THE PHOTOCHEMISTRY OF VISION

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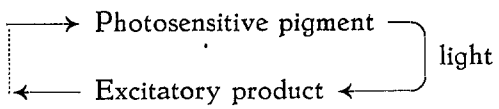
A ray of light entering the eye penetrates cornea and lens, aqueous and vitreous humours, and finally the nervous tissue of the retina itself to its furthermost boundary. Here it encounters a thin mosaic of cellular organelles, the plain of outer segments of the rods and cones. These accomplish the transmutation upon which vision depends. The light ceases to exist as such — we say it has been absorbed — having been transformed into the internal energy of retinal molecules. Up to this point we have to deal with light, a form of radiant energy. From here on it has become part of the substance of the organism, generating within it the train of complex changes by which we see.

It is this transformation with which we are concerned in the photochemistry of vision. Necessarily it is accomplished by pigments; for light, to have any effect, physical or chemical, must first be absorbed, and substances which absorb *visible* light are by that token pigments. It is not enough that the retinal pigment absorb light. It must also be changed thereby in some special way that results ultimately in a nervous excitation. This having occurred in a rod or cone, the excitation can be transmitted from cell to cell to the brain.

The pigment changed by the absorption of light must be rapidly restored to its original state; otherwise one could not go on seeing. The state of excitation also must be rapidly undone; otherwise one should continue to see after the light had gone off. The processes which initiate vision must therefore include these three reactions: the modification of a photosensitive pigment by light so as to yield an excitation; the removal of excitation; and the regeneration of the original pigment:



It would aid the economy of such a system if the restitution of photopigment were coupled somehow to the removal of photoproducts, so that the system operated as a cycle, with no loss of material. This possibility is indicated in the diagram by a broken

line. It represents an advantage, yet not a necessary condition in the organization of the photoreceptor process.

It is implicit in this formulation that the sensitivity of the eye depends upon the capacity of the photopigment to absorb light. In a constant retinal state, the visual sensitivity rises and falls in the various regions of the spectrum with the absorption spectrum of the photopigment. When light is turned on, the concentration of photopigment falls to a steady-state level, at which the rate of its photochemical decay is balanced by its rate of resynthesis. The associated fall of visual sensitivity to a steady, depressed value is light-adaptation. In the dark only regenerative reactions remain; the concentration of photopigment, and hence the visual sensitivity, rise to maximal values. This is dark-adaptation.

Within such a context of ideas one can bring together the chemistry of the retina and the physiology of vision. The notion that visual excitation is founded upon some reversible, or better, pseudo-reversible photochemical system, the properties of which are reflected in the sensory response, is the product of a gradual development, to which notable contributions were made by Kühne, the ophthalmologist Garten, the photochemist Luther, and the botanist Blaauw. But it was Selig Hecht who throughout his lifetime developed these ideas systematically, gave them precise form, and taught them to our generation.

Rhodopsin

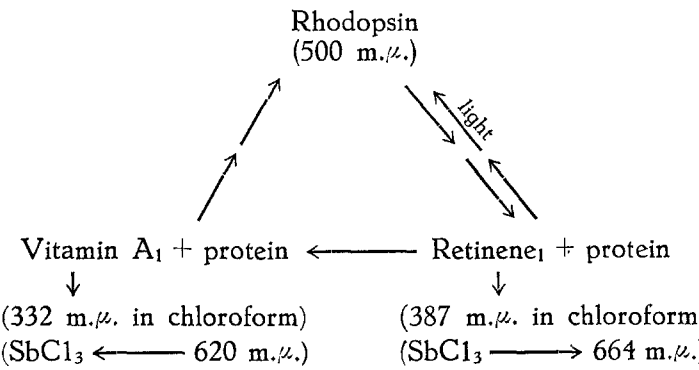
The first photosensitive pigment was discovered in the rods of the frog retina by Franz Boll (1877). It bleaches in the light and is restored in darkness, and so fulfils the elementary requirements of a *visual* pigment. Boll remained in some doubt whether this was truly a substance or merely a physical appearance. Its status as a substance was settled in the following year by Kühne (1878). [1])

[1]) It is not generally realized that Kühne, whose work on retinal chemistry was so comprehensive that half a century passed before it was significantly extended, entertained serious doubts of the role of rhodopsin in vision, and failed generally to grasp the theoretical necessity for visual *pigments*. Since highly light-adapted animals whose rhodopsin seems wholly bleached still continue to see, as do also animals possessing only cones, in which no pigment is visible, Kühne concluded that photosensitive pigments are not needed in vision, and that rhodopsin itself plays a problematical role. Toward the close of his great pioneer investigations he stated: „Fasst man die Frage allgemeiner, ob es Sehorgane gebe ohne alle Farbstoffe, so muss auch diese bejaht werden, weil die Zapfen in der Thierreihe zum grossen Theile pigmentlos sind... Die optochemische Hypothese

Boll called this material *Sehrot,* or visual red. Kühne re-named it *Sehpurpur,* which Michael and Mrs. Foster immediately translated into English as 'visual purple' (Kühne, 1878b). This was an unhappy choice among the meanings of *Purpur,* which include scarlet and red as well as purple; and does some violence to the colour of the substance, which is red. I shall refer to it hereafter by Kühne's other name for it, rhodopsin.

Rhodopsin is a conjugated protein, which owes its colour and light-sensitivity to a special prosthetic group. This is derived from the large class of fat-soluble, highly unsaturated, animal and plant pigments known as carotenoids.

When rhodopsin is exposed to light in the retina, two major changes occur: the carotenoid is cleaved from protein, and is degraded through orange intermediates, first to the yellow retinene$_1$, then to colorless vitamin A$_1$. The retina can also resynthesize rhodopsin in two ways: rapidly by reversion from retinene$_1$, and relatively slowly from vitamin A$_1$. The rhodopsin system as a whole therefore constitutes a cycle of the form:



It must be emphasized that this is a skeletal formulation, which does no more than express in terms of three most stable components what is, in fact, a more complex succession of substances and

ist hiernach unbedingt auf die Annahme auch farbloser Sehstoffe angewiesen" (1879, p. 329). And following a further discussion, „Dies dürfte es sehr wahrscheinlich machen, dass auch Stäbchen ohne Sehpurpur sehen und man würde daher in diesem Augenblicke niemanden wiederlegen können, der behauptete der Sehpurpur sei gar kein Sehstoff. Die optochemische Hypothese ist also genötigt auch in den Stäbchen farblose Sehstoffe anzunehmen....." (p. 331). This is a curious confusion in Kühne's otherwise magnificent achievement. Grotthus and Draper had long before expressed the requirement that light be absorbed in order that it act; but this idea had not yet attained the easy currency it now possesses, and Kühne for all his genius had failed to grasp it.

processes. One can not yet be sure for example which among the orange and yellow products of bleaching is the immediate precursor of the resynthesis of rhodopsin, or at which point carotenoid becomes altogether disjoined from protein (cf. Wald, 1937—'38b, p. 828).

Nor is it intended that retinene$_1$ be regarded literally as the product responsible for neural excitation. Carotenoids are by nature bland substances to which it is difficult to impute strong pharmacodynamic effects. A simple computation shows that rhodopsin bulks large in the total composition of the rod outer limb; and I should prefer to look for the source of excitation in the direct physical attack on the microstructure of the rods implicit in the bleaching process itself, rather than in the liberation of a specific stimulant (cf. Wald, 1944 a).

The appearance of a vitamin in the equations of the rhodopsin cycle introduces a series of special relationships. By definition this component cannot be synthesized by the organism, and so must be made available in the diet. At this point the visual processes make contact with the general metabolism and transport of vitamin A throughout the body, and with an external factor, its nutritional supply. These relations become manifest clinically in the appearance of night blindness as one of the earliest symptoms of vitamin A deficiency, and in certain chronic liver diseases in which the absorption and utilization of vitamin A are impeded (Wald, Jeghers & Arminio, 1938; Patek & Haig, 1939; Hecht & Mandelbaum, 1939).

It is important also that the rhodopsin cycle contains two independent reactions for forming rhodopsin, since this synthesis is the basis of dark-adaptation in the rods. The synthesis from retinene$_1$ is a relatively fast process, that from vitamin A_1 much slower. The corresponding interplay of fast and slow components in human dark-adaptation can be demonstrated clearly in suitably designed experiments (Wald & Clark, 1937—38). A short exposure of the eye to intense light bleaches a large quantity of rhodopsin to retinene$_1$ but allows too little time for the comparatively slow conversion of retinene$_1$ to vitamin A_1. Directly following such short, intense exposures, dark-adaptation is found to be very rapid. On the other hand, long illumination of the eye brings the visual cycle into a steady state; subsequent dark-adaptation depends largely upon the synthesis of rhodopsin from vitamin A_1, and hence is relatively slow.

The measurements of Schouten & Ornstein (1939) and Crawford (1946) reveal a similar complication in the adaptation of human cones, implying that here also two processes exist for synthesizing photopigment. This arrangement may be very general, for it is found again in the porphyropsin system (see below): and Hartline & McDonald (1947) have observed similarly fast and slow components in the dark-adaptation of single visual cells of the horseshoe crab, *Limulus*.

As a consequence therefore of this particular feature in the photoreceptor cycle, two independent parameters are found to govern light- and dark-adaptation. The *threshold* of vision rises and falls with the fall and rise of photopigment concentration. Simultaneously the *rate of change* of threshold alters with the changing proportions of precursors for resynthesis of photopigment.

It was Kühne who, after a long and discouraging search, discovered how to bring rhodopsin into aqueous solution. This requires the aid of substances which we now recognize to be detergents. Kühne used bile salts for this purpose; Tansley (1931) has introduced the more convenient reagent digitonin.

In aqueous solutions of rhodopsin as ordinarily prepared, one finds only the bleaching to retinene$_1$ and protein proceeding to completion. A very small reversion to rhodopsin can also sometimes be detected (Ewald & Kühne, 1878; p. 266; Chase & Smith, 1939—40); but there is virtually no formation of vitamin A_1. In such solutions one can study with precision the spectrum of rhodopsin and the progress of its bleaching.

Spectra of rhodopsin and of the final product of its bleaching in aqueous solution are shown in Fig. 1. The rhodopsin spectrum possesses three absorption bands. Of these the most familiar and important is the broad α-band, maximal at 500 $\pm$ 2 mμ., upon which the photosensitivity of the pigment and of rod vision principally depends.

The α-band falls to very low values in the red, but the absorption is maintained at a high level in the violet and on into the ultraviolet. The reality of this low-wave-length absorption is now conceded by all, though it was held in question for a considerable period. It makes an important contribution to the light-sensitivity of rhodopsin; and under certain circumstances to be described below it is realized physiologically as a high visual sensitivity in the near ultraviolet.

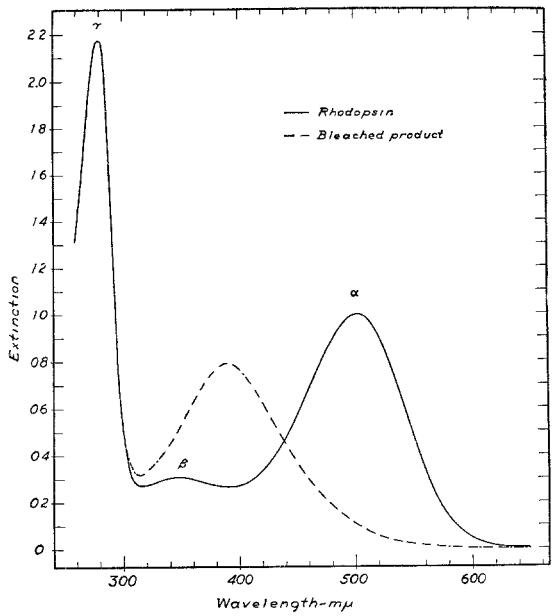


Fig. 1.

