

THE METAMORPHOSIS OF VISUAL SYSTEMS IN THE SEA LAMPREY

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The lampreys are members of the most primitive living group of vertebrates, the cyclostomes, last survivors of the ancient class Agnatha, the jawless vertebrates. Having evolved in directions peculiarly their own, they have also preserved many primitive characters which other vertebrates have lost. This is as true biochemically as it is anatomically (*cf.* Florkin, 1949). For example, lamprey hemoglobin is unique among vertebrates, possessing properties found otherwise only among invertebrates (*cf.* Wald and Riggs, 1951-52).

Some years ago I examined the visual system of the sea lamprey, *Petromyzon marinus* (Wald, 1941-42 *b*). The retinas were found to contain both vitamins A₂ and A₁ in the approximate proportion 9:1. The pigment epithelium and choroid layers of the eye also contained a predominance of vitamin A₂, about 4 times as much as A₁. The livers, however, contained vitamin A₁ alone. I made no attempt at that time to extract visual pigment from the retina; but on the strength of the great preponderance of vitamin A₂, and Walls's report (1935) that dark-adapted retinas of this species are "truly purple" in color, I concluded that this retina contains porphyropsin. This lent some support to the view that porphyropsin rather than rhodopsin may have been the primitive vertebrate visual pigment.

Recently Crescitelli (1955-56) has extracted the visual pigment from retinas of *P. marinus*, and found it to be rhodopsin. This occurrence of rhodopsin in a vertebrate which spawns in fresh water—indeed, Crescitelli's animals had never left fresh water—seems at variance with the observation that such animals in general possess predominantly porphyropsin (Wald, 1945-46; 1952). Crescitelli noted also that the occurrence of rhodopsin in the lamprey favors the view that rhodopsin rather than porphyropsin was the primitive vertebrate visual pigment.

On reexamination it has come out that both Crescitelli's observations and

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mine were correct. The examples of the sea lamprey with which Crescitelli worked were newly metamorphosed animals, on their journey downstream which on the sea coast would have led them to the ocean, and in the case of his animals would have led them into Lake Huron. The animals with which I had worked were sexually mature adults, on their migration upstream from the ocean. The downstream migrants have, as Crescitelli found, the rhodopsin system alone. The mature upstream migrants, however, possess the porphyropsin system virtually alone. The sea lamprey exhibits a biochemical metamorphosis of visual systems, comparable with that previously observed among amphibia (Wald, 1945-46; 1952). The significance of this metamorphosis in terms of the lamprey's life cycle and phylogeny will be discussed below.

Material

Adult, sexually mature lampreys were taken from the Oyster River in Durham, New Hampshire, on the night of May 22, 1956.¹ These animals had collected below a dam on their spawning migration upstream from the ocean. Twenty-four of them were brought alive to a laboratory at the University of New Hampshire, and the eyes were taken out at once under red light. The retinas (46 in all) were dissected out and frozen immediately on dry ice. The remaining eye tissues—sclera, choroid, and pigment epithelium—were frozen separately. The dissection had been performed in frog-Ringer solution, and this also was saved.

One of the retinas, looked at in white light, appeared almost colorless. It has been noted earlier that the retina of the adult sea lamprey contains very little visual pigment (Wald, 1941-42). With the idea that perhaps considerable numbers of outer segments might have broken off the retina into the Ringer solution, or might have remained embedded in the pigment epithelium, we saved all these materials for extraction.

Back at the Harvard Laboratory, the retinas were ground by mortar and pestle in 40 per cent sucrose dissolved in phosphate buffer, pH 7. The suspension, in a small plastic centrifuge tube, was layered over with phosphate buffer, and centrifuged for 10 minutes at 40,000 R.P.M. in the Spinco preparative ultracentrifuge. On such treatment, the rod outer segments, in large part fragmented, collect as a thin layer in the interface between the sucrose solution and buffer. The entire supernatant, containing the suspended rods, was poured off, and the sediment was reground in 40 per cent sucrose. In all, three such flotations were performed. On pouring off the supernatants, the sucrose was diluted by mixing with buffer solution. As a result, on recentrifuging the mixed supernatants, all the rod outer segments sedimented. They were washed once in distilled water, and then were left to harden in 4 per cent alum solution for 35 minutes. They were collected by centrifuging, washed again in distilled water, and in phosphate buffer of pH 6.9, and finally were stirred into 0.55 ml. of 2 per cent digitonin solution and left for $1\frac{3}{4}$ hours in the refrigerator

¹ I am grateful to Professors Paul A. Holle and Edwin Scheier of the University of New Hampshire for help with procuring and handling these animals; and to my coworkers, Ruth Hubbard, Norman Krinsky, and Timothy Goldsmith, with whose help this expedition was brought to a successful outcome.

to extract the visual pigment. This suspension was centrifuged for 20 minutes at 25,000 R.P.M., and the clear extract of visual pigment poured off.

