THE PORPHYROPSIN VISUAL SYSTEM

By GEORGE WALD*

(From the Biological Laboratories, Harvard University, Cambridge)

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Certain fishes have evolved a visual system different from that common to all other types of vertebrate. In place of the rose-colored rhodopsin, their rods contain a purple photolabile pigment which I have suggested be called porphyropsin (Wald, 1937 *a*). This peculiarity was first noted in a number of fresh-water fishes by Kühne and Sewall (1880), and later was confirmed spectrophotometrically by Köttgen and Abelsdorff (1896).

Following the work of the latter authors it has been believed that fish retinas in general contain porphyropsin. Recently, however, a number of marine fishes have been shown to possess visual systems identical with those of frogs and mammals, in which rhodopsin participates in a retinal cycle with the carotenoids retinene and vitamin A (Wald, 1936–37; 1937–38).

This suggested the curious possibility that porphyropsin is restricted to fresh-water fishes. And in fact a preliminary survey indicates that the retinas of most permanently marine fishes contain rhodopsin, those of permanently fresh-water fishes porphyropsin; while those of euryhaline[1] fishes contain either predominantly or exclusively that photopigment ordinarily associated with the environment in which the fish is spawned (Wald, 1938–39).

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[1] It has been found convenient to divide these fishes into four classes: two groups of stenohaline fishes, restricted permanently to marine or fresh-water environments; and two groups of euryhaline fishes, capable of existence in a wide range of salinities: those spawned in fresh water (anadromous), and those spawned in the sea (catadromous).

The present paper is concerned with the chemistry of the "pure" porphyropsin system as found in permanently fresh-water and some anadromous fishes; specifically with the behavior of porphyropsin in the retina and in aqueous solution, and its interaction with carotenoids in the retinal cycle. In this work I have followed closely the design of previous experiments on the rhodopsin system in marine fishes (Wald, 1936–37; 1937–38), in order to demonstrate clearly that both systems, though distinctly different in composition, are remarkably parallel in behavior. To aid in this comparison, I have included in the present paper new observations on the marine scup and on two members of the previously unexplored group of marine elasmobranchs.

Methods

Porphyropsin Solutions.—Retinas of dark adapted fishes were prepared in dim red light, as free as possible of the underlying pigmented epithelium and choroid layer. After soaking for about 3 hours in a 4 per cent solution of aluminum potassium sulfate (alum), they were washed twice in distilled water and twice in phosphate buffer solutions, usually neutral. Finally they were ground in 2 per cent aqueous digitonin, and allowed to leech at room temperature overnight. The mixtures were centrifuged for about 20 minutes at about 3000 R.P.M. and the clear solutions stored at 2°C. Generally these solutions were recentrifuged just before their spectra were recorded to dispose of colorless precipitates which sometimes developed during storage.

Antimony Chloride Tests.—Retinal extracts in dry chloroform were tested by mixing with saturated solutions of antimony trichloride in chloroform in the proportion 0.3 cc. of extract to 3.2 cc. of reagent. Spectra of these mixtures were recorded immediately, since the test color fades and changes qualitatively within 10 to 15 minutes.

Spectrophotometry.—Spectra in the visible region were measured with the recording photoelectric spectrophotometer of A. C. Hardy at the Color Measurements Laboratory of the Massachusetts Institute of Technology (Hardy, 1935). The special advantages of this instrument for the type of work with which we are concerned have already been indicated (Wald, 1937–38). Figs. 1, 2, 3, and 6 are direct recordings drawn by the instrument itself; Fig. 8 is a tracing from such a record. In each case the difference in absorption between the test solution and a blank of pure solvent of equal depth was measured. The absorption is recorded either as percentage of incident light absorbed ($1\text{-}I/I_o$), or as extinction (optical density), log I_o/I, in which I_o is the incident and I the transmitted intensity.

Absorption spectra in the ultra-violet were measured with a Hilger Intermediate quartz spectrograph, equipped with a Spekker Photometer, and using a tungsten spark as source.

Animals.—The fishes which have been examined include the fresh-water calico bass (*Pomoxis sparoides*), carp (*Cyprinus carpio*), blue-gill (*Lepomis pallidus*), and pickerel (*Esox reticulatus*); the anadromous white perch (*Morone americana*); and the marine scup (*Stenotomus chrysops*), smooth dogfish (*Galeorhinus laevis*), and spiny dogfish (*Squalus acanthias*).

The Retinal Cycle

Isolated retinas of dark adapted fresh-water fishes are deep purple in color. On exposure to bright daylight they bleach almost instantly to a russet hue, which at 0°C. is maintained for hours, even in bright light. This russet material represents the end-product of the photochemical reaction.

At room temperatures in moderate light the initial russet color fades slowly to faintest greenish-yellow, virtually to colorlessness. This process occupies about ¾ of an hour at 25°C. It occurs in the dark as well as in the light, and possesses a high temperature coefficient. It is an ordinary thermal reaction.

A russet retina replaced in darkness at room temperatures, in addition to fading, also regenerates some porphyropsin, by rough visual estimate perhaps ⅓ of its original content. Retinas in intermediate stages of fading regenerate less porphyropsin, in amounts roughly proportional to their content of russet products. The completely faded retina does not synthesize porphyropsin at all.