Spectra of rhodopsin and of the product of its bleaching in aqueous solution. pH 5.55. The absorption is plotted as extinction, log I_0/I, in which I_0 is the incident and I the transmitted radiation

The impurities found in rhodopsin preparations absorb mainly in this low-wave-length region. It therefore has become useful to gauge the purity of such solutions by the ratio of extinctions at 400 and 500 mμ. (400/500 ratio). In general, the lower this ratio, the purer is the preparation. About a decade ago, rhodopsin solutions were prepared in a number of laboratories with 400/500 ratios of 0.30—0.35. At this time I was able to show that the 400/500 ratio of pure rhodopsin could not be less than about 0.22 (Wald, 1937—38 b). The ratio in the preparation shown in Fig. 1 is 0.26; and this must be close to the value in the pure pigment.

After passing through a minimum at about 390 mμ., the spectrum of rhodopsin rises to a low peak, the β-band, maximal at about 350 mμ. This absorption also is effective in disrupting the molecule, for a corresponding maximum appears in the bleaching spectrum of rhodopsin in solution (cf. Fig. 14 below). [2])

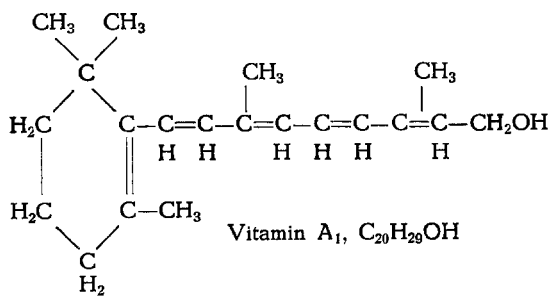
[2]) The β-band of rhodopsin has every appearance of being what Zechmeister has called a '*cis*-peak', an indication that the chromophore of rhodopsin possesses a *cis* configuration such as is rarely encountered among natural carotenoids.

Beyond a further minimum at about 310—320 mμ., the rhodopsin absorption rises to the high, sharp γ-band, maximal at about 278 mμ. This is the protein band, associated generally in proteins with their content of aromatic amino-acids. In pure rhodopsin the γ-band may not be as high as shown in Fig. 1. Light absorbed in this portion of the molecule apparently is not available for bleaching. for throughout the region of the γ-band the photosensitivity of rhodopsin continues to fall (cf. Fig. 14). It is possible therefore that only radiation absorbed directly by the chromophore of rhodopsin is effective in bleaching the molecule.

The conversion of rhodopsin to retinene$_1$ and protein can be examined in detail in aqueous solutions. On exposure to light, the red rhodopsin is transformed to a highly unstable orange-red product, called by Lythgoe 'transient orange' (Wald, 1937; Lythgoe, 1937; Lythgoe & Quilliam, 1938). This is the only photochemical step in the entire rhodopsin cycle. It was isolated by Broda & Goodeve (1941—42) by irradiating rhodopsin in glycerol-water mixtures at —73° C. Its effect on the α-band is to move the maximum about 5 mμ. toward shorter wave-lengths. In light or darkness this initial product breaks down in the course of about an hour at 25° C. (neutral solutions) to a relatively stable yellow product which changes in colour with pH, going from near-colourlessness in alkaline solution to deep yellow in acid (Lythgoe's 'indicator yellow'). Our experiments have shown this to be retinene$_1$ and protein, still loosely coupled to each other in some way that scarcely alters the retinene$_1$ spectrum (Wald, 1937—38 b).

The spectrum of this final product of bleaching in solution is shown in Fig. 1. It possesses two bands, that derived from retinene$_1$, which may be compared with the spectrum of impure retinene$_1$ shown in Fig. 2; and the protein γ-band. The latter, as is to be expected, is not changed by the bleaching process.

The further chemistry of the rhodopsin system can be understood with reference to a component of known structure, vitamin A$_1$:



Vitamin A$_1$, $C_{20}H_{29}OH$

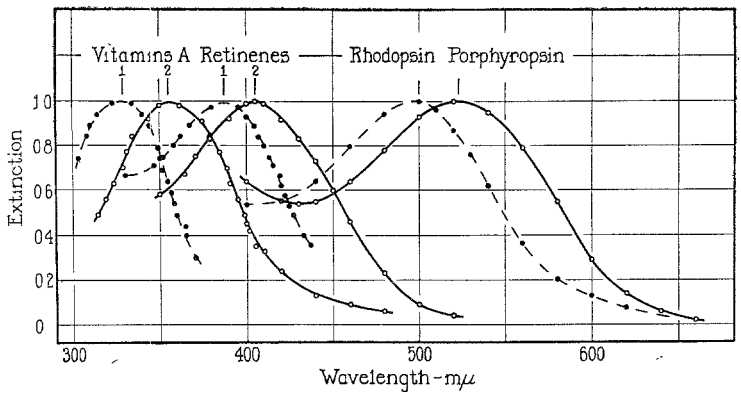


Fig. 2.

Spectra of components of the rhodopsin and porphyropsin systems. Crude preparations from retinae of the freshwater calico bass (solid lines) and the marine scup (broken lines). Rhodopsin and porphyropsin are dissolved in 1 % aqueous digitonin, the retinenes and vitamins A in chloroform. All maxima have been brought to the same height to facilitate comparison
(From Wald, 1938—39)

This is a polyene alcohol, characterized by an absorption band in the ultraviolet at about 325 mμ. in hexane or ethanol, and at about 332 mμ. in chloroform (Fig. 2). When mixed with antimony chloride reagent, vitamin A_1 yields a deep blue product with an absorption maximum at about 620 mμ. (615—620 mμ. in crude extracts). The most distinctive feature of this molecule, common to all the carotenoids, is the regular alternation of single and double bonds forming what is called a conjugated system. Upon this arrangement the spectrum depends.

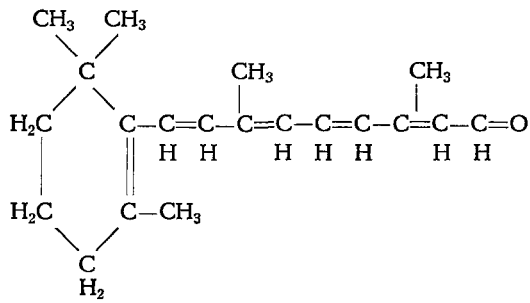
Retinene$_1$ has its maximal absorption in the near ultraviolet at about 365 mμ. in hexane, about 387 mμ. in chloroform (Fig. 2). With antimony chloride it too forms a blue product, with an absorption maximum at 664 mμ. A third property is regularly associated with crude natural preparations of retinene$_1$: they change colour with pH, much as does the whole product of bleached rhodopsin. In default of thorough purification I have expressed some uncertainty that this property belongs to retinene$_1$ itself; but concluded that there existed no better alternative than to ascribe it tentatively to this substance (Wald, 1937—38 b, p. 810).

Recently R. A. Morton and his colleagues in Liverpool have discovered that by simple exposure of vitamin A_1 to solid man-

ganese dioxide it is oxidized to a product virtually identical with natural retinene$_1$ in its absorption maximum and antimony chroride reaction (Ball, Goodwin & Morton, 1946). I have confirmed these observations. In a modified chromatographic procedure, the manganese dioxide is used in the form of a short column. A solution of vitamin A_1 is poured in at the top, and a solution of retinene$_1$ drawn off in the filtrate (Wald, 1947—48).

This synthetic product, however, does not change colour with pH. Indeed, neither does natural retinene$_1$ after adsorption on a solid powder and elution. The crude retinene$_1$ derived from bleaching rhodopsin clearly differs in some way from the synthetic product. It is certain, however, that both types of preparation stand in the closest chemical relationship.

Morton et al. have provided evidence that synthetic retinene$_1$ is the simplest oxidation product of vitamin A_1, its aldehyde:



Vitamin A_1-aldehyde, $C_{20}H_{28}O$

In all probability this is the correct structure of the synthetic substance (see also Wald, 1947—48); and it may be taken also to be at least the prototype of the natural material. Morton's synthesis has therefore reversed one of the reactions in the rhodopsin cycle, the reduction of retinene$_1$ to vitamin A_1, which in the retina proceeds irreversibly.

Recently this retinal process also has been accomplished *in vitro*. The conversion of retinene$_1$ to vitamin A_1 by a cell-free brei from cattle retinae is shown in Fig. 3. Bliss has lately reported that this process can occur also in solution. I had found some time ago that fresh rhodopsin solutions from frog retinae exhibit a special type of bleaching which goes further than in old solutions (Wald, 1937—38 b). Bliss (1948) has now shown that this is associated with the conversion of retinene$_1$ to vitamin A_1.

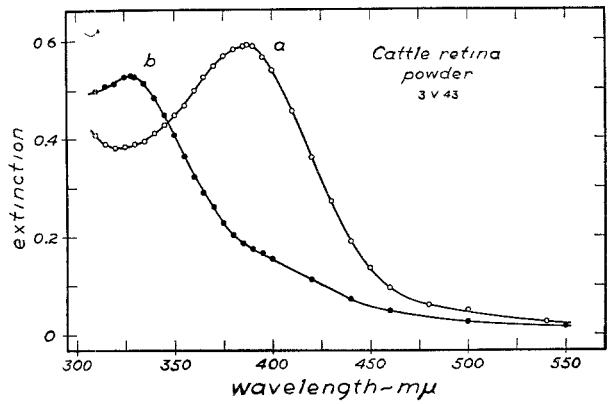


Fig. 3.

The conversion of retinene$_1$ to vitamin A_1 by a cell-free brei from cattle retinae. The retinal tissue had been frozen, dessicated at low temperature, and exhaustively extracted with petroleum ether, all in darkness. The dry powder was stirred with phosphate buffer, pH 6.8. The suspension was divided into halves, and irradiated. One half, extracted immediately, yielded retinene$_1$ (open circles). In the other half, extracted an hour later, the retinene$_1$ had been converted quantitatively to vitamin A_1 (solid circles). Spectra measured in chloroform

We have noted above that the spectra of such polyenes as retinene and vitamin A depend primarily upon conjugated systems of alternating single and double bonds. Within this family of substances it is known that the addition of one conjugated ethylene group (—C=C—) shifts the absorption spectrum about 20—25 mμ. toward the red. Vitamin A_1 possesses 5 conjugated double bonds, and an absorption maximum at about 330 mμ. Retinene$_1$ possesses in addition the conjugated double bond of the carbonyl group (—C=O). This shifts the spectrum much further toward the red than does an added ethylene, bringing the maximum to about 380 mμ. (in neutral aqueous digitonin).

To go from here to the α-band of rhodopsin requires a further shift of spectrum of about 120 mμ. In terms of simple increase in length of the conjugated system this would correspond to the addition of another 5—6 double bonds; that is, to an approximate doubling of the retinene$_1$ structure.

To be sure this is a very crude statement of the situation. To refine it adequately would require more space than should be taken here, and more information than we now possess. Yet even

this superficial consideration makes it seem highly probable that to make the rhodopsin chromophore requires two such molecules as retinene$_1$ or vitamin A_1.

