker, we do not find a single instance in which the strength of the absorption at any wavelength greater than 500 m μ exceeds that at 500 m μ . Nor do we find a single instance in which the authors of that treatise thought it necessary to extend the scale of wavelengths beyond 600 m μ . The reason for this is that in nearly all cases, the absorption ceases to be significant beyond 550 m μ . In a majority of cases, also, the absorption reaches its maximum at or near a wavelength of 500 m μ . As has already been remarked on an earlier page, the wavelengths and intensities of the maxima in the absorption spectra of both natural and synthetic polyenes increase with the number of conjugated ethylenic bonds. The absorption spectra are therefore an indication of the number of such bonds contained in the molecule.

In view of what has been stated above, it may justifiably be inferred that the visual pigment functioning in dim light vision is a carotenoid having the same number of ethylenic bonds as β -carotene. We shall also be justified in inferring that it is a derivative of β -carotene in which the two groups appearing at the ends of each molecule have been so modified as to render its absorption spectrum generally similar to that of astacene represented above in Fig. 2.

Carotenoid chemistry makes extensive use of oxidative and reductive processes in which suitable reagents are employed. Numerous

examples of this will be found set out in the chapter on the synthesis of carotenoids in the treatise of Karrer and Jucker. There is present in the retina a substance, *viz.*, oxyhemoglobin, which can transfer its oxygen content to other materials, being itself reduced to hemoglobin in the process. One may, therefore, venture to put forward the suggestion that the transformation of β -carotene to a derivative having an altered spectroscopic behaviour is effected through such oxidation. The pigment thus formed could scarcely be expected to be light-fast. In other words it would break up and result in other substances being formed when exposed to strong light. Its formation can therefore take place only in dim light or in complete darkness. These are, in fact, the characteristic features of the visual pigment functioning in dim light.

Night-Blindness and Its Origin.—The β -carotene that enters the human body by way of the food-stuffs consumed has to play a dual role. It has, in the first place, to function as the parent of Vitamin-A, and in the second place to provide the material needed for vision in dim light. As the supply of β -carotene is limited by the quantity and by the quality of the food-stuffs consumed, it is scarcely surprising that in certain circumstances it may prove insufficient to meet the requirements. As is well known, Vitamin-A is stored up in the liver and also elsewhere in the human body and that the reserves can be drawn upon when necessary. A deficiency in the carotenic content of food would therefore in the first instance result in an inadequate replenishment of the visual pigment which is destroyed by exposure to bright light. This would produce a condition of partial or complete night-blindness, which can, of course, be set right by an increased consumption of food-stuffs containing β -carotene. Alternatively, the addition to the food of material with a large content of Vitamin-A would serve the same purpose. For, this would reduce the major demands for a supply of β -carotene and enable more to be available for vision. It is even possible that the transformation of β -carotene to Vitamin-A in the human body is a reversible process, and that doses of Vitamin-A may remedy the deficiency in the carotenoid input needed for vision.