It may be concluded that in the isolated retina light bleaches porphyropsin to a russet pigment, which is removed by thermal processes in two directions: (*a*) by reversion to porphyropsin; and (*b*) by irreversible formation of a faint yellow product.

Bleaching of the isolated retina ends, therefore, in the faintly yellow, almost colorless condition. The retinas of living fish which had been exposed to direct sunlight for 15 minutes and then dissected out either in the light or immediately on removal to darkness, possess the same virtually colorless appearance. Yet *in vivo* such retinas rapidly regain their original content of porphyropsin in darkness. Though the isolated retina cannot synthesize porphyropsin from the faintly yellow product, it is clear that this process occurs readily in the intact animal.

The porphyropsin, like the rhodopsin system, therefore, constitutes a cycle within a cycle. Porphyropsin bleaches in the light reversibly to a russet pigment. This in turn fades irreversibly to a light yellow

material, which in the intact eye, though not in the isolated retina, is re-synthesized to porphyropsin.

Vitamin A_2 and Retinene$_2$

Rhodopsin is a conjugated protein bearing a carotenoid prosthetic group. The color changes which characterize the rhodopsin cycle depend primarily upon alterations in its carotenoid components. The remarkably parallel gross behavior of porphyropsin in the retina suggests that it may be some simple chemical variant of rhodopsin, involving the carotenoid or the protein residue, or the linkage between them. The first of these alternatives proves to be correct, and provides a sufficient basis for all the observations. The porphyropsin cycle, though identical in form with that of rhodopsin, contains new carotenoids in the positions of retinene and vitamin A.

This situation is revealed in a simple procedure originally applied to marine fishes (Wald, 1936–37; see particularly Fig. 1). Its application to the smooth dogfish, a marine elasmobranch, is shown in Fig. 1; and to the permanently fresh-water calico bass and pickerel in Figs. 2 and 3.

Right and left retinas of several dark adapted fish are isolated separately in dim red light, so that two symmetrical sets of retinas from the same animals result.

One group of these is centrifuged, the clear Ringer's solution decanted, and the tissue extracted by shaking for 20 minutes by machine *in darkness* with benzine (petroleum ether, boiling range 30 to 60°C.). This treatment does not disturb the retinal photopigments. The mixture is centrifuged, the benzine extract poured off, and transferred to 0.3 cc. of chloroform. It forms a virtually colorless solution. The whole of this solution is mixed with 3.2 cc. of antimony chloride and the absorption spectrum of the faintly blue product measured immediately. Marine fishes yield in this test a low absorption band, maximal at about 618 mμ, showing the dark adapted retina to contain a small quantity of vitamin A (Fig. 1 *a*). Extracts from fresh-water fishes yield a similar band; its maximum, however, lies at 696 mμ (Figs. 2 and 3, curves *a*).

Following extraction in the dark with benzine, the retinal tissue is still deeply colored with photopigment. On exposure to bright light

it bleaches quickly to orange or russet. It is immediately re-extracted with benzine, exactly as before. The extract, transferred to 0.3 cc.

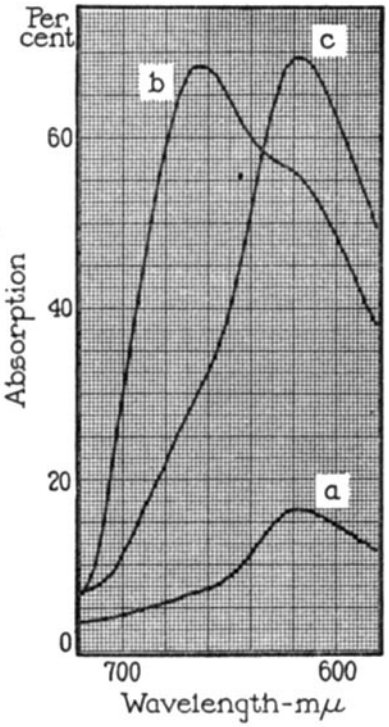


FIG. 1. Antimony chloride reactions with benzine extracts of retinas of the smooth dogfish. (*a*) Dark adapted retinas yield a small amount of the 618 mμ chromogen, vitamin A. (*b*) Irradiated retinas; a large quantity of the 664 mμ chromogen, retinene, has been liberated. (*c*) Completely faded retinas; practically all retinene has been converted to vitamin A.

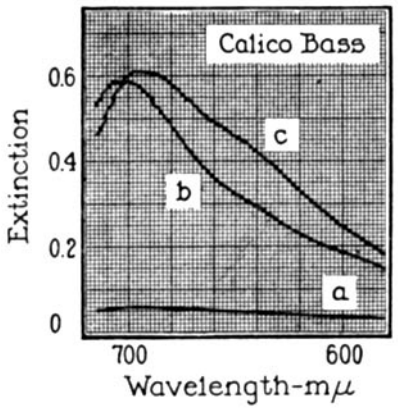


FIG. 2. Antimony chloride reactions with benzine extracts of retinas of the calico bass. (*a*) The dark adapted retina contains a trace of the 696 mμ chromogen, vitamin A_2. (*b*) Irradiated retinas; a large amount of the 704 mμ chromogen, retinene$_2$ has been liberated. (*c*) Faded retinas; the retinene$_2$ has been converted to vitamin A_2.