We are left with the following concept of the workings of the rhodopsin system. On absorption of light within the chromophore of the rhodopsin molecule its binding to protein is loosened, and simultaneously it is made highly unstable. It breaks down to molecules of retinene$_1$, probably to two such molecules of about half the original size. These are then reduced to vitamin A_1. The vitamin in turn is coupled with the appropriate protein to regenerate rhodopsin.

Porphyropsin

In the course of his pioneer researches in visual chemistry, Kühne took every opportunity to examine the retinae of new animals in the dark-adapted state. In this way he recorded the appearances of an extraordinary variety of retinae, from the lamprey to man. Those of most animals he found to be red, owing to their content of rhodopsin. The retinae of fishes however, — and indeed that of the lamprey — he described as purple in color.

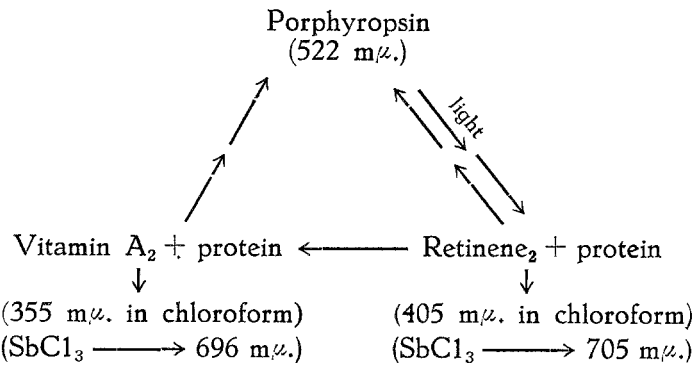
Some time later, Köttgen & Abelsdorff (1896) studied spectrophotometrically solutions of photosensitive pigment extracted from a variety of vertebrates. Those from frogs, owls and mammals all displayed the characteristics of rhodopsin. Those from eight species of fish, however, were purple in colour and had quite different spectral characteristics.

This purple photosensitieve pigment, now called porphyropsin, appears to be confined to rods. Unfortunately Köttgen & Abelsdorff measured, not its absorption spectrum, but only the differences in absorption between unbleached and bleached solutions ('difference spectra'). These differences go through a maximum at about 535—540 mμ., which is still confused occasionally with the true absorption maximum of porphyropsin. The latter lies at 522 $\pm$ 2 mμ. (Fig. 2).

The distribution of porphyropsin among vertebrates is peculiar and significant. This has been reviewed elsewhere (cf. Wald, 1934; 1945—46). It will be enough here to note that this photopigment is rigorously associated with fresh-water existence, or more specifically with freshwater origins, throughout the vertebrate stock. Marine fishes, like true land vertebrates, possess the rhodopsin

system (Fig. 2). Freshwater fishes have porphyropsin. Fishes which spawn in fresh water but are free to migrate to sea thereafter — as, for example, the salmons — have either porphyropsin alone, or mixture of porphyropsin and rhodopsin in which the former predominates. The sea lamprey, a cyclostome, representative of the most primitive of living vertebrates, spawns in fresh water and has a great predominance of porphyropsin. A number of amphibia, all freshwater spawners, have porphyropsin. The adult frog has rhodopsin; but it has recently been shown that the tadpole of the bullfrog has porphyropsin in its retina, and that precisely at metamorphosis the visual system metamorphoses from porphyropsin to rhodopsin. To summarize our present information, true land and sea vertebrates have the rhodopsin system; true freshwater vertebrates have porphyropsin; while those equivocal forms which can distribute their lives between freshwater and one of the other environments frequently possess both photopigments, mixed or in temporal succession (cf. Fig. 7).

It has emerged that the porphyropsin system constitutes a cycle of precisely the same form as the rhodopsin system, in which, however, vitamin A_1 and retinene$_1$ are replaced by new carotenoids Shortly after the properties of these new substances were first announced (Wald, 1937 b) it was suggested that the vitamin A_1-analogue be called vitamin A_2 (Edisbury, Morton & Simpkins, 1937). The retinene-analogue in the porphyropsin cycle may correspondingly be called retinene$_2$. The porphyropsin system then has the form (Wald, 1938—39):



Porphyropsin is therefore a conjugated protein which contains a new carotenoid chromophore, displaced in spectrum about 22 mμ. toward the red from that of rhodopsin. A comparable displacement

of spectrum divides all components of the porphyropsin system from their analogues in the rhodopsin cycle (Fig. 2). As already noted above, such a displacement would correspond to the addition to the polyene chain of one conjugated double bond. In one way or another this change seems to have occurred in forming the porphyropsin system, and it appears to constitute the only difference between it and the rhodopsin cycle.

Just how this extra double bond is inserted in the porphyropsin system is still very incertain. The difficulty centres in the structure of vitamin A_2. The chemical properties and the biological activity of this substance as a growth factor in rats have both been subjects of wide disagreement in the literature. At least four formulations of vitamin A_2 have been proposed, and none yet adequately founded. One prime difficulty is that this molecule has not yet been prepared in unequivocally pure condition. (cf. Karrer, Geiger & Bretscher, 1941; Karrer & Bretscher, 1943; Jensen, Shantz, Embree, Cawley & Harris, 1943; Morton, Salah & Stubbs, 1947).

On the other hand, it is now generally agreed that vitamin A_2 possesses 20 carbon atoms as does A_1; that both molecules are very closely related in structure, as shown by the failure to separate them by even the very searching procedures of chromatographic adsorption; and that some small modification of the vitamin A_1 structure, including almost surely the addition of a double bond, accounts for the displacement of spectrum in vitamin A_2 toward the red.

This small change in structure is accompanied by extraordinarily little change in chemical behavior. The rhodopsin and porphyropsin systems not only are identical in form, but all components of both cycles exhibit parallel behaviour in all reactions so far investigated. Recently Morton et al. (1946) have reported that the same treatment which converts vitamin A_1 synthetically to retinene$_1$ also transforms vitamin A_2 to retinene$_2$. We can also apply the argument advanced above in connection with rhodopsin to conclude that to form the porphyropsin chromophore probably requires two molecules of vitamin A_2 or retinene$_2$.

In the rhodopsin and porphyropsin systems, therefore, some small change in polyene structure displaces the spectrum without notably altering the chemical behavior. Both systems are found in rods, and probably neither has anything to do with colour vision. But the kind of molecular adjustment which these systems exemplify

is exactly that needed to meet the chemical requirements of colour vision. This matter, however, can be treated in better perspective below.

Iodopsin

Cones, no less than rods, must possess light-sensitive pigments. Yet Kühne, in spite of many careful trials with a variety of cone retinae, was unable to see any bleaching of pigment in the dark adapted tissues on exposure to light, and was willing to believe that the cones lack photosensitive pigments. This is clearly an

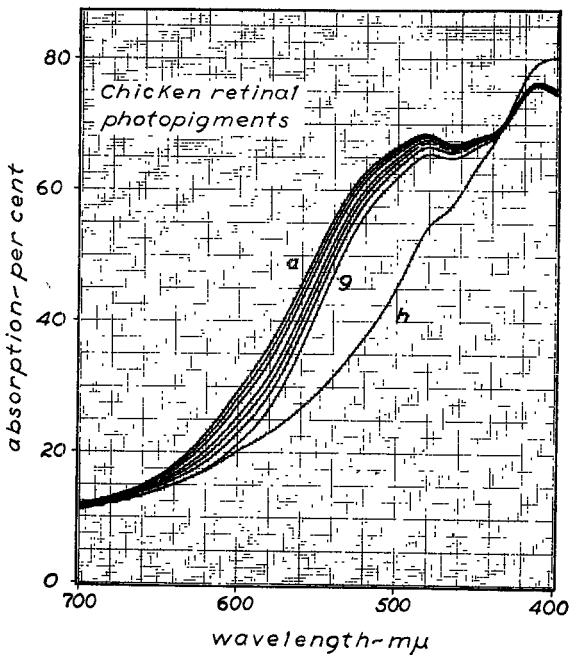


Fig. 4.

Bleaching of an extract of dark-adapted chicken retinae in red and white light. The retinae had been frozen, dried at low temperature, and exhaustively extracted with petroleum ether in darkness. They were then leached three times with neutral phosphate buffer. Finally they were extracted with aqueous digitonin solution. The spectrum of this extract is shown in (a). On repeated irradiation with deep red light the spectrum fell to (g). At this point red light had no further effect; but on exposure to white light the spectrum changed to (h). The bleaching from (a) to (g) is due to iodopsin, that from (g) to (h) to rhodopsin. The spectra are automatically recorded with the Hardy spectrophotometer

unacceptable inference. We are forced instead to conclude that cone photopigments, since they are invisible, must occur in very low concentrations.

For this reason in our first attempt to extract photopigments from cones we used the chicken retina, which contains a few rods among a large predominance of cones (Wald, 1937 c). Whole extracts of these retinae have complex spectra, due to contamination with light-stable carotenoid pigments and other coloured substances. They are, however, light-sensitive, and display a type of bleaching in white light which in characteristic neither of rhodopsin nor of

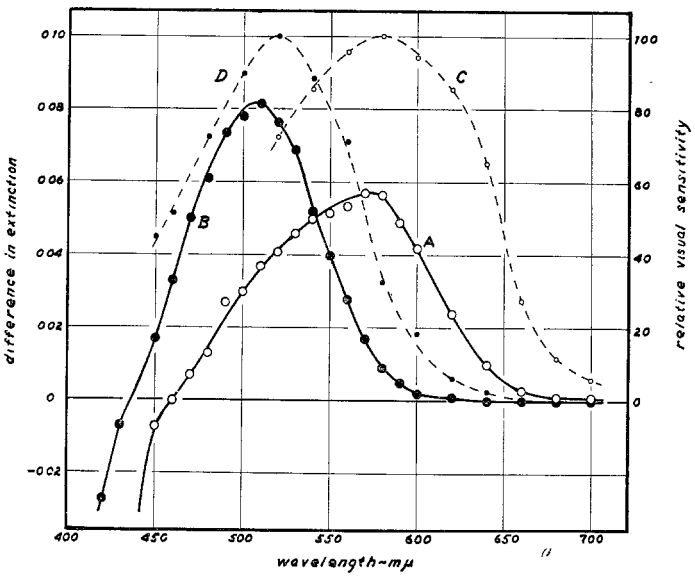


Fig. 5.

The 'difference spectra' of rhodopsin and iodopsin, and the Purkinje shift in the chicken. The solid lines show the changes in extinction of an extract of chicken retinae due to (A) irradiation with red light (iodopsin), and (B) subsequent irradiation with white light (rhodopsin; compare Fig. 4). The broken lines show Honigmann's measuremen's of the spectral sensitivity of the light-adapted (C) and the dark-adapted (D) chicken. (From Wald, 1937 c).

what is to be expected of a cone photopigment, but of something about halfway between. It seemed possible therefore that such extracts contain mixtures of rhodopsin and the cone pigment. This was demonstrated to be the case by use of a procedure based upon the Purkinje phenomenon.

In extreme red light, to which rods and rhodopsin are relatively insensitive, cones, and hence necessarily cone photopigments, still respond strongly. Chicken retinal extracts were therefore irradiated with deep red light of wave-lengths longer than about 650 mμ. A distinctive type of bleaching occurred, which could be pursued to completion in red light (Fig. 4, a-g). On subtracting the spectrum of the product of this bleaching (g) from that of the original extract (a), one obtains the difference-spectrum of the red-sensitive pigment. This is shown in Fig. 5, A. It is maximal at 565—575 mμ., depending upon the pH.

