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Cyanopsin, A New Pigment of Cone Vision

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THE three visual pigments known heretofore are formed by combinations between two retinenes and two visual proteins or opsins. Retinene$_1$ combines with rod opsin (scotopsin) to make rhodopsin, or with cone opsin (photopsin) to make iodopsin. Retinene$_2$ combines with rod opsin to make porphyropsin (*1–3*). Clearly a fourth combination is possible: retinene$_2$ with cone opsin. This has now been prepared. It is a blue, light-sensitive pigment, with an absorption maximum at 620 mμ. We propose to call it *cyanopsin.*

It is synthesized as follows. An extract of dark-adapted rods and cones from the chicken retina contains a mixture of rhodopsin and iodopsin. With deep red light, to which rhodopsin is insensitive, the iodopsin alone is bleached irreversibly to a mixture of all-trans retinene$_1$ and cone opsin. To this is added a small amount of the specific cis isomer of retinene$_2$ which, when mixed with rod opsin, forms porphyropsin (*2, 3*). Added in this instance to cone opsin, it forms cyanopsin.

The synthesis of cyanopsin is completed within 5 minutes in the dark at room temperature. The absorption spectrum of the resulting solution is measured in the dark, and again after bleaching in deep red light. The former spectrum minus the latter is the difference spectrum of cyanopsin. This is shown in Fig. 1.

In red light, cyanopsin bleaches to a straw-colored mixture of all-trans retinene$_2$ and cone opsin. The absorption falls in the red and yellow, simultaneously rising in the blue and violet. At about 502 mμ—the "isosbestic" point—the extinction does not change. Above this wavelength the difference spectrum is positive, with a maximum at 620 mμ; below this wavelength it is negative, with a minimum at about 407 mμ, the absorption maximum of retinene$_2$.

Such a difference spectrum is defined by the equation

(Spectrum of cyanopsin) − (Spectrum of all-trans retinene$_2$ + opsin) = Difference spectrum

Our preparation of cyanopsin was formed in a chicken retinal extract in the presence of a variety of impurities. A difference spectrum, however, is by its nature "pure." It represents only the change in spectrum of the photosensitive material bleached by light—in this case cyanopsin—regardless of whatever else is present. We can measure also the spectrum of a mixture of crystalline all-trans retinene$_2$ and opsin. The sum of these spectra, according to the above equation, should represent the absorption spectrum of pure cyanopsin.

Such an estimate of the spectrum of cyanopsin is shown in Fig. 2. It was obtained by adding together the difference spectrum of Fig. 1 and the spectrum of retinene$_2$ plus opsin shown in Fig. 2. This procedure has two arbitrary aspects. Not having a pure preparation of chicken cone opsin, we have substituted here a good preparation of cattle rod opsin. The opsins possess only the protein absorption band at about 280 mμ, and have very low extinctions at wavelengths longer than 310 mμ. Their own absorption therefore does not affect appreciably the spectra shown in Fig. 2. They do, however, have an effect upon the spectrum of retinene$_2$. The retinenes couple spontaneously in solution with the amino groups of opsins and other proteins to yield loosely bound complexes

[1] We should like to dedicate this paper to Professor Otto Loewi on his eightieth birthday. The chemistry of excitation owes much to him; and in a broader sense excitation and vision are very much his province.

[2] This research has been supported in part by funds from the Rockefeller Foundation and the Office of Naval Research. We are grateful also to the Organic Research Laboratory of Distillation Products Industries of Rochester, N. Y., for a gift of crystalline retinene$_2$.