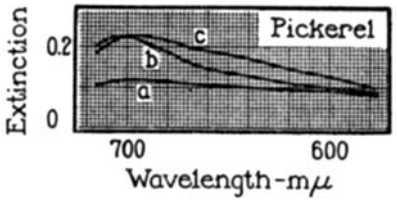


FIG. 3. Antimony chloride reactions with benzine extracts of pickerel retinas. Otherwise as in Fig. 2.

chloroform, is golden in color. Tested as before with antimony chloride, marine fish extracts yield a high band, maximal at 664 mμ; the irradiation of rhodopsin has liberated a large quantity of

retinene (Fig. 1 *b*). From fresh-water fishes a parallel result is obtained; but in this case the antimony chloride maximum lies at 703–706 mμ (Figs. 2 and 3, curves *b*).

The symmetrical set of retinas is exposed to bright light in Ringer's solution and is left at room temperatures in moderate light for about an hour. During this period the retinas fade almost to colorlessness. Finally they are collected by centrifuging and extracted with benzine exactly as were the others. The extracts, transferred to 0.3 cc. chloroform, are virtually colorless. Tested with antimony chloride, they yield in the case of marine fishes a high band at about 618 mμ with little or no specific absorption at 664 mμ. The fading process has transformed the retinene liberated on irradiation quantitatively to vitamin A (Fig. 1 *c*). Fresh-water fishes yield a parallel result, but the antimony chloride band of the final product lies at 696 mμ (Figs. 2 and 3, curves *c*).

This final state is qualitatively identical with that obtained by high light adaptation of the living fish; *in vivo* it reverts in darkness to the original condition represented by curves *a* of Figs. 1 to 3. The transitions revealed in this procedure therefore characterize in the intact animal three stages in the retinal cycle. The cycle is identical in form in both fresh-water and marine fishes; but in the former a 703–706 mμ chromogen replaces retinene, and a 696 mμ chromogen vitamin A. No trace of either vitamin A or retinene has yet been found in a fresh-water fish retina.

The data of Fig. 1 were obtained from four smooth dogfish about 25 inches in length. Each curve, therefore, was derived from the whole extract of four retinas. Similarly the data of Figs. 2 and 3 were obtained from eight calico bass about 8 inches in length, and from eight pickerel 10 inches in length.

Similar experiments have been performed with the anadromous white perch (*cf.* Wald, 1937 *a*) and with the carp, with results essentially identical with those of Figs. 2 and 3.

These observations were first reported early in 1937. Almost simultaneously Lederer and Rosanova (1937) reported that though the liver oils of a number of Russian marine fishes yield the familiar antimony chloride band of vitamin A, those of most fresh-water fishes which were examined yield instead an "abnormal reaction" consisting of a dominant band at about 690 mμ. It was clear that the substance

which replaces vitamin A in the fresh-water fish retina may do so also in the liver.

The retinal activity of the new substance demonstrated its vitamin nature, while its discovery in liver oils provided a rich source for its further investigation. This has proceeded with great rapidity. The 696 mμ chromogen was shown to possess a direct absorption band in ethanol at 345–350 mμ. It is probably a homologue of vitamin A, containing an added ethylene group and the empirical formula $C_{22}H_{32}O$ (Gillam, Heilbron, Jones, and Lederer, 1938). A pike-perch liver oil which probably contained little if any vitamin A displayed growth-promoting activity in rats about equal to that of a halibut liver oil of the same Lovibond value (Gillam *et al.*). Rats and frogs, which normally contain little or no 696 mμ chromogen, accumulate this substance in their livers when fed fresh-water fish liver oil concentrates (Lederer and Rathmann, 1938). A similar accumulation may occur naturally in certain fish-eating mammals (Gillam, 1938). The distribution of vitamin A and the 696 mμ chromogen in a wide variety of animals and tissues has been reported in the papers cited, by Edisbury, Morton, Simpkins, and Lovern (1938), and by Wald (1938–39).

Since the 696 mμ chromogen replaces vitamin A in the retinal cycle of fresh-water fishes, Edisbury, Morton, and Simpkins (1937) have suggested that it be called *vitamin* A_2.[2] The retinene-analogue in these fishes may correspondingly be called *retinene*$_2$. These carotenoid substances with porphyropsin constitute the known components of the fresh-water fish visual system.

The direct spectra of rhodopsin and porphyropsin in aqueous solution and of the retinenes and vitamins A in chloroform, all brought to the same height to facilitate comparison, are shown in Fig. 4. They were derived from crude untreated preparations resulting from precisely parallel procedures in both systems. The photopigment solutions were aqueous digitonin extracts of alum-treated dark adapted retinas. The retinenes were obtained by immediate benzine extraction of irradiated retinas, from which free vitamins A had previously been removed by benzine extraction in darkness. The vitamins A

[2] *Cf.* footnote in Wald, 1938–39, page 392.

were obtained by simple benzine extraction of completely bleached retinas. The solutions of retinenes and vitamins A were therefore precisely similar to those which yielded the antimony chloride tests shown in curves *b* and *c* of Figs. 1 to 3. It is clear that the direct absorption spectra throughout both systems are single-banded, and maintain a consistent parallelism. The significance of these observations is discussed below.