When red light produces no further effect upon such extracts, the residue can be exposed to white light. It undergoes a renewed bleaching (Fig. 4, g-h), associated with a difference-spectrum maximal at about 510 mμ. (Fig. 5, B). This is characteristic of rhodopsin.

It is reasonably certain that the substance which bleaches in red light is the photopigment of the cones. From the position of its absorption spectrum one can conclude that it is violet in colour. I have therefore called it iodopsin (i.e., visual violet).

These observations on chicken retinal extracts have since been confirmed in detail by Bliss (1945—46 a). It is curious that such experiments have not yet succeeded in other types of retina. I have attempted several times to extract photopigments from the all-cone retinae of turtles, so far without success. The principal difficulty in such experiments is the very low concentration in which iodopsin normally occurs.

Figs. 4 and 5 show that even in spite of the great predominance of cones in chicken retinas they contain larger amounts of rhodopsin than of iodopsin. It is probably true that the concentration of iodopsin in a single cone is hundreds of times less than that of rhodopsin in a rod. This fact in itself probably accounts for the very much lower sensitivity of cones than rods, and hence the peculiar status of cones as the organs of vision in bright light.

Honigmann (1921) has measured the spectral sensitivity of chickens in bright and dim light. He found that light-adaptation shifts the maximum sensitivity from about 520 mμ. to about 580 mμ. (Fig. 5; C, D). This displacement is closely matched by the difference in spectrum between rhodopsin and iodopsin. The Purkinje shift in the chicken is accounted for completely by a transfer from

dependance upon the absorption spectrum of rhodopsin in dim light to that of iodopsin in bright light.

We still have little information regarding the further chemistry of iodopsin. Similarities in its behavior with that of rhodopsin suggest strongly that it too is a conjugated carotenoid-protein. From the displaced position of its spectrum one might suppose that the same type of change that has produced porphyropsin from rhodopsin has been extended further to produce iodopsin. One might therefore look here for a third vitamin A and a third retinene.

It is significant however that in human vitamin A deficiency cone vision deteriorates simultaneously with rod vision, and both recover together on administration of vitamin A. Bliss (1945—46 b) has also reported that irradiation of chicken retinae with red light liberates retinene$_1$. I too have found this result in a number of trials; but I am not yet convinced that the retinene$_1$ originates in iodopsin, and that the latter is therefore derived from the same polyenes as rhodopsin. This is however possible, for the shift of spectrum between rhodopsin and iodopsin could be obtained with the same or very similar chromophores, differently attached to protein.

The Problem of Colour Vision

In addition to special central nervous arrangements whose nature is still obscure, colour vision makes specific chemical demands upon the photoreceptor processes in the cones themselves. These are of two kinds:

1. *Differential sensitivity.* The cones must vary in spectral sensitivity, to provide a peripheral sorting-out mechanism for colour differentiation. Chemically this means that the cones must contain either different photosensitive pigments, or auxiliary pigments for modifying the spectral sensitivity of a single photopigment.

2. *Parallel kinetics.* However differential sensitivity is achieved in the cones, it must not disturb an essential parallelism in the changes which all the cones undergo in light and darkness. Otherwise hue would vary widely with the conditions of stimulation and the state of adaptation, and it would be impossible to associate a specific hue with any perceived object. Colour vision systems can and do depart from this condition in detail, but they must adhere to it in gross, and the more closely they do so the more efficiently can they differentiate colours. This requirement implies that the

rates of bleaching and resynthesis of photopigment must be closely similar in all the cones.

The chemistry of visual systems has revealed two ways in which these conditions can be fulfilled.

The retinae of certain birds and turtles contain a system of coloured oil globules, situated at the junction of the inner and outer segments of the cones, so that light must penetrate them just before entering the photosensitive outer limbs. They are therefore in the position of colour filters. One can distinguish four distinct groups of globules, red, orange-yellow, greenish yellow and colourless. It was suggested long ago that they form an arrangement for colour differentiation. This is a highly reasonable assumption, though it still lacks direct demonstration. Such a system should require only one photopigment, just as three-filter systems of colour photography operate with the single photosensitive substance silver bromide.

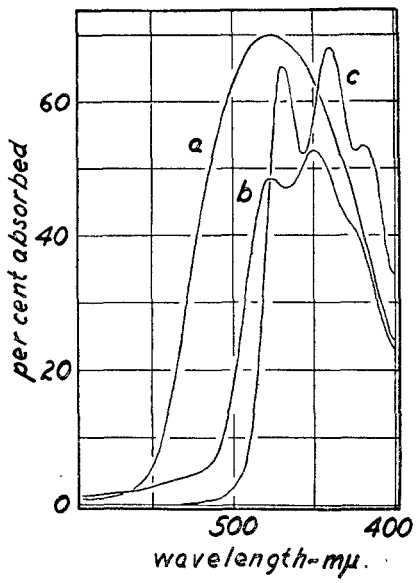


Fig. 6.
Filter pigments of the chicken retina. Spectra of crystalline carotenoids dissolved in castor oil to simulate the condition in the retinal oil globules. (a) astacene; (b) xanthophylls; and (c) an unidentified carotene. (From Wald & Zussman, 1938)

The filter pigments of the chicken retina have been isolated in crystalline condition (Wald & Zussman, 1938). They are all carotenoids: the red astaxanthin (isolated originally as its autoxidation product astacene); a golden mixture of xanthophylls like that found in chicken egg yolk; and a greenish yellow carotene. Their spectra are shown in Fig. 6. Together with iodopsin they appear to constitute a complete peripheral apparatus for colour vision. In this system the colour filters provide differential sensitivity, while complete kinetic parallelism in all the cones is ensured by the presence of a single photopigment.

One cannot appeal to such devices as this in human colour vision where no such filter pigments appear, and we are forced instead to the assumption of a variety of cone photopigments. The chemical problem in this case is to vary the spectrum of the individual photopigments without changing very much their chemical behaviour. We have as yet only a model of how this can be done, derived from the rods, and applicable only by analogy to cones. In the rhodopsin and porphyropsin systems just such a change has been effected, apparently by some small alteration in the number and arrangement of double bonds in the photopigment chromophore. By analogous changes a large number of photopigments might be constructed, differing in spectral sensitivity, yet closely similar in chemical behaviour.

The model provided by rhodopsin and porphyropsin has special force in those organisms which possess mixtures of both pigments, among them certain euryhaline fishes which are restricted neither to fresh water nor the sea, but can migrate freely as adults between both environments. Photopigment extracts from the retinae of such animals present a graded series of spectra, correlated closely with environmental habit (Fig. 7). It seems clear that even two photopigments, together with suitable mixtures, might meet the peripheral needs of relatively elaborate systems of colour vision.

There may exist a third way of fulfilling these requirements which we are just beginning to explore. It seems possible to obtain a wide array of protein pigments with a single carotenoid chromophore by varying the protein or the nature of the carotenoid-protein linkage. Thus we now know a series of astaxanthin-proteins, ranging in colour from orange-red as in lobster blood (max. 480 mμ.; Wald, unpublished observations); through green as in the ovoverdin of lobster eggs (max. 470, 645 mμ.; Stern & Salomon, 1938); to

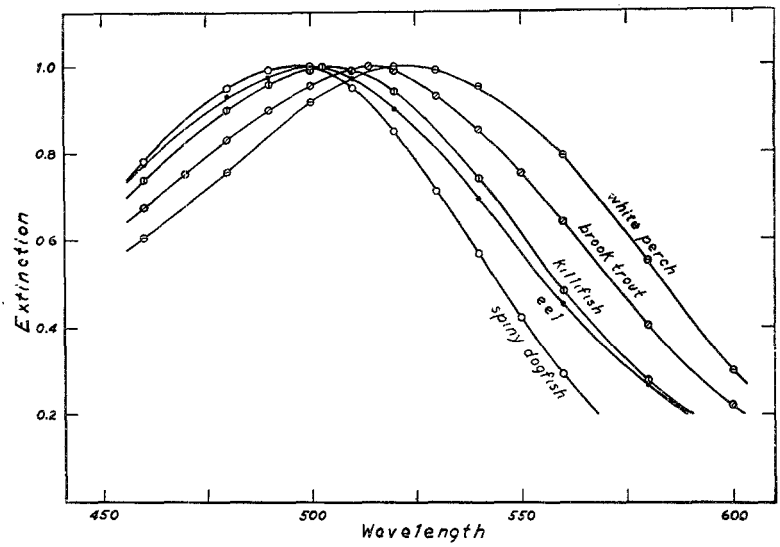


Fig. 7.

Spectra of photopigments from the rods of various fishes, illustrating the transition from an exclusively rhodopsin to an exclusively porphyropsin system. The permanently marine dogfish possesses rhodopsin alone; the eel, killifish and trout — which can exist in either fresh water or the sea — possess mixtures of rhodopsin and porphyropsin; and the white perch possesses porphyropsin alone. (From Wald, 1943; 1945—46)

the blue crustacyanin of lobster shells (max. 625 mμ.; Wald, Nathanson, Jencks & Tarr, 1948). There is as yet no evidence that this device for creating new pigments is used in retinae, or that it could fulfil for colour vision the requirement of parallel kinetics. Yet this is an interesting possibility, and it merits careful examination.

The Spectral Sensitivity of Human Vision

The spectral sensitivity of the eye, and such differences in the spectral sensitivities of individual cones as underly colour vision, have their origins in the absorption spectra of retinal photipigments. In human vision, however, the intrinsic sensitivities which photopigments confer on the rods and cones are realized only partly and in distorted form, for coloured structures intervene between the light and the receptors.

Principal among these is the lens, which is yellow in colour and grows more deeply pigmented with age. The cornea and

ocular humours also have small absorptions in the violet, increasing into the ultraviolet. The spectral transmissions of these structures affect the sensitivity of the whole eye.

In man and certain other primates, alone among mammals, the retina itself is pigmented in a zone subtending a visual angle of some 5°—10°, centred about the fovea — the yellow patch or *macula lutea*. This pigmentation imposes special differences in spectral sensitivity upon the central as compared with the peripheral retina.

The measurements to be described were performed in our laboratory with a specially designed spectral adaptometer (Wald, 1945a). This uses as source a high pressure mercury arc, from the radiation of which 10 spectral regions are isolated with filters. In all cases the absolute threshold of vision was measured in a test field subtending a visual angle of 1° and exposed for $^1/_{25}$ second. The data are presented in terms of sensitivity — the reciprocal of the relative energy at the threshold — and are plotted logarithmically to do justice to the wide range of sensitivities encountered. A more complete account of these measurements has been presented earlier (Wald, 1945 b).

Foveal cones. The central fovea of the human retina, which subtends a visual angle of about 1.5°, contains only cones. Within this area, therefore, no Purkinje phenomenon is observed. Even in the wholly dark-adapted eye, in which all extrafoveal responses are dominated by rods, the fovea retains the characteristics of purely cone vision.

The image of a 1° test field, fixated centrally, falls entirely within the central fovea, and stimulates cones alone. As one indication of this the field appears coloured at the threshold at all wavelengths. Measurements made with such a stimulus in the completely dark-adapted eye are shown in Fig. 8 (open circles). They represent averages for 22 observers of average age 20.