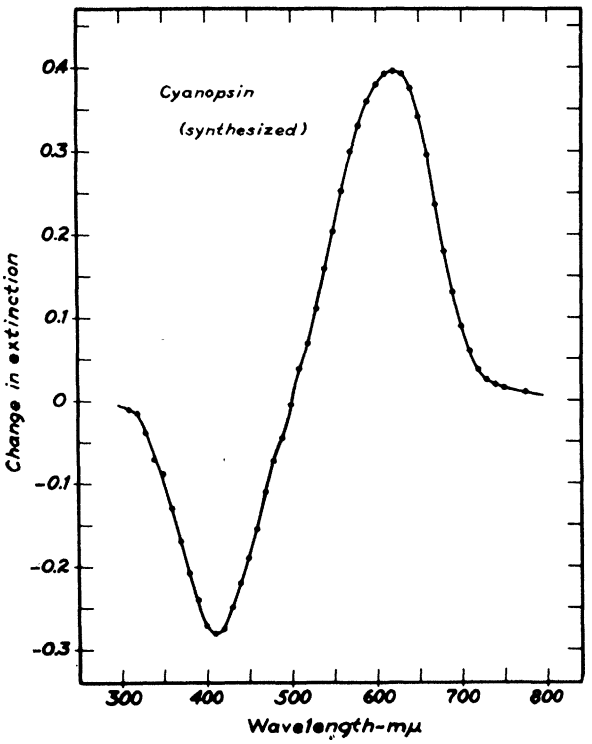

FIG. 1. The difference spectrum of cyanopsin. An aqueous digitonin extract of dark-adapted chicken rods and cones, containing a mixture of rhodopsin and iodopsin, was exposed for 20 minutes to the deep red light of a 160-watt microscope lamp passing through a Wratten 89 filter. This bleached the iodopsin alone to a mixture of retinene$_1$ and cone opsin. A small amount of cis-retinene$_2$ in digitonin solution was added, and the solution was left dark for 8 minutes. Its absorption spectrum was measured in the dark, and again after bleaching for 4.5 min in the same red light as used before. The difference in extinction between these two spectra is shown in the figure. 23° C, pH 6.8.

which vary in spectrum with pH ("indicator yellow") (*4, 5*). At any given pH the absorption spectra of such complexes vary somewhat with the nature of the proteins. The substitution of opsins in the present instance may therefore involve a small change in the spectrum of the retinene$_2$–opsin complex.

We have also, as shown in Fig. 2, arbitrarily set the maximum extinction of the mixture of retinene$_2$ and opsin equal to that of cyanopsin. We believe this to be approximately correct. Relatively pure solutions of rhodopsin bleach at neutrality to yield an almost equal extinction of retinene$_1$ + opsin (*6*). Here we assume the same to be true of cyanopsin. Any large departure from this assumption in either direction leads to an absurd result.

Neither of these reservations involves the spectrum above 560 mμ, since here the absorption of retinene$_2$ is so small that the absorption spectrum of cyanopsin is nearly identical with its difference spectrum. Only the dashed portion of the curve is in any doubt; the solid portion is unequivocal. Within these limitations, the spectra of Fig. 2 can be taken to represent reliably cyanopsin and the product of its bleaching in neutral solution.

Has cyanopsin a place in vision? Always heretofore our chemical knowledge of a visual pigment has developed in the sequence: extraction, analysis, synthesis. With cyanopsin this process is reversed. Retinene$_2$ and cone opsin were available to synthesize a pigment which has not yet been extracted from a retina.

One might, however, expect to find cyanopsin in a retina which possesses both vitamin A$_2$—and hence retinene$_2$—and cones. There is good evidence that it occurs in such situations.

Some years ago Granit measured electrophysiologically the spectral sensitivity of photopic vision in a freshwater fish, the tench (*Tinca*) (*7*); and in the European tortoise, *Testudo graeca* (*8*). In both cases the spectral sensitivity curves were maximal at about 620 mμ. These data are shown as the points of Fig. 3. It should be noted that they represent, in Granit's terminology, "dominators," not "modulators." It is clear that they resemble closely the absorption spectrum of cyanopsin.