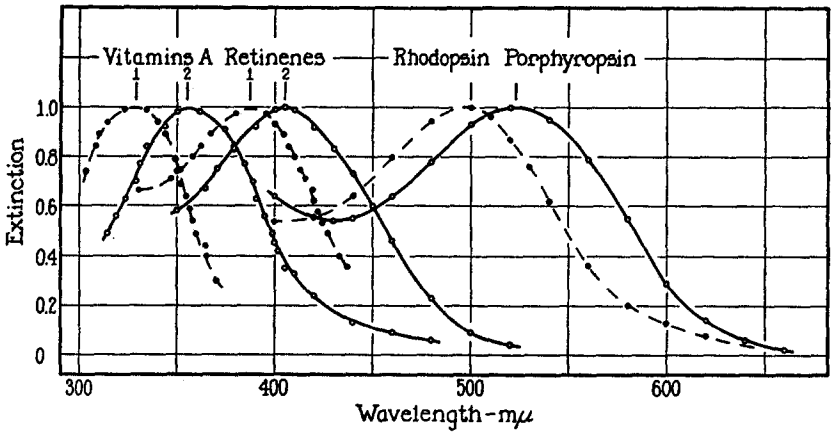


FIG. 4. Direct spectra of retinal extracts of the calico bass (circles, solid lines) and of the marine scup (broken lines). The rhodopsin and porphyropsin preparations are digitonin extracts of alum-treated retinas, measured in 1 per cent aqueous digitonin solution. The retinene preparations are crude untreated benzine extracts of irradiated retinas, measured in chloroform solution; the vitamin A preparations are similar solutions of extracts of completely faded retinas. All maxima have been brought to the same height to facilitate comparison.

Vitamin A_2 is a light yellow pigment found in the non-saponifiable fraction of tissue oils. It partitions about equally between 90 per cent methanol and benzine, and is about 70 per cent hypophasic when shaken with 95 per cent methanol and benzine. In chloroform it displays a broad absorption band maximal at about 355 mμ, accompanied in all preparations so far examined by a low band at about 290 mμ. Tested with antimony chloride it yields a high band at 696 mμ, accompanied by a small, broad hump at about 645 mμ. In crude liver oils the 696 mμ band may be displaced to positions as low as 685 mμ.

It is not yet known whether the satellitic direct absorption at about 290 mμ and in the antimony chloride test at 645 mμ are due to vitamin A_2 itself or to a contaminant.

Retinene$_2$ is deep yellow in color. It is non-saponifiable and partitions principally epiphasically between 90 per cent methanol and benzine. In chloroform it possesses a broad band maximal at about 405 mμ, and with antimony chloride the best preparations yield a band at 706 mμ. In other preparations the antimony chloride band may be displaced as low as 702 mμ. Retinene$_2$, like retinene, appears to change color markedly with hydrogen ion concentration, tending toward very light yellow in alkaline and deep orange in acid solution.

Porphyropsin in Aqueous Solution

Porphyropsin, like rhodopsin, may be extracted into aqueous solutions of bile salts or digitonin. Such solutions are deep purple in color. A collection of spectra of porphyropsin preparations is shown in Fig. 5, and for comparison the spectrum of a rhodopsin solution from the spiny dogfish, prepared in the same manner. Above 470 mμ the porphyropsin spectra form a homogeneous group, maximal at 522 ± 2 mμ. Below 470 mμ they diverge, due to the presence of varying amounts of impurities. A minimum has always appeared in these spectra, varying in position with the degree of purity, but approaching a low wavelength limit at about 430 mμ in the best preparations. It is not yet known whether this is characteristic of porphyropsin itself, or whether it is an artefact, and the rise in absorption below 430 mμ due to contaminants. The porphyropsin band is wider than that of rhodopsin, its displacement varying from about 20 mμ on the low wavelength side of the maximum to about 30 mμ on the high wavelength side.

These spectra are not to be confused with the reputed absorption spectra of fish photopigments measured by Köttgen and Abelsdorff (1896) and since grown familiar in the literature. The latter consist of a single broad band, falling almost symmetrically away from a maximum at about 540 mμ to negligible absorptions at about 440 and 700 mμ. Köttgen and Abelsdorff reported in these observations only the *differences* in absorption between unbleached and bleached porphyropsin solutions ("difference spectra"). These should be