The maximum sensitivity of the fovea, here arbitrarily set at 1 (log sensitivity = 0) occurs at about 562 mμ. in these measurements. To both sides of this wave-length the sensitivity declines. reaching about 1/10,000 of its maximal value at 750 mμ. and about 1/40,000 at 365 mμ.

A deep inflection appears in the foveal function at 400—500 mμ. The reason for this will become apparent below. In this regard the present measurements agree very well with others which have

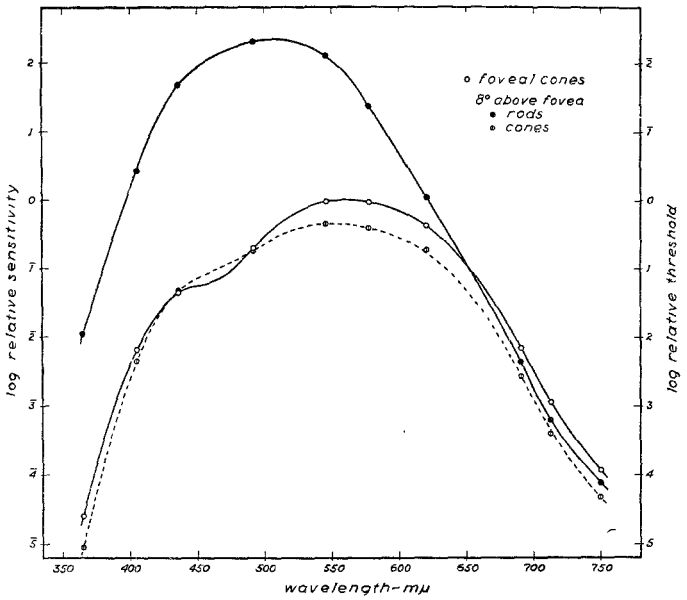


Fig. 8.

Spectral sensitivities of foveal cones (open circles); and of rods (solid circles) and cones (broken line) in an extra-macular area of retina. All sensitivities are expressed relative to the maximum sensitivity of the fovea. The relative positions of these functions on the ordinates are therefore those observed in the eye. (From Wald, 1945 b)

penetrated into the blue and violet (Coblentz & Emerson, 1918—19; Gibson & Tyndall, 1923; Stiles, 1944). They depart widely however from the photopic luminosity function standardized by the International Commission on Illumination at Geneva in 1924 (the I.C.I. function). At 436 mμ. they reveal a sensitivity about 2.4 times, and at 405 mμ. about 9 times as high as the I.C.I. factors indicate.

The I.C.I. function at low wave-lengths has a peculiar history, in which the rigours of standardization came eventually to outweigh the visual measurements. In the blue and violet the standard function steers a course approximately midway between the data of Nutting (1915) and of Hartman (1918), which themselves disagree widely with each other. It is a curious fact that no set of measurements yet made in the human eye conforms at low wave-lengths with the International Standard.

Peripheral rods. When the image of the test field is made to fall outside the macula in the dark-adapted eye, the reaction at

the threshold is due to rods. The field is seen as colourless at all wave-lengths by about 9/10 of the observers. The remainder see the neighbourhood of 700 mμ. as reddish, some cone activity apparently entering their threshold responses in this spectral region.

Measurements of the spectral sensitivity of rod vision in a peripheral retinal area are shown in Fig. 8 (solid circles). They are averages from the same 22 observers who yielded the foveal data. The form of this function agrees very well with the measurements of Abney & Watson (1915), who used a similar procedure. It is narrower than the scotopic sensitivity curves of Hecht & Williams (1922—23) and of Weaver (1937), obtained by brightness matching in large central areas at illuminations well above the threshold. The relatively higher sensitivities in the red, found in the latter researches, were almost certainly caused by a widespread participation of cones in the measurements.

The rod sensitivity is maximal at about 505 mμ., and falls at 365 mμ. to about 1/20,000, at 750 mμ. to about 1/2,500,000 of its maximal value. At wave-lengths below 550 mμ. the dark-adapted peripheral retina is 100 to 1000 times as sensitive as the fovea. At higher wave-lengths the peripheral and foveal functions draw together, and under the conditions of the present measurements the curves cross at about 650 mμ.; in the further red the fovea is the more sensitive. These relations change somewhat in test fields of other sizes and for other durations of exposure, since the thresholds of rod and of cone vision vary differently with changes in the field area and the exposure (cf. Griffin, Hubbard & Wald, 1947).

Peripheral cones. In order to measure the thresholds of cone vision in a peripheral area of retina it is necessary to circumvent the activity of the rods. This is done in the following way.

It is well known that after high light-adaptation, the human eye adapts to darkness in two stages. The threshold first falls rapidly to a plateau, held constant from about the fourth to the eighth minute or longer, depending upon the wave-length of the test stimulus. During this period the test field continues to look coloured at all wave-lengths. This is cone-adaptation; the plateau represents the threshold of the completely dark-adapted cones. Later the dark-adaptation of the rods supervenes; the threshold begins to fall again and reaches a lower and final level, that of the wholly dark-adapted rods. By repeatedly light-adapting the eye and measuring the cone plateaux in subsequent dark-adaptations at one wave-

length after another, one can determine the spectral sensitivity of dark-adapted cones without interference from rods in any area of retina desired.

Such measurements, performed with 10 of the 22 subjects who yielded the foveal and peripheral rod data, are shown as a broken line in Fig. 8. The same retinal area was employed for them as in the rod measurements.

The maximum sensitivity of peripheral cone vision is found at about 550 mμ., about 12 mμ. below that of foveal cones. Also over most of the spectrum cone vision in the periphery is less sensitive than in a corresponding area of the fovea. The reason for this is probably the lower density of cones in the peripheral retina. It is well recognized that the visual sensitivity falls with decrease in the number of receptors in the stimulus field (Wald, 1937—38 a).

The macular pigment. The curves which describe the spectral sensitivities of foveal and peripheral cones have the same shape

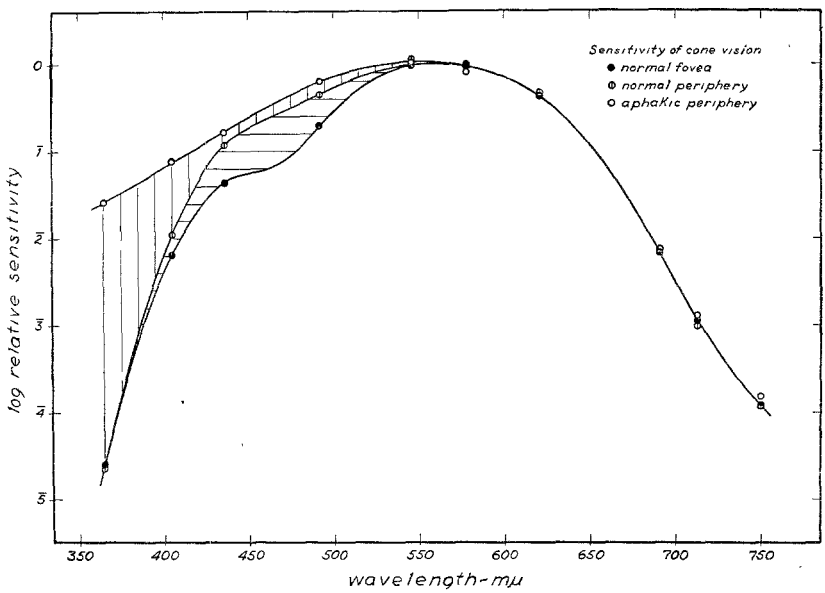


Fig. 9.

Spectral sensitivities of cone vision in the normal fovea, and in an area 8° above the fovea in the normal and aphakic eye. All the functions have been brought together above 578 mμ., where all of them are parallel. The horizontally hatched area represents the optical density (log 1/transmission) of the macular pigmentation in the central fovea, the vertically hatched area the optical density of the lens. (From Wald, 1945 b)

at wave-lengths above 578 mμ. If they are brought together in this region of the spectrum, as in Fig. 9, the foveal cones are seen to have a relatively lower sensitivity in the blue than those of the peripheral retina. This is the effect on foveal vision of the macular pigmentation.

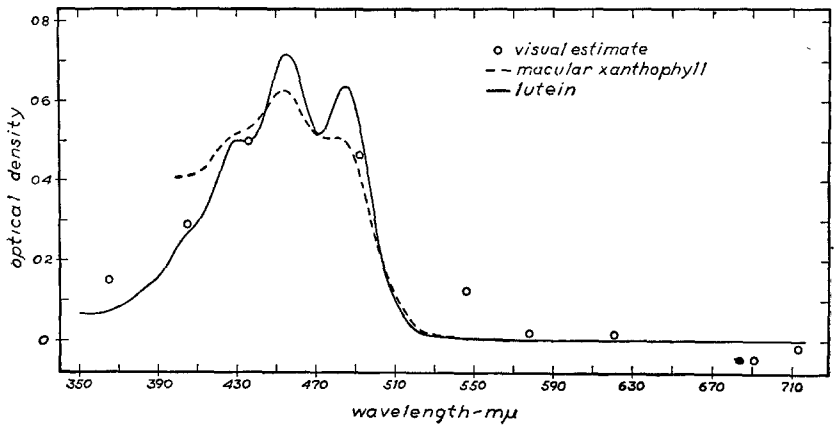


Fig. 10.

Absorption spectrum of the human macular pigment. The open circles represent visual estimates of this, differences in log sensitivity of peripheral and foveal cones, comparable with the horizontally hatched area of Fig. 9. The broken line is the absorption spectrum of a preparation of xanthophyll extracted from human maculae, dissolved in chloroform. The solid line is the spectrum of crystalline lutein or leaf xanthophyll in chloroform. (Compare Wald, 1945 b)

The macular pigment lowers the sensitivity of the central retina by a factor equivalent to the fraction of incident light which it transmits. This is equal to the foveal divided by the peripheral cone sensitivity. Consequently the difference between the logarithms of the peripheral and foveal sensitivities — the horizontally hatched area of Fig. 9 — represents directly log (1/transmission) or the optical density of the macular pigment in the living retina.

Such differences are plotted as the open circles of Fig 10. They are averages taken from foveal and peripheral cone measurements on the same 10 observers. The macular extinction rises steeply from about 550 mμ. to a broad maximum in the region 430—490 mμ., then falls again in the violet and near ultraviolet.

The form of this absorption suggested that the macular pigment may be a carotenoid, for this is the typical character of a carotenoid

spectrum. To explore this possibility I accumulated 9 human maculae. They yielded a yellow fat-soluble pigment which displayed the properties of a hydroxy-carotenoid or xanthophyll, and which, in all probability, was lutein or leaf xanthophyll ($C_{40}H_{54}(OH)_2$) the commonest member of the class.

The absorption spectrum of a partly purified preparation of human macular xanthophyll is shown in Fig. 10 (broken line). It has been brought to the height indicated by the visually estimated absorption. The spectrum of pure crystalline leaf xanthophyll dissolved in chloroform is also shown in the figure. The differences between it and the macular pigment are no greater than is to be expected in the comparison of a crude tissue extract with the pure substance.

In our 10 subjects the macular pigment absorbed on the average about 70 % of light of wave-lengths 430—490 mμ. incident on the fovea. This pigmentation is responsible for the deep inflection in the foveal sensitivity function between 400 and 500 mμ. The depth of pigmentation, however, varies greatly from one observer to another. In two of our subjects — who, as it happens, were siblings — no macular pigmentation at all was perceptible; in two others more than 90 % of the incident light was absorbed at 436 mμ.