The Ringer washings were centrifuged, and the sedimented material mixed with the remaining eye tissues. This material was ground thoroughly, and treated exactly as described above.

We then had to wait for 7 months, until Dr. Vernon C. Applegate, who had obtained animals also for Crescitelli, could send us downstream migrants.² Fourteen such animals were sent us from Rogers City, Michigan, on the shore of Lake Huron, by Air Express on December 5, 1956. They were dark-adapted overnight in running tap water and the eyes removed under red light. After cutting away the cornea and lens, the whole fundus of each eye was frozen. We also dissected out and froze the livers of all these animals. The whole fundi of twenty-seven eyes were ground with mortar and pestle and suspended in 40 per cent sucrose. They were treated exactly as has already been described, the rod outer segments being floated out in three successive grindings and centrifugings, washed with distilled water, hardened in alum solution, washed again with distilled water and neutral phosphate buffer, and finally stirred into 0.5 ml. of 2 per cent digitonin solution to extract in the cold for 2 hours.

Observations

The absorption spectrum of the visual pigment of the downstream migrants is shown in curve *a* of Fig. 1. It is a typical rhodopsin spectrum, displaying an α -band with λ_{\max} 500 $m\mu$, and an unusually prominent β -band with λ_{\max} about 340 $m\mu$. Between these maxima lies a typical minimum at 400 $m\mu$.

This pigment was bleached by exposure to the concentrated light of a 160 watt microscope lamp passing through a yellow Corning 3482 filter, which transmits only wavelengths longer than 555 $m\mu$, and so fails to isomerize the retinene which is formed. The first such exposure, for 3 minutes, yielded curve *b*. This was followed by a second, 5 minute exposure, which yielded curve *c*. Curve *c* possesses λ_{\max} about 373 $m\mu$, typical of the bleached all-*trans* retinene₁-protein complex at the pH at which these measurements were made (7.5); and a slightly higher maximal extinction than rhodopsin itself, also typical at this pH. Apparently lamprey rhodopsin possesses very nearly the same molar extinction as cattle rhodopsin (*i.e.*, about 40,000 per retinene equivalent (Wald and Brown, 1953-54).

A small granule of potassium borohydride was stirred into this solution. The spectrum immediately afterward had changed to *d*. The borohydride had reduced the retinene formed by bleaching to vitamin A, with λ_{\max} about 328 $m\mu$, the correct position for all-*trans* vitamin A₁ in digitonin solution. There is no suggestion of the presence of vitamin A₂, which possesses an absorption maximum at about 355 $m\mu$.

To the solution which yielded curve *d*, methanol was added to a concentration of 60 per cent, and the mixture was extracted with petroleum ether. The petroleum ether extract was transferred to 0.5 ml. of chloroform. To this

² I am greatly indebted to Dr. Applegate, Director of the Hammond Bay Fishery Laboratory in Rogers City, Michigan, for providing these animals.

in an absorption cell in place in the spectrophotometer, 2 ml. of saturated antimony chloride solution in chloroform was added, and the absorption spectrum of the resulting blue product recorded at once. This is shown in Fig. 3. It exhibits the single absorption band maximal at about $618\text{ m}\mu$ typical of vitamin A_1 in this test.

These observations therefore confirm and extend those of Crescitelli. The downstream migrant sea lamprey possesses in its retina the rhodopsin system, based upon retinene₁ and vitamin A_1 alone.

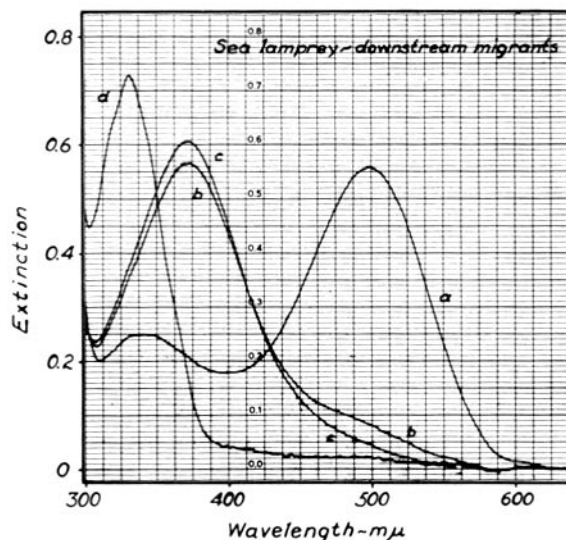


FIG. 1. The visual pigment of the downstream migrant sea lamprey, and the products of its bleaching. (a) Absorption spectrum of the visual pigment, a typical rhodopsin, with α -band at $500\text{ m}\mu$ and β -band at $340\text{ m}\mu$. After exposure for 3 minutes to a yellow (non-isomerizing) light, the spectrum has changed to (b); and following a second, 5 minute exposure to the same light, to (c). This is the spectrum of all-*trans* retinene with opsin. On stirring with potassium borohydride, the retinene is reduced to all-*trans* vitamin A (d). pH 7.5.