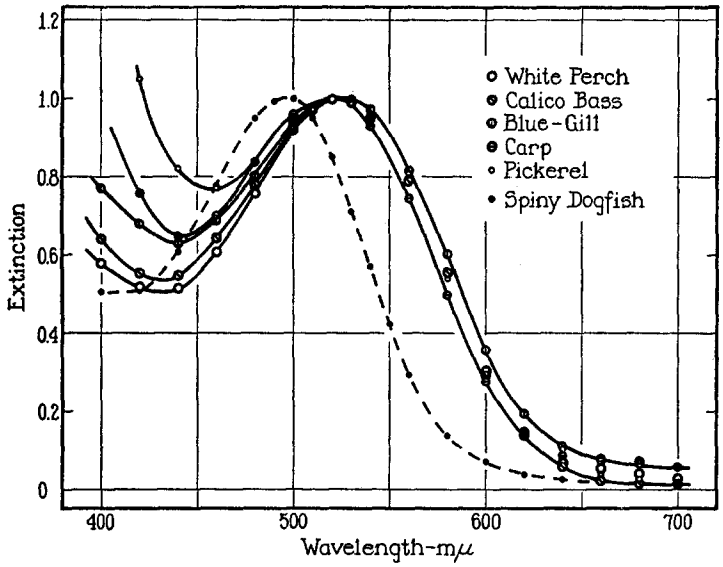


FIG. 5. Spectra of porphyropsin preparations from anadromous and fresh-water fishes, and of a typical rhodopsin preparation from the marine spiny dogfish. Digitonin extracts of retinas pre-treated with alum, measured in 1 per cent neutral aqueous digitonin solution. The porphyropsin spectra form a homogeneous group, with maxima at 522 ± 2 mμ; the rhodopsin band is narrower, and maximal at about 500 mμ.

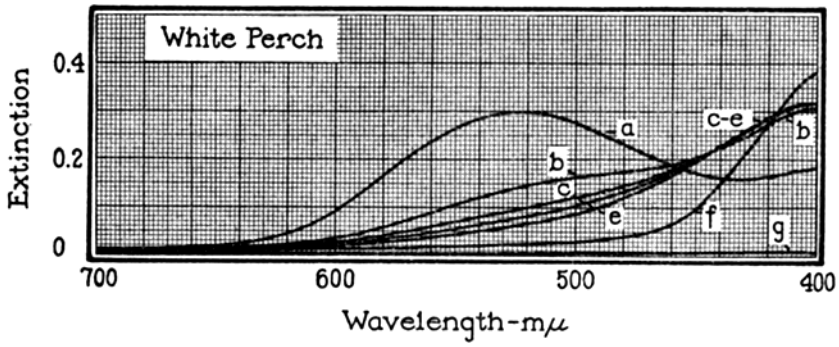


FIG. 6. The bleaching of neutral porphyropsin. Extract of eight alum-treated white perch retinas in 2 per cent aqueous digitonin, measured against an equal depth (5 mm.) of distilled water. The unbleached solution (*a*) was exposed to bright light (about 700 foot candles) for 30 seconds. It was then left in darkness and its spectrum re-measured periodically, curve (*b*) at 1.2, (*c*) at 4.6, (*d*) at 12.5, and (*e*) at 64.5 minutes from the onset of irradiation. The final yellow product, brought to pH 11 with sodium carbonate, yielded curve (*f*). Curve (*g*) is the spectrum of the 2 per cent digitonin alone.

equivalent to true absorption spectra only if porphyropsin bleaches to colorless products. However, as shown below, under all known circumstances porphyropsin bleaches to new pigments; and since at low wavelengths the absorption invariably *rises* on bleaching, difference spectra over this region possess negative values. Had Köttgen and Abelsdorff's measurements extended further into the violet, the confusion of their difference spectra with true absorption spectra could not have occurred. As it was, they observed negative values in the majority of their measurements at 420 mμ, but dismissed them as "physically meaningless." Actually the difference spectra of porphyropsin like those of rhodopsin, though on occasion useful for descriptive or analytic purposes, are physically meaningless.

On exposure to bright light, porphyropsin solutions bleach very quickly to deep orange, then more slowly to a straw color which, unless exposed to intense irradiation, is maintained indefinitely. I have examined the bleaching processes spectrophotometrically at various pH's, using procedures applied previously to the study of rhodopsin preparations.

Neutral rhodopsin bleaches in a succession of light and "dark" (photic and thermal) processes, of which the latter may account for about half the total fall in extinction at the maximum. I have distinguished three dark components in the bleaching of rhodopsin, only one of which appears regularly in neutral preparations from alum-treated retinas, while a second becomes prominent in acidic solutions (Wald, 1937–38; dark processes II and I respectively).

The bleaching of a neutral solution of porphyropsin from white perch is shown in Fig. 6. The spectrum of the unbleached preparation (*a*) possesses the characteristic maximum at 522 mμ. The first recorded product of irradiation (*b*) possesses a broad maximum at about 510 mμ, and higher absorption than porphyropsin itself below about 460 mμ. Following irradiation the preparation was left in complete darkness. The absorption continued to fall, maximally in the region of 515–520 mμ, simultaneously rising below a node at 444 mμ. This thermal change was virtually complete in about 1 hour (*e*). Dark reactions account in this instance for 34 per cent of the total fall in extinction at 520 mμ. The behavior of this preparation is precisely parallel to that of rhodopsin in comparable circumstances (*cf.* Wald, 1937–38, Fig. 3B).

The final product of bleaching (*e*) possesses a spectrum similar to that of retinene$_2$. It varies in color reversibly with pH, tending toward orange in acid and pale yellow in alkaline solution. The present residue, brought to pH 11 with sodium carbonate, yielded curve *f*. Such bleached residues yield all their color on extraction with benzine. The benzine extract, transferred to methanol or aqueous digitonin, exhibits a spectrum similar to that of the whole residue, and to retinene$_2$. It varies in color with pH as does the whole residue. It apparently owes its spectral properties to retinene$_2$.