Some question has persisted, associated principally with Gullstrand and his followers (cf. Polyak, 1941, p. 223) whether the macular pigmentation exists in the living eye or is merely a *post mortem* phenomenon. These doubts can be considered resolved with the measurement of the pigment in the living eye and the demonstration that it is xanthophyll. The sudden infiltration of xanthophyll into a restricted area of the human retina at death is an altogether unlikely phenomenon.

With xanthophyll as the macular pigment one might expect some evidence of the xanthophyll bands to appear in foveal vision. They do so very clearly.

The most careful measurements of photopic spectral sensitivity that extend into the blue and violet reveal inflections in this region which greatly disturbed the workers who observed them. They were especially evident to those workers who used the step-by-step method, setting a wave-length in one half of a comparison field, a neighbouring wave-length in the other half, and determining the ratio of energies at which both sides looked equally bright. Gibson & Tyndall, Federova, Plakhov & Seletzkaya (1940) used this method,

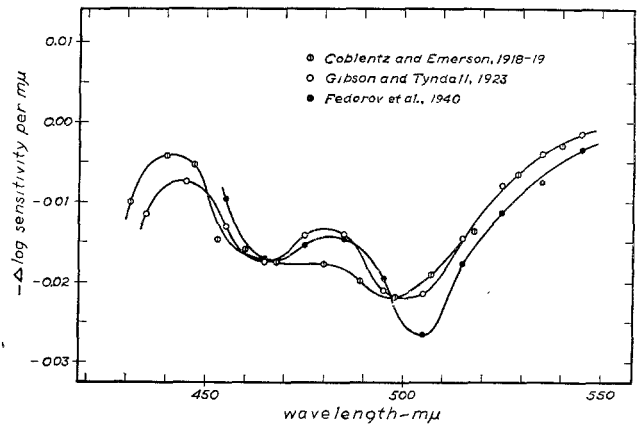


Fig. 11.

First differential (Δ log sensitivity Δ wave-length) of the spectral sensitivity data of various observers for the photopic eye. The measurements of Coblentz & Emerson (1918—19) were obtained by flicker photometry, the others by step-by-step brightness matching. All these functions exhibit maxima and minima in the blue and violet, introduced by the absorption bands of macular xanthophyll

and found the energy ratios to rise and fall in an unaccountable way throughout the blue and violet. Actually such ratios have virtually the force of the first differential of the log sensitivity: wave-length function (Δ log sensitivity/Δwave-length) [3]). If this differential is plotted as in Fig. 11 — whether the data are obtained by the step-by-step method or by heterochromic flicker photometry — it passes through a series of maxima and minima, which follow the bands of the xanthophyll spectrum.

This type of plot greatly magnifies the xanthophyll inflections in the photopic sensitivity function. On careful examination, however,

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[3]) The argument runs as follows. The ratios plotted in the step-by-step method may be expressed as $\frac{V_\lambda + \Delta V_\lambda}{V_\lambda}$, in which V_λ is the sensitivity at wave-length λ, $V_\lambda + \Delta V_\lambda$ the sensitivity at the neighbouring wave-lenght $\lambda + \Delta\lambda$. For small values of ΔV_λ, the ratio $\frac{V_\lambda + \Delta V_\lambda}{V_\lambda}$, or $1 + \frac{\Delta V_\lambda}{V_\lambda}$, becomes equivalent to $1 + \Delta \log V_\lambda$.

the bands themselves are evident in the direct plot of the log sensitivity data. They appear as a pair of small depressions at about 455 and 495 mμ., superimposed upon the main course of the function. (Fig. 15).

What is perhaps more striking is that these bands can be seen in one's own eye by looking into a spectroscope of small dispersion (Keilin & Smith, 1939). Diffuse bands are visible at about 455 and 495 mμ., which these workers suggested might be caused by flavin or β-carotene in the macula. They are in fact the main bands of the xanthophyll spectrum. Their presence in the eye makes it perilous to attempt to identify xanthophyll or related carotenoids in tissue extracts by looking for their absorption bands with a pocket spectroscope. With care one can always find them.

Aphakic vision: the lens. The principal colour filter in the human eye is the lens, and this of course affects the spectral sensitivity of all vision, rod and cone, central and peripheral. To investigate this influence one must turn to a special class of subjects whose lenses have been removed in the operation for cataract. With the loss of the yellow lens the eye gains enormously in sensitivity in the far violet and near ultraviolet.

Such aphakic persons have in fact a very high order of ultraviolet vision. This was discovered accidentally by the young English spectroscopist Gaydon (1938) who had lost one eye and the lens of the other in an explosion. On returning to the laboratory Gaydon found he could see portions of the ultraviolet spectrum down to 309 mμ., for which previously he had needed a fluorescent screen. The sensitivity of Gaydon's rod vision was measured at 365 and 546 mμ. and found to be nearly equal at those wave-lengths (Goodeve, Lythgoe & Schneider, 1941—42).

We have found that aphakic subjects are on the average about 1000 times as sensitive as normal persons at 365 mμ. In radiation confined to this wave-length an aphakic person can read a Snellen chart from top to bottom under conditions in which a normal person is in complete darkness. On one occasion when we were trying to make photomicrographs at 365 mμ. and had great difficulty in focussing the camera, a young aphakic readily focussed it for us. using an ordinary ground glass screen. When in this arrangement, we switched him back and forth between the mercury 365 mμ. radiation and the green line, he declared that the ultraviolet looked much the brighter.

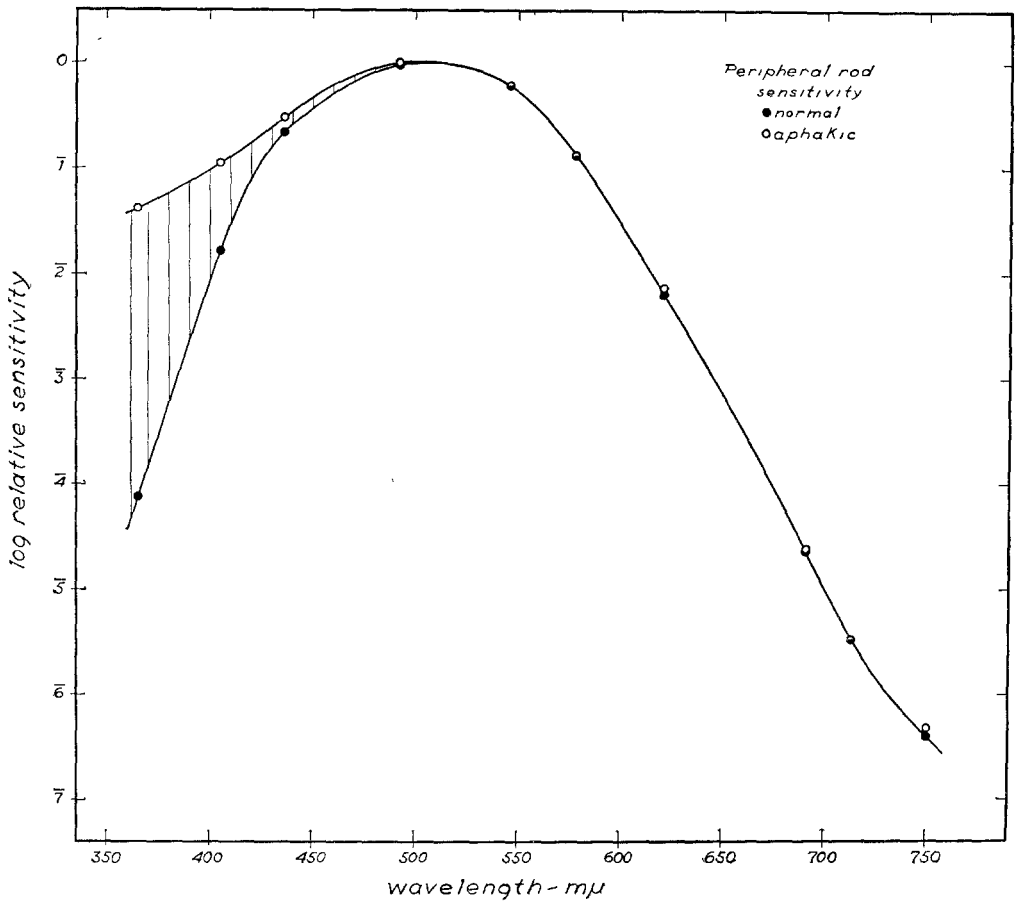

Fig. 12.

Spectral sensitivities of rod vision in an area 8° above the fovea in the normal and aphakic eye. Both functions have been brought together above 546 mμ., where they are parallel. The vertically hatched area represents the optical density of the lens.
(From Wald, 1945 b)

Measurements on aphakic cone vision (6 subjects) are shown in Fig. 9, and on aphakic rod vision (24 subjects, 39 eyes) in Fig. 12. In both cases the image of the test field was made to fall 8° above the fovea, hence well outside the yellow patch. In both cases also the aphakic data have been brought together with the corresponding normal functions above 550 mμ., where all these curves have the same form.

In the aphakic eye the sensitivity falls off much less steeply at

low wave-lengths than in the normal. At 365 mμ. the sensitivity is still about 1/30 as great as at the maximum, rod or cone. At this wave length, aphakic rod vision is as sensitive as in the yellow, cone vision as sensitive as in the near red.

The differences in log sensitivity of normal and aphakic eyes, shown as vertically hatched areas in Fig. 9 and 12, represent directly the optical densities of the human lens *in situ*. These values are for subjects averaging 20 years of age. In older persons the lens becomes more deeply pigmented.

The density of lens absorption rises abruptly from about 430 mμ. to very high values in the near ultraviolet. At 405 mμ. the average lens transmits about 15 %, at 356 mμ. about 0.1 % of the incident radiation. The lens is in fact an excellent cut-off filter, comparing favorably with the very best commercial products. It sets a sharp boundary at about 400 mμ. which excludes the near ultraviolet from normal vision.

The pigmentation of the lens varies greatly in different persons even within a narrow age-group. In young, normal subjects this factor alone adds more than a logarithmic unit to the range of variation of sensitivity at 365 mμ.

We have measured directly the absorption spectra of a number of isolated lenses from persons 63—68 years of age. Such a spectrum is shown in Fig. 13, together with the visually estimated absorption of 21 year old lenses obtained from Fig. 12. As is expected, the older lens displays much higher absorption, and a much greater intrusion of the absorption into the visible spectrum, accounting for its pronounced yellowness. We did not have access to a young isolated human lens. We were able, however, to obtain the lenses of a young rhesus monkey, and their absorption spectrum proved to be remarkably close to that of the lenses of our young human subjects, visually estimated (Fig. 13).

Rhodopsin and Spectral Sensitivity. Measurements made in peripheral areas of the aphakic eye come as close to the intrinsic spectral sensitivities of the rods and cones as it is possible to penetrate *in vivo*. The lens is absent, the macular pigmentation has been avoided. Only the minor absorptions of the cornea, ocular humours and inner layers of the retina itself remain to screen the receptors.