Comparable data from the mature upstream migrants are shown in Fig. 2. The visual pigment (curve a) has its absorption maximum at about $518\text{ m}\mu$. The porphyropsins of fresh water fishes lie characteristically at about $522 \pm 2\text{ m}\mu$. The visual pigment of the upstream migrant, therefore, lies just lower in wavelength than a typical porphyropsin, either because of a small intrinsic difference, or a very small admixture of rhodopsin (see below).³

³ We have obtained a porphyropsin with $\lambda_{\text{max}} 517\text{ m}\mu$ synthetically, by incubating the hindered *cis*-isomer neo-b retinene₂ with cattle opsin (Wald, Brown, and Brown, unpublished experiments).

This pigment was bleached by exposure to the concentrated light of a 160 watt microscope lamp passing through Corning filter 2412, which passes only wavelengths longer than about 610 $m\mu$. After 19 minutes' exposure, the spectrum had changed to curve *b*. The solution was exposed 2 minutes longer through a Jena OG 2 filter, which transmits only wavelengths longer than 550 $m\mu$. This yielded curve *c*. It possesses the maximal absorption at about 401 $m\mu$ characteristic of all-*trans* retinene₂ in digitonin solution at this pH (7.5).

A droplet of potassium borohydride solution was now stirred into this solu-

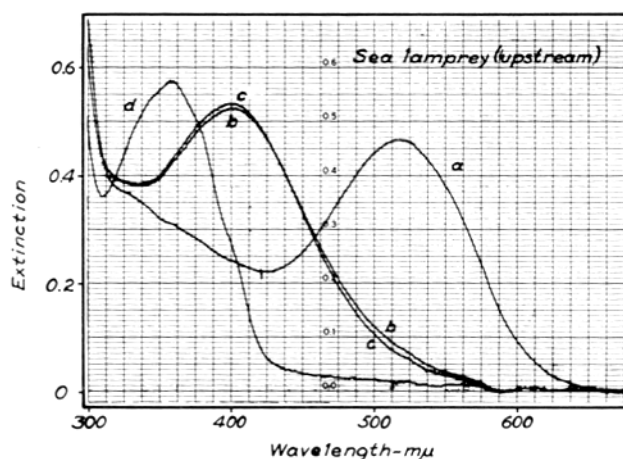


FIG. 2. The visual pigment of the upstream migrant sea lamprey, and the products of its bleaching. (a) Absorption spectrum of the visual pigment, a porphyropsin with λ_{\max} 518 $m\mu$. On exposure to orange light for 19 minutes, the spectrum has changed to (b); and after 2 minutes' further exposure to yellow light, to (c). This is the spectrum of all-*trans* retinene₂ and opsin. Stirring with potassium borohydride reduces the retinene₂ to vitamin A₂ (d). pH 7.5.

tion. The spectrum changed to curve *d*. This possesses a maximum at about 357 $m\mu$, characteristic of all-*trans* vitamin A₂ in digitonin solution.

The solution which yielded curve *d* was diluted with methyl alcohol to a concentration of 60 per cent, and extracted with petroleum ether. The petroleum ether extract was transferred to 0.5 ml. chloroform. To this in place in the spectrophotometer about 2 ml. of antimony chloride reagent was added, and the spectrum was recorded immediately. This is shown in Fig. 4. It exhibits predominantly the absorption maximum at about 690 $m\mu$ characteristic of vitamin A₂. There is in addition a small inflection in the neighborhood of 615 to 620 $m\mu$, caused by the presence of a small amount of vitamin A₁.

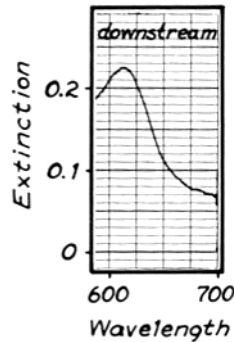


FIG. 3

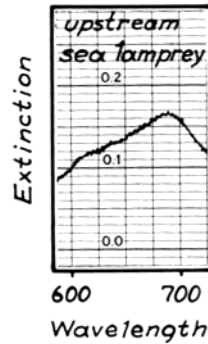


FIG. 4

FIG. 3. Antimony chloride test with an extract of the bleached visual pigment from downstream migrant sea lampreys (Fig. 1 *d*). The test displays only the absorption band maximal at $618\text{ m}\mu$ characteristic of vitamin A_1 .