In the tench this result is to be expected. As in other freshwater fishes, the rods of this animal contain porphyropsin—hence vitamin A$_2$ (*9, 10*). In the dark-adapted fish the spectral sensitivity is maximal at about 530 mμ (*7*), and is based upon porphyropsin. On light adaptation, the spectral sensitivity shifts toward the red—the familiar Purkinje shift—to the function shown in Fig. 3, based evidently upon cyanopsin.[3]

The retina of the tortoise possesses only cones. In this animal there is no Purkinje shift; the sensitivity remains maximal at about 620 mμ in all states of light or dark adaptation (*8*). Again it appears to be based upon cyanopsin.

We have no chemical data from the tortoise. Some years ago, however, we attempted several times to extract light-sensitive pigments from the all-cone retinas of two American turtles, *Pseudemys scripta* and *P. mobilensis*. No photosensitive pigment was obtained; but in these retinas we found considerable amounts of vitamin A$_2$. This was identified by its absorption maximum in ethyl alcohol at 355 mμ, and by the blue product which it yields with antimony chloride, with an absorption maximum at 693 mμ (Fig. 4).

Vitamin A$_2$ is found characteristically in the retinas of vertebrates which live, or at least begin their lives, in fresh water (*11, 12*). Turtles, whatever their later habitat, originate on land, and constitute a clear exception to this rule. They are not the first exception, for it had been found earlier that the marine fishes of the family Labridae, or wrasses, also possess vitamin A$_2$ and porphyropsin in their retinas.

It seems probable now that in the turtle and tortoise, as in the tench, the pigment of cone vision is

[3] Grundfest (*J. Gen. Physiol.*, **15**, 307, 507 [1931–32]) made the first quantitative measurements of the Purkinje phenomenon in a freshwater fish, the sunfish *Lepomis*, using a behavioral method. His spectral sensitivity curves are somewhat distorted in shape. The maximum sensitivity was found at about 540 mμ in the dark adapted fish, and shifted to about 600 mμ on light adaptation.

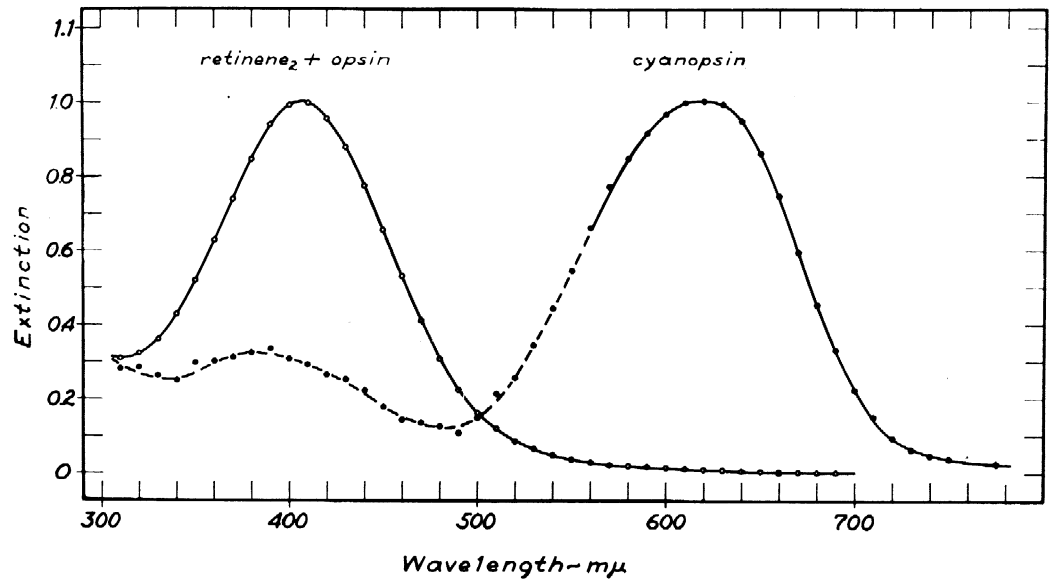

FIG. 2. The absorption spectrum of cyanopsin. On the left is the absorption spectrum of a mixture of crystalline all-trans retinene$_2$ and cattle rod opsin, comparable with the mixture of all-trans retinene$_2$ and chicken cone opsin which results from the bleaching of cyanopsin. On the right is the spectrum of cyanopsin obtained by adding the left-hand curve to the difference spectrum of Fig. 1. Both spectra have been made equal to 1.0 at the maximum. The solid portion of the right-hand curve represents accurately the absorption spectrum of pure cyanopsin, for in this region the absorption spectrum does not depart appreciably from the difference spectrum. The dashed portion of the curve is in some measure of doubt, owing to arbitrary elements in its derivation, discussed in the text.