The supposed similarity of the absorption spectrum of rhodopsin to the spectral sensitivity of human rod vision has provided one

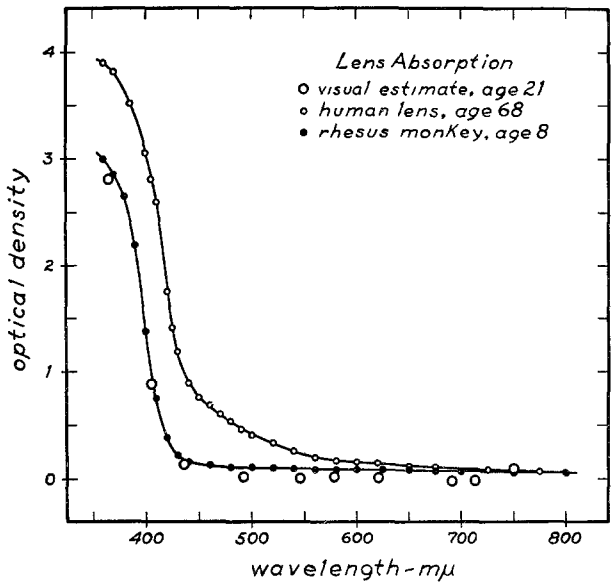

Fig. 13.

Absorption spectrum of the lens. The large open circles represent visual estimates of this, taken from Fig. 12. Direct measurements of the absorption spectrum of the lens of a 68 year old person are also shown; as also the spectrum of a monkey lens. All these measurements display the same general character, a strong absorption rising sharply and without incident into the near ultraviolet

of the strongest arguments that rhodopsin is the photopigment of the rods. This comparison, which originated qualitatively with Kühne (1878) and quantitatively with König & Köttgen (1894), has had a complicated history. Until lately it was pursued with what were in fact inappropriate data: on the one hand the 'difference spectrum' of rhodopsin — the difference in spectrum between unbleached and bleached rhodopsin — mistakenly taken to be its absorption spectrum (König & Köttgen; Köttgen & Abelsdorff); on the other hand the sensitivity of rod vision measured at the *corneal* surface, and taking no account therefore of the screening effects of the intraocular filters.

It is now recognized that this comparison must be made between the absorption spectrum of purified rhodopsin, measured directly; and a 'retinal' spectral sensitivity function — one corrected for the transmission of light by the ocular tissues. Two relatively minor considerations may be added. Rhodopsin absorbs light in discrete

quanta which vary in energy content inversely with the wave-length. It seems probable that the quanta are equally efficient at all wave-lengths in the ordinary visible spectrum; their effect on rhodopsin then depends simply on their number, not on their total energy content. It probably is preferable therefore to convert the spectral sensitivity from a relative energy basis, as in the foregoing figures, to a quantum basis. This is done by dividing the sensitivity at each wave-length by the wave-length. The effect is to shift the sensitivity maximum slightly toward the violet (Dartnall & Goodeve, 1937).

Finally, the spectral sensitivity should be compared with the percentage absorption of rhodopsin. A spectrum expressed as percentage absorption, unlike one expressed as extinction, varies in shape with the value of the absorption. For this comparison therefore one should know the real absorption of rhodopsin in the human retina. This has been estimated to be about 20 % at 500 mμ. It must be conceded, however, that a percentage absorption curve made up to this maximal value differs in shape only slightly from an extinction spectrum.

This argument has been presented in detail by Wald (1937—38 b; 1944 b). It is possible to demonstrate a very satisfactory agreement between the percentage absorption spectrum of rhodopsin in solution and the spectral sensitivity of rod vision, quantized and corrected for ocular transmission. The most questionable element in this computation, however, involves the available information on the spectral transmission of the human eye (Ludvigh & McCarthy, 1938).

The measurements on aphakic eyes now permit a more direct approach to this comparison. As already remarked, the aphakic data come as close to the intrinsic sensitivities of the rods and cones as one can come in the intact eye. There is a special interest therefore in comparing the data of aphakic rod vision directly with the absorption spectrum of rhodopsin, without appeal to extraneous sources of information.

Such a comparison is shown in Fig. 14. To it have been added data showing the effectiveness of the spectrum in bleaching rhodopsin in solution (Schneider, Goodeve & Lythgoe, 1939; Goodeve, Lythgoe & Schneider, 1941—42).

In this figure the absorption spectrum of rhodopsin is plotted as extinction, and is therefore equivalent in shape to the percentage

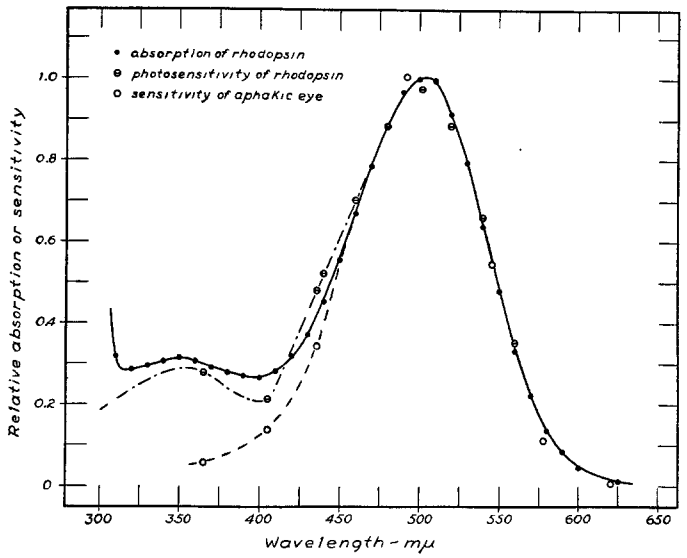

Fig. 14.

The absorption spectrum of frog rhodopsin in aqueous solution, compared with its bleaching spectrum and with the spectral sensitivity of rod vision in the aphakic human eye. The bleaching spectrum is from Schneider et al. (1939) and Goodeve et al. (1941—42); at low wave lengths it descends toward a point at 254 mμ., not shown in the figure

absorption curve for a very dilute solution. The photosensitivity of rhodopsin in solution is expressed in terms of the absorption coefficient multiplied by the quantum efficiency. The spectral sensitivity of aphakic rod vision is taken from Fig. 12, but has been converted to an arithmetic scale and quantized.

Fig. 14 reveals a very close correspondence between the absorption spectrum of rhodopsin and its bleaching spectrum in solution. Both functions follow the same course not only through the main α-band but also the small β-peak at 350 mμ. At still lower wave-lengths these functions diverge; as the absorption rises to the high protein γ-band (cf. Fig. 1 above), the photosensitivity falls to a low value at 254 mμ., not shown in the figure.

The spectral sensitivity of aphakic rod vision is identical in form with the absorption spectrum of rhodopsin from the red to the violet. From here into the near ultraviolet the visual sensitivity falls below the rhodopsin spectrum. This departure is caused by the absorption of light at low wave-lengths by the tissues which

still screen the receptors in the aphakic eye — the cornea, ocular humours, and inner retinal layers.

The Visual Domain

It is customary to define the visible spectrum as the range of wave-lengths between 380—400 and 700—750 mμ. Actually, however, human vision has no sharp spectral limits. It is bounded only by spectral regions of rapidly declining sensitivity, in which more and more energy is needed to stimulate the eye. It is clear from Fig. 8 that 400 and 700 mμ. mark only the wave-lengths at which the cone sensitivity has declined to roughly 1/100 of its maximal value. This is an entirely arbitrary figure. Goodeve has explored the visibility of radiations in the ultraviolet to 312.5 mμ. (1934) and has measured the sensitivity in the infrared to 900 mμ. (1936).

Lately we have measured human visual sensitivity in the infrared to 1000 mμ. in the fovea and 1050 mμ. in a peripheral area of retina (Griffin, Hubbard & Wald, 1947). These data, appended to measurements in the more visible regions of the spectrum, are shown in Fig. 15. Exposures of 1 second were used; under these circumstances the difference between foveal cone and peripheral rod sensitivity in the region 675—740 mμ. (cf. Fig. 8 above) is almost obliterated. At longer wave-lengths these functions diverge again, the peripheral rods becoming increasingly more sensitive. This accounts for the fact that, at the threshold of vision for the free, unfixated eye, wave-lengths in the neighborhood of 700 mμ. are seen as red, while longer wave-lengths appear less saturated and the infrared looks colourless.

To see with the fovea at 1000 mμ. requires about 10^{10} times as much energy as at 562 mμ., the wave-length of maximum sensitivity. To see at 1050 mμ. with the dark-adapted peripheral retina requires more than 10^{12} times the energy needed at 505 mμ., the rod maximum.

One may ask whether there is any end to extending the range of the visible spectrum by increasing the energy of the stimulus. Ultimately we depend upon the absorption of radiation by rhodopsin and the cone photopigments, and certainly a major factor in the decline of sensitivity at the ends of the spectrum is the decreasing capacity of the photosensitive pigments or of their chromophores to absorb light.

An added factor of considerable interest arises in the infrared. Light is absorbed by photopigment molecules in discrete quanta,

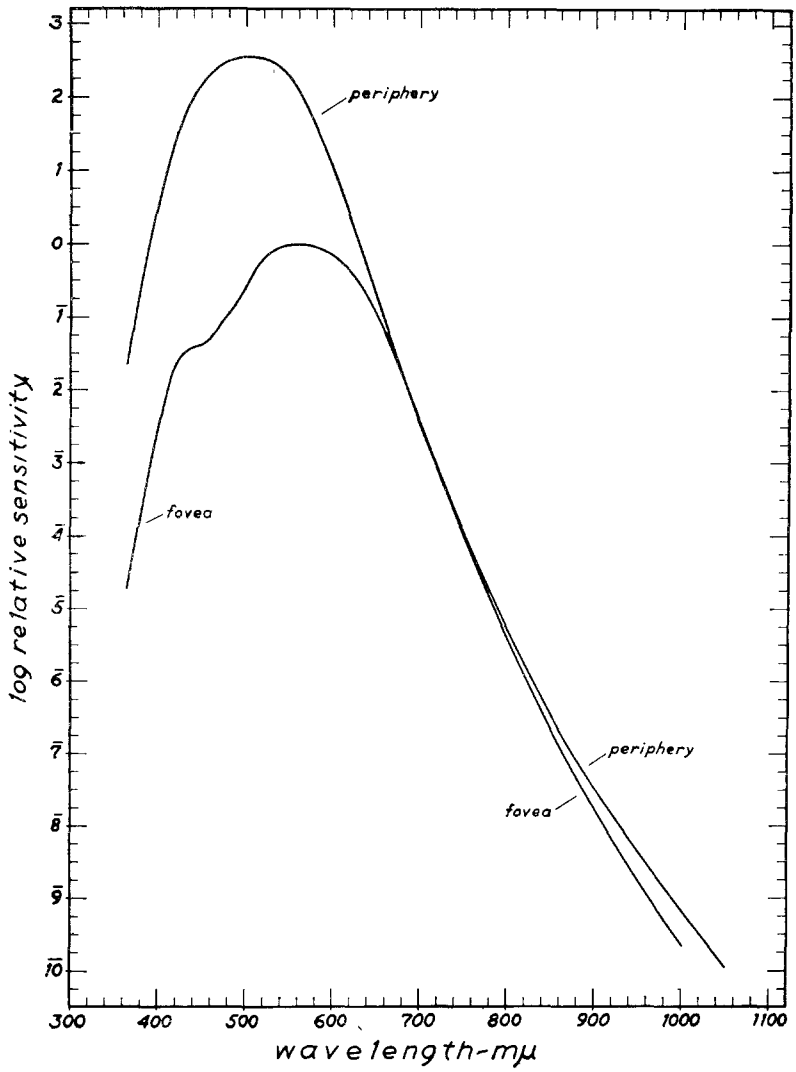

Fig. 15.