The light and dark components in the bleaching of porphyropsin differ markedly in their effects. This is most conveniently shown by computation of the difference spectrum which each of these components contributes (Fig. 7). The fall in extinction due to the photic reaction is maximal at about 540 mμ, and possesses negative values below about 460 mμ. The difference spectrum of the thermal component is maximal at about 515–520 mμ, possesses negative values below about 445 mμ, and a distinct minimum at about 420 mμ. A similar displacement of maximum distinguishes the photic and thermal components of bleaching in rhodopsin solutions (compare Wald, 1937–38; Fig. 4).[3]

The sum of the curves shown in Fig. 7 represents the total change in absorption on bleaching neutral porphyropsin. This possesses a maximum at 535–540 mμ. The difference spectra of fish photopigments reported by Köttgen and Abelsdorff consist simply of the positive portions of this composite function.

In alkaline solution the thermal components of bleaching are greatly accelerated. At pH 9 they are of much the same form as in neutral solution, except that the nodal point below which the absorption rises is displaced to about 430 mμ. Probably due to further increase in its velocity, no dark component of bleaching has yet been recorded at pH 10.5 or 11. Within 1 minute from the beginning of irradiation at about 25°C. the spectrum of the product has reached a stable condition. It is similar to that of the final product of bleaching in neutral solution, brought to the same pH (Fig. 6*f*), and does not change further in darkness (compare Wald, 1937–38, Fig. 7).

[3] It should be noted that in the earlier figure the ordinates of the dark component of bleaching of rhodopsin had been multiplied throughout by 4.

In acid solution the thermal component of bleaching is greatly retarded and probably for this reason a second dark reaction appears. The bleaching of a blue-gill preparation at pH 3.65 is shown in Fig. 8. The first recorded product of irradiation possesses a broad hump max-

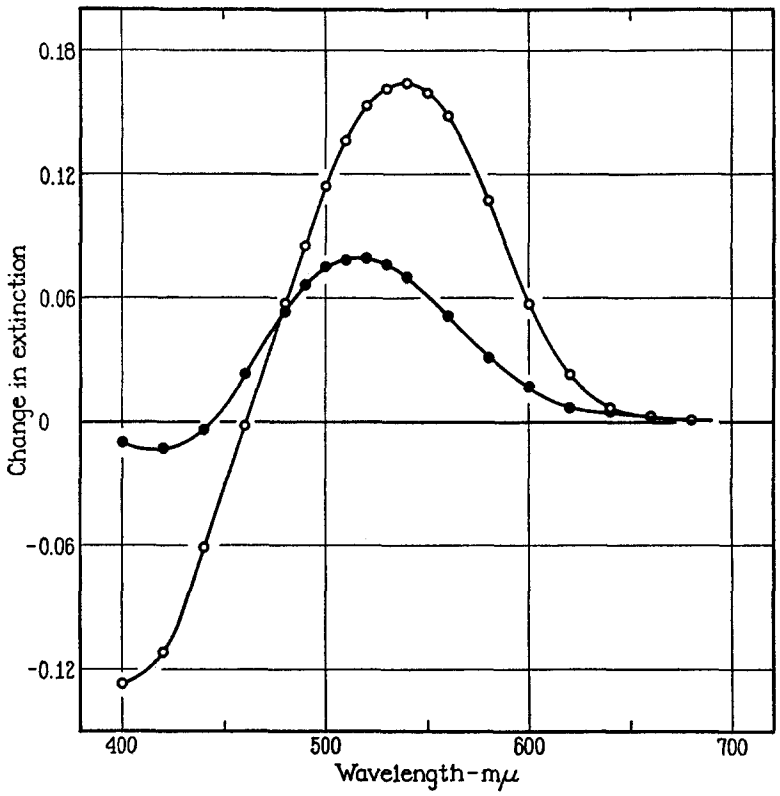


FIG. 7. Changes in absorption which accompany the bleaching of neutral porphyropsin ("difference spectra"), taken from the data of Fig. 6. Positive values indicate decrease and negative values increase in absorption. Open circles show the effect of the light reaction (Fig. 6, *a–b*), closed circles those of the "dark" processes (Fig. 6, *b–e*). Bleaching due to light is maximal at about 540 mμ, that due to thermal reactions at about 515 mμ.

imal at about 510 mμ and higher absorption than porphyropsin itself below about 470 mμ (*b*). Thereafter in darkness the absorption *rises* slowly in the region of 460 mμ, simultaneously falling to either side of nodes at about 540 and 425 mμ (*c*, *d*). This change is precisely

analogous to dark process I in rhodopsin solutions. Within 10 to 15 minutes this development reaches its peak and the absorption begins to fall over the entire spectrum above about 410 mμ. After many hours it reaches a stable condition which is the acidic analogue of the final product in neutral and alkaline solutions (*e*). All these changes are precisely parallel to the behavior of rhodopsin in comparable circumstances (Wald, 1937–38, Figs. 5 and 6).