cyanopsin. Our failure to extract it from the turtle retina in the past must be ascribed in part to our practice of making all retinal preparations in red light.

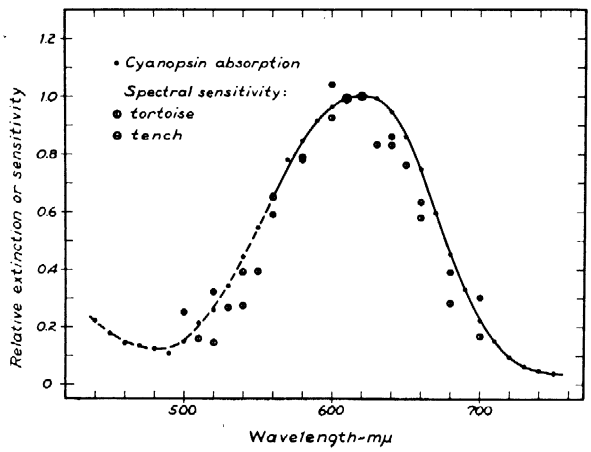

FIG. 3. The absorption spectrum of cyanopsin, compared with Granit's electrophysiological measurements of the spectral sensitivity of cone vision in a freshwater fish, the tench, and in the European tortoise, *Testudo graeca*.

This is proper for the other visual pigments, but the worst possible light for cyanopsin. Judging from Fig. 2, cyanopsin should be least sensitive in the blue-green, and this is the light in which one should attempt to extract it. If this for any reason proves too difficult, it would be nearly as satisfactory to demonstrate in a retina the presence of both vitamin A_2 and cone opsin, the ingredients from which cyanopsin is synthesized. Such experiments are now in progress.

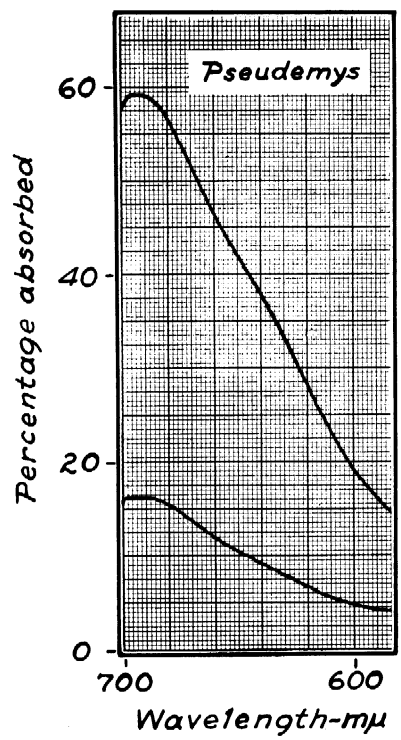

FIG. 4. Absorption spectra of antimony chloride tests with extracts from the retina of the turtle. *Pseudemys mobilensis*, drawn with a recording photoelectric spectrophotometer. Both spectra show the absorption maximum at about 693 mμ characteristic of vitamin A_2.