Relative spectral sensitivities of the dark-adapted human fovea and peripheral retina. The spacing of these curves is appropriate for a 1° test field exposed for 1 second. Below 750 mμ., the foveal curve is a composite function based on the original data of a number of workers; the peripheral function is from Wald (1945 b). To these, the data for the far red and infrared have been joined. The large depression and minor inflections in the region 440—520 mμ. mark the absorption bands of macular xanthophyll. (From Griffin, Hubbard & Wald, 1947)

which bring them into the excited state which initiates bleaching. The energy contained in a quantum of radiation is inversely proportional to the wave-length. The further one penetrates the infrared therefore, the smaller are the quanta and hence the energies available for photopigment excitation. At 550 mμ. the photopigments in absorbing a mol of quanta acquire about 52,000 calories of energy; at 1000 mμ. they obtain only about 28,500 calories. Since one can see at 1000 mμ., this must still be enough to excite the photopigment. One wonders nevertheless whether the quantum efficiency — the probability that a molecule having absorbed a quantum reacts — has not declined considerably by this wave-length. If so, this is an added factor decreasing the visual sensitivity in the infrared.

Finally we must reckon with the absorption of radiation by ocular tissues, preventing it from reaching the outer segments of the rods and cones. In the ultraviolet, in addition to the specific yellow pigmentations already discussed, all the eye tissues possess strong protein absorption, rising to a sharp maximum in the region about 280 mμ. This probably makes it impossible to see below about 310 mμ. with any sources of radiation now available.

In the infrared a similarly general absorption is encountered, that of the water of the eye tissues. This is small below about 950 mμ., but rises to about 84 % at 1150 mμ., to about 92 % at 1200 mμ. (Hartridge & Hill, 1915). It seems probable that this absorption, joined to the rapid decline in sensitivity of the receptors, sets an absolute limit to human vision at about 1150 mμ.

The infrared is of course the adequate stimulus for a second group of sensory receptors, the warm spots of the skin. During our experiments on infrared vision we became accustomed to feeling the warmth of the stimulus as well as seeing it. My colleague Professor Griffin calculated that at just about 1150 mμ. radiation should be more readily felt as heat than seen as light. This is the wave-length at which vision ends and another sensory modality takes over.

So much for the absolute limits of vision. We have seen however that at low wave-lengths, long before the decline in absorption by the retinal photopigments and the cut-off in transmission by ocular proteins puts an end to spectral sensitivity, it is brought low by the special yellow pigmentations of the lens and macula. The lens alone excludes human vision from the near ultraviolet; the macula

in addition greatly depresses the sensitivity to blue and violet light in the area of fixation and most acute pattern vision. This is their negative aspect; do they also have some positive role? We know of no inner necessity for a lens to be yellow; and the macular pigmentation is even a great rarity, reserved for the primate eye. Is it not probable that these are adaptive structures, the products of some evolutionary *quid pro quo* by which the eye loses in sensitivity to gain in some other aspect of vision?

I think it is now clear that the function of the yellow pigmentations of the macula and lens is to counteract the chromatic aber-

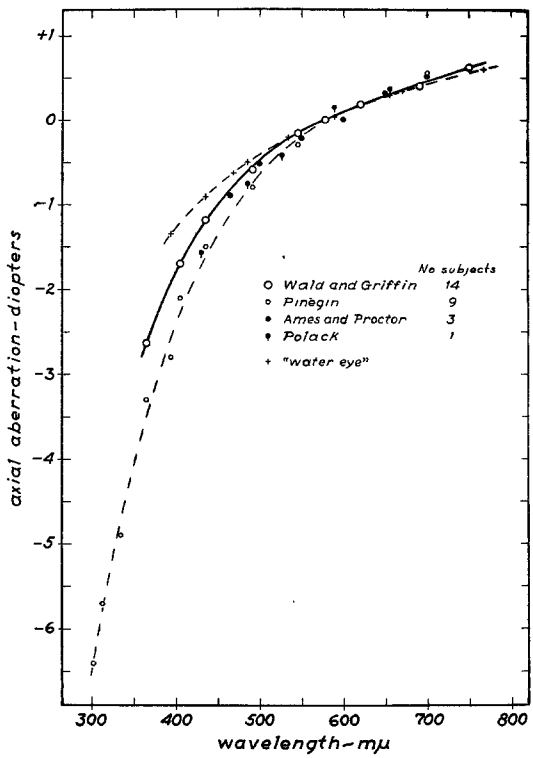

Fig. 16.

Chromatic aberration of the human eye. The axial aberration is expressed as the lens correction in dioptres that must be applied to an eye to bring it into correct focus at various wave-lengths. Data of a number of observers are shown; and also the computed chromatic aberration of an eye presenting a single refracting surface and consisting entirely of water. (From Wald & Griffin, 1947)

ration of the eye. Though the human eye is reasonably well corrected for spherical aberration, it has been known since Newton to possess a large chromatic aberration. The axial aberration as a function of wave-length is shown in Fig. 16 (Wald & Griffin, 1947). Between 750 and 500 mμ. the chromatic aberration increases moderately and linearly. Below 500 mμ. however the function accelerates markedly, and below 400 mμ the aberration becomes very large. If the eye dealt with the entire range of wave-lengths to which its photopigments, and hence the rods and cones, are sensitive, the chromatic aberration would produce a very considerable blurring of the perceived image..

It seems probable that the organism is unable to elaborate the materials needed to produce an achromatic lens combination. The only defense it has against chromatic aberration therefore is to exclude those regions of the spectrum which would cause the greatest visual disturbance.

Precisely this is accomplished by the yellow filters of the human eye. The lens eliminates for the whole eye the near ultraviolet, for which the chromatic aberration is especially large. In the central area of the primate retina the absorption of the macular pigmentation rises just as that of the lens falls off, and continues a filtering action that cuts heavily into the violet and blue. This is the retinal area in which the organism achieves its most acute form vision; one reserved, moreover, for use in bright light, since it contains predominantly cones. In this region of the retina the eye sacrifices sensitivity in order to achieve the highest pattern resolution.

It is interesting to note that the only class of animals in which a high capacity for ultraviolet vision has been demonstrated — the insects (Bertholf, 1931; 1932—33) — have eyes which evaluate images on an entirely different principle from the lens eye. The image is received as a pattern of discrete points by a mosaic of independent receptor organs — the ommatidia — each equipped with its own dioptric system. Lenses play no part in pattern vision in such eyes, and lens aberrations are of no consequence. Such eyes can therefore deal without embarrassment with ultraviolet radiation.

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As a brief postscript to Professor Wald's very interesting paper I may add that, *retinene*$_1$ has been prepared in my laboratory by Mr. Ball and Mr. Goodwin as a pure crystalline substance. It is the aldehyde of vitamin A_1 and it may be prepared in good yield from vitamin A (free alcohol) by leaving a solution in light petroleum to stand in the dark over solid pyrolusite (MnO_2) at room temperature. The final purification by chromatography and crystallisation is tedious but the purest material shows the same maximum molecular extinction coefficient as vitamin A.

The positions of the absorption maxima for solutions in different solvents and for the antimony trichloride colour test agree perfectly with Wald's criteria.

Retinene$_2$ has been similarly prepared by Mr. Salah and Dr. Stubbs in a state approaching purity by using a mixture of vitamin A_1 and A_2 from ling cod liver oil. The mixed aldehydes are separated chromatographically. Retinene$_2$ and Vitamin A_2 aldehyde appear to be synonymous. Retinene$_2$ (or a substance very difficult to distinguish from vitamin A_2 aldehyde) can be prepared from vitamin A_1 or retinene$_1$ by the Oppenauer reaction (aluminium isopropoxide). This implies that A_2 differs from A_1 by having one additional conjugated double bond located in the ring.

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Prof. Wald's derivation of the spectral absorption of the macular pigment by comparison of the spectral sensitivities of the cones (a) at the fovea (b) at an 8° parafoveal point, rests on the assumption that the cone mechanism beneath the pigment layer has the same relative spectral sensitivity curve in the fovea and parafovea. There is considerable evidence, some of which is reviewed in my paper to the Conference, that the spectral sensitivity curve of the cones is a resultant of three sensitivity curves associated respectively with three cone mechanisms, and that the form of the resultant curve may be modified by changes in the relative sensitivities of the three mechanisms produced either by an adapting field, or by changes in the characteristics of the test stimulus, for example its angular size, or in its position on the retina. The good agreement of Wald's result with his direct measurements of the absorption of the pigment extracted from human maculae, and with the properties of macular pigment to be expected from other sensory observations, suggests that for his conditions (zero adaption field, test stimulus of diam. 1° and exposure 0.04 sec.) the resultant sensitivity curve of the cone mechanisms beneath the pigment layer was substantially the same in fovea and parafovea. A derivation, similar to Wald's, of the spectral absorption of the macular pigment can be made by comparing the rod sensitivity of the dark-adapted eye (a) in the macula but outside the fovea (say 2½° out) (b) in the parafovea (say 10° out). Provided we exclude the red end of the spectrum where cone vision may play a part, the difficulty mentioned above will not apply because the rods beneath the pigment layer behave, we believe, as a single mechanism with a unique spectral sensitivity curve. Analysis on these lines of the threshold data obtained by Abney &

Watson (1915) leads to a spectral absorption curve for macular pigment which is generally similar to Wald's in the more limited range of the Abney & Watson data ($\lambda \geq$ 425 mμ.).

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As suggested by Prof. Wald, the bleaching of rhodopsin to vitamin. A includes several intermediate steps other than retinene formation. Further description of these steps is desirable because they show that colour vision is chemically similar to night vision (Bliss, 1946 a, 1947). In the terminology of Lythgoe, bleached rhodopsin liberates transient orange, which rapidly decomposes at room temperature to indicator yellow, a striking colorimetric pH indicator which is almost colourless in base, and bright orange-red in acid chloroform, when it has an absorption maximum about 477.5 mμ. Acid indicator yellow is converted in a few hours to retinene, a pH-stable carotenoid with an absorption maximum at 385 mμ. in chloroform. It must be emphasized that indicator yellow is not the same substance as retinene. The conversion of indicator yellow to retinene has been demontrated in chloroform solutions of the products of bleaching both rhodopsin and iodopsin.

There is some evidence which suggests that vision, like photosynthesis, does not require that the absorption of light be followed by bleaching of the absorbing pigment. Thus the eye of the squid and of certain other invertebrates contains a light-stable, non-melanoid red pigment which becomes photosensitive in the presence of low concentrations of formalin, and bleaches with the release of indicator yellow and retinene (Bliss, 1943, 1946 b). This pigment has an absorption spectrum identical with that of rhodopsin, but is chemically more labile, and releases retinene even in the dark (Wald, 1941). The results of experiments now in progress are not in agreement with the view that the squid retina contains a primarily photosensitive pigment of the rhodopsin type.

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It was pointed out by Wald that it is undesirable to move the sensitivity curves further to the red. I might draw attention to the fact that this would involve a decrease of the energy necessary for decomposition of the visual substances and therefore an increase of spontaneous decomposition by thermal motion. As the rods and cones can be excited by only one quantum of light or by the decomposition of one molecule, this would involve too large a number of spontaneous excitations.