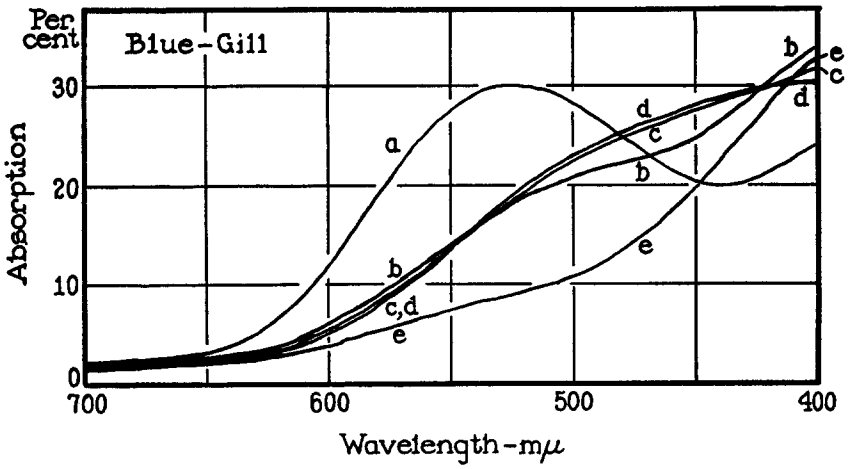


FIG. 8. Bleaching of porphyropsin at pH 3.65. The unbleached solution (*a*) was irradiated with about 500 foot candles for 30 seconds, and the spectrum thereafter recorded periodically in darkness, *b* at 2.5, *c* at 5.5, and *d* at 8.5 minutes (retraced at 15.5 minutes). 26.5°C. After 20 hours more in darkness, the spectrum had fallen to *e*.

It may be concluded that in every detail so far examined the behavior of porphyropsin solutions parallels that of rhodopsin, but the spectra of porphyropsin and of all its products of bleaching are displaced uniformly 20–30 mμ toward the red. Porphyropsin in solution bleaches in a sequence of light and dark processes, a final product of which is retinene$_2$.

Porphyropsin as Protein

Many of the observations of Ewald and Kühne on rhodopsin show it to be protein in nature (Wald, 1935–36). Recently this aspect of rhodopsin chemistry has been further investigated. Hecht and

Pickels (1938) have examined frog rhodopsin in the ultracentrifuge, and conclude from its diffusion and sedimentation properties that it is monodisperse and possesses an apparent molecular weight of 200,000–270,000. Lythgoe and Quilliam (1938) have re-examined the heat destruction of frog rhodopsin in digitonin solution and find an apparent activation energy of about 45,000 calories per mol. Electrophoretic measurements on rhodopsin solutions from grass and bull frogs (*Rana pipiens and catesbiana*) by the moving boundary method show this pigment to be amphoteric and to possess an isoelectric point in the neighborhood of pH 4.5 (Wald and Raymont, unpublished observations). It may be concluded that rhodopsin is a conjugated carotenoid-protein.

The qualitative observations which led to this conclusion have been repeated with calico bass porphyropsin. This pigment does not diffuse through a wet collodion membrane when dialyzed against solutions of phosphate buffer (pH 5.9), digitonin, or egg albumin. It is not precipitated from dilute solution by half-saturation with ammonium sulfate, but does precipitate at about 0.8 saturation. Porphyropsin in the retina is destroyed almost instantly by exposure to normal solutions of KOH, HCl, or H_2SO_4. In concentrated ammonia water (25 per cent NH_3) it is decolorized within about 2 minutes, in methanol within 15–30 seconds, in ethanol, chloroform, or acetone within 2 to 3 minutes (19°C.). The color of the final product of these treatments in the retina displays the reversible pH-lability of retinene$_2$; it is deep orange in acid solution, straw-colored in neutral, and faint greenish-yellow in alkaline media.

Dark adapted retinas retain an intense purple color in concentrated zinc chloride solution; on irradiation they bleach to a bright orange color which is maintained indefinitely. In mercuric chloride solution (pH 3.2) dark adapted retinas turn bright pink, and this color does not change appreciably on irradiation. In mercuric acetate (pH 3.7) retinas turn deep yellow in the dark within 20 minutes at about 20°C., and are not further affected by exposure to light.

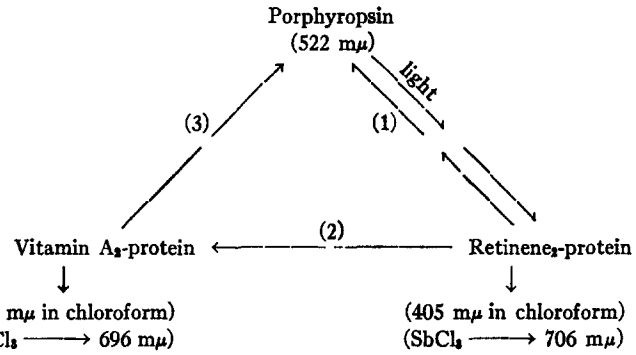
Porphyropsin in the retina is destroyed by warming to temperatures above 62°C. At 66 to 67° it is decolorized within 3 to 4 minutes, at 72 to 73° within 30 seconds. The product of heat, like that of chem-

ical destruction, is light straw in color when neutral, and changes in color reversibly with pH.

Its non-diffusibility, salting-out properties, and sensitivity to common protein denaturants and to heat indicate that porphyropsin, like rhodopsin, is a conjugated carotenoid-protein. On denaturation, as on irradiation, it yields orange or yellow products and eventually retinene$_2$. But the further conversion of retinene$_2$ to vitamin A$_2$ characteristic of the "native" retina has not been observed in retinas treated with denaturants. Apparently denaturation, either of the protein residue of porphyropsin itself or of some essential retinal component, perhaps an enzyme, blocks this process.