The synthesis of a visual pigment demands not merely retinene$_1$ or retinene$_2$, but a specific cis iso-

mer of each of these substances. The cis isomer of retinene$_1$ called neoretinene *b* is the precursor of rhodopsin (λ_{max} 500 mμ) and iodopsin (λ_{max} 562 mμ). A second cis isomer of retinene$_1$, called iso-retinene *a*, forms the isomeric light-sensitive pigments, iso-rhodopsin (λ_{max} 487 mμ) and iso-iodopsin (λ_{max} 515 mμ), the absorption spectra of which are displaced toward shorter wavelengths (*2, 3, 13*). In the same way a specific cis isomer of retinene$_2$ forms porphyropsin (λ_{max} 522 mμ), while a second cis isomer forms iso-porphyropsin (λ_{max} 507 mμ) (*3*). With cone opsin these retinene$_2$ isomers yield a parallel result: the first forms cyanopsin (λ_{max} 620 mμ), the second iso-cyanopsin (λ_{max} 575 mμ). None of the iso-pigments has yet been identified in a retina, and for the present all of them must be regarded as artifacts.

As originally iodopsin, so now cyanopsin raises in chemical form the problem of color vision (*1, 14*). There is as yet no evidence that either pigment takes part in color differentiation in any retina in which it has been encountered. Yet each addition to the array of visual pigments, particularly in cones, seems to bring this function closer. Cyanopsin extends the spectral range of the known visual pigments far into the red; it is the first such pigment that could serve efficiently in a "red receptor." Indeed rhodopsin, iodopsin, and cyanopsin form a trio of visual pigments regularly spaced about 60 mμ apart throughout the visible spectrum, and well suited in spectral sensitivity to provide the basis of a system of trichromatic vision.[4, 5] Iodopsin alone may suffice for color vision in the chicken, and cyanopsin in the turtle, since the cones of both animals contain systems of colored oil globules which could serve for color discrimination (*19, 20*). In the absence of such special devices, however, color vision requires the presence of at least two photosensitive pigments in the cones of a single animal. This condition is probably fulfilled in certain euryhaline fishes—salmon, trout, eel—in lampreys, and in certain amphibia which are known to contain both rhodopsin and porphyropsin in their rods, and very likely therefore contain both iodopsin and cyanopsin in their cones (*11, 12*); yet again there is no evidence that this has anything to do with color vision. The chemistry of color vision all lies before us, and the growing accumulation of visual pigments and iso-pigments does little more as yet than offer increasing promise of eventually approaching it.

[4] The slow rate of synthesis of rhodopsin compared with cone pigments in mammals, chickens, and frogs is no barrier to its finding a place eventually in cone vision. We have recently observed that retinene$_1$ and retinene$_2$ combine nearly as rapidly with the rod opsins of certain fishes, both freshwater and marine, to form rhodopsin and porphyropsin, as with the cone opsin of the chicken to form iodopsin and cyanopsin.

[5] A number of recent investigations of the spectral sensitivity of human cone vision indicate a small subsidiary peak near 620 mμ, which is thought to mark the position of the red color vision primary (*15–18*). This could involve cyanopsin itself, though the sensitivity peak seems narrow compared with the absorption spectrum of cyanopsin.

Summary

A new visual pigment has been synthesized by the combination of chicken cone opsin with retinene$_2$. It is a blue, light-sensitive substance with maximum absorption at 620 mμ, called *cyanopsin*. It has not yet been extracted from a retina, but would be expected to occur in any retina which contains vitamin A$_2$ or retinene$_2$ and cone opsin. Freshwater fish retinas possess these ingredients; and Granit has shown the spectral sensitivity of cone vision in a freshwater fish, the tench, to be maximal near 620 mμ, resembling closely in form and position the absorption spectrum of cyanopsin. Granit has found the same type of spectral sensitivity function also in the all-cone retina of the tortoise *Testudo*. This is in good accord with our observation that the all-cone retinas of two American turtles contain vitamin A$_2$. It is concluded that in these animals cyanopsin is a pigment of cone vision. To make porphyropsin and cyanopsin demands a specific cis isomer of retinene$_2$. A second cis isomer forms the isomeric iso-porphyropsin and iso-cyanopsin. The absorption maximum of iso-cyanopsin is at 575 mμ. Cyanopsin extends the range of visual pigments far into the red, and may in some animals play a part in the mechanism of color vision.

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