CONCLUSIONS

All the foregoing observations may be united in the following diagram of the porphyropsin cycle:



Isolation of the retina cuts this cycle at point (3); the processes initiated by light end in the production of a quantity of vitamin A$_2$. Denaturation of the retina, or extraction of porphyropsin into aqueous solution virtually eliminates in addition processes (1) and (2), and leaves only the succession of photic and thermal reactions which form retinene$_2$. This formulation is not intended to present the actual course of these changes rigorously. Certain of them may be further complicated by intermediate and side reactions. The equations are designed specifically to represent the known empirical relations among the three most stable loci of the porphyropsin system.

In every particular so far examined, in the retina and in solution,

this and the rhodopsin system exhibit parallel behavior. Yet a uniform displacement in spectrum divides all components in each cycle from their analogues in the other. The significance of this relationship is now reasonably clear.

Vitamin A_2 appears to be the next higher homologue of vitamin A, possessing one added ethylenic linkage, and hence one added double bond (Gillam *et al.*, 1938). Hausser, Kuhn, and Smakula (1935) have shown that in a homologous series of synthetic polyenes in benzol solution the addition of a conjugated ethylene group shifts the absorption maximum 20 to 27 mμ toward the red. The vitamin A_2 maximum in chloroform lies just 27 mμ from that of vitamin A. This displacement is therefore consistent with the proposed structure.

However, this same range of displacements separates the absorption maxima of all components of the rhodopsin and porphyropsin systems (Fig. 4). The maxima of the retinenes are about 18 mμ, those of the photopigments about 22 mμ apart. Similar differences divide the intermediates of bleaching in solution in the two systems.

We may conclude, therefore, that the primary chemical difference between the rhodopsin and porphyropsin systems as a whole is the possession by the latter of one added ethylene group. This accounts not only for all the observed spectral displacements, but explains the otherwise extraordinary parallelism in chemical behavior in the two systems. For the introduction of an added ethylene in the polyene chain should affect the ordinary chemical performance almost negligibly. By this very simple variation of a structure common to all other types of vertebrate, the fresh-water fishes have evolved their distinctive visual system.

This situation offers an important commentary on the general problem of color vision. It has been recognized since Helmholtz that trichromatic vision demands three types of retinal elements which vary in spectral sensitivity. In animals like the chicken, pigeon, and certain turtles, whose cones are equipped with three groups of color filters, this requirement can be met with a single cone photopigment (Wald and Zussman, 1938). In other animals including man, where there is no evidence of similar structures, one has been led to assume the existence of three distinct photopigments which vary in their absorption spectra (*cf.* Hecht, 1937). It seems clear from a

wide variety of physiological data that none of these pigments is rhodopsin; and the recent extraction of a distinct cone photopigment, iodopsin, from chicken retinas has confirmed this belief (Wald, 1937 *b*; see also Chase, 1938).

I think it has not previously been stated that in addition to differences in absorption spectra, the hypothetical cone photopigment systems must satisfy a further requirement. Each system must maintain its position relative to the others in all illuminations and at all stages of adaptation. If this condition were not fulfilled, the quality of the sensation would vary widely with adaptation, and the association of a specific color with an illuminant would be impossible. To form a consistent color vision system, therefore, the cone photopigments must exhibit precisely parallel chemical behavior, even to the extent of possessing roughly equivalent kinetics in all their retinal reactions.

Comparison of the rhodopsin and porphyropsin systems shows how both these criteria may be fulfilled simply and elegantly. By small variations in the number or arrangement of double bonds in the polyene nucleus, a number of photopigments might be obtained, varying slightly or widely in their spectral characteristics and consistently parallel in all their chemical activities.

SUMMARY

1. In the rods of fresh-water and some anadromous fishes, rhodopsin is replaced by the purple photolabile pigment porphyropsin. This participates in a retinal cycle identical in form with that of rhodopsin, but in which new carotenoids replace retinene and vitamin A.

2. Porphyropsin possesses a broad absorption maximum at 522 $\pm$ 2 mμ, and perhaps a minimum at about 430 mμ. The vitamin A-analogue, vitamin A_2, possesses a maximum in chloroform at 355 mμ and yields with antimony trichloride a deep blue color due to a band at 696 mμ. The retinene-analogue, retinene$_2$, absorbs maximally in chloroform at 405 mμ and possesses an antimony chloride maximum at 706 mμ.

3. Its non-diffusibility through a semi-permeable membrane, salting-out properties, and sensitivity to chemical denaturants and to heat, characterize porphyropsin as a conjugated carotenoid-protein.

4. The porphyropsin cycle may be formulated: porphyropsin $\underset{(1)}{\overset{\text{light}}{\rightleftarrows}}$ retinene$_2$-protein $\underset{(2)}{\rightarrow}$ vitamin A$_2$-protein $\underset{(3)}{\rightarrow}$ porphyropsin. Isolation of the retina cuts this cycle at (3); denaturation procedures or extraction of porphyropsin into aqueous solution eliminate in addition (1) and (2).

5. The primary difference between the rhodopsin and porphyropsin systems appears to be the possession by the latter of an added ethylenic group in the polyene chain.

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