FIG. 4. Antimony chloride test with an extract of the bleached visual pigment from upstream migrant sea lampreys (Fig. 2 *d*). The test shows the presence of vitamin A_2 ($\lambda_{\text{max}} 690\text{ m}\mu$), accompanied by a trace of vitamin A_1 ($\lambda_{\text{max}} 615\text{ m}\mu$).

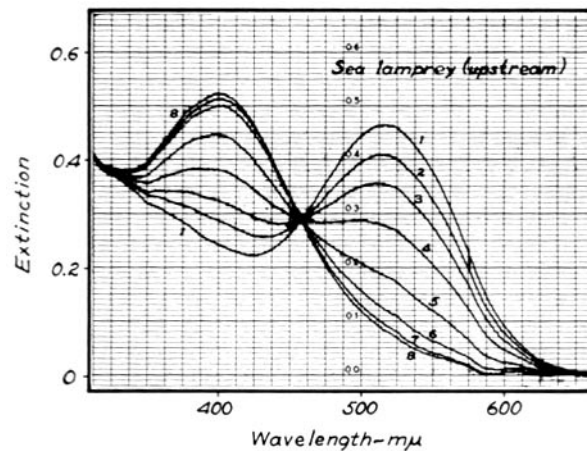


FIG. 5. Test for homogeneity of the visual pigment of upstream migrants. The pigment (1) was exposed repeatedly to a non-isomerizing orange light, containing only wavelengths longer than $610\text{ m}\mu$, and the spectrum recorded (2) after 15 seconds' exposure, (3) after a total of 30 seconds, (4) 1 minute, (5) 2 minutes, (6) 4 minutes, (7) 9 minutes, and (8) a total of 19 minutes' exposure. Such a deep orange light should have bleached porphyropsin much faster than rhodopsin, if both pigments were present. The sharp isosbestic point at $457\text{ m}\mu$ is good evidence that a single pigment (porphyropsin) is bleaching.

The proportions are much as we have found before in the retina of the sexually mature lamprey.

Do the upstream migrants possess porphyropsin alone, or mixed with a very small amount of rhodopsin? We attempted to test the visual pigment for homogeneity by bleaching it by stages in the orange light passing through a Corning 2412 filter. Since this transmits only wavelengths longer than about $610\text{ m}\mu$, it should bleach porphyropsin much more rapidly than rhodopsin.

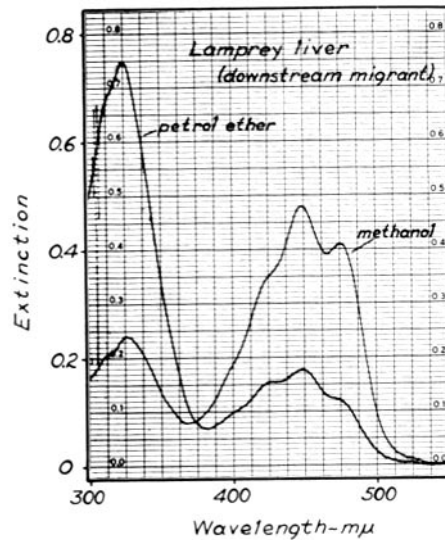


FIG. 6. Carotenoids of the liver of the downstream migrant sea lamprey. The absorption spectra show the presence of vitamin A₁ (λ_{max} $325\text{ m}\mu$) and a xanthophyll (λ_{max} 423 , 448 , and $476\text{ m}\mu$). The spectra show the fractions obtained by partitioning the extract between petroleum ether and 90 per cent methyl alcohol. The xanthophyll enters primarily the methanol layer, showing it to be mainly unesterified; whereas the vitamin A enters primarily the petroleum ether, showing it to be present mainly as esters.

One might expect, therefore, that the porphyropsin would bleach first, and that the spectra in the later stages of bleaching would shift more and more toward those typical of rhodopsin. This test is shown in Fig. 5. Its most striking result is that bleaching under these conditions exhibits every sign of complete homogeneity. The best evidence for this is the sharp common or isosbestic point at about $457\text{ m}\mu$, showing that one is dealing with the transformation of a single molecular species into another. From these data one would infer that the upstream migrants possess porphyropsin alone, with little or no admixture of rhodopsin.

It appears from our measurements that the concentration of visual pigment is considerably greater in the retinas of downstream than in upstream migrants. In both we find the extinction of visual pigment per eye nearly the same: 0.012 when the total visual pigment from one retina is dissolved in 1 ml., and measured in a 1 cm. layer. Since, however, the adult retina has about 4 times the area of that of a downstream migrant, the *concentration* of visual pigment in the adults is only about one-fourth of that in downstream migrants. Indeed I have not otherwise seen a retina well supplied with rods that contains so little visual pigment as the upstream migrant sea lamprey; and Kühne (1878) has much the same thing to say of the retinas of (presumably mature) *Lampetra fluviatilis*.

In my earlier paper (1941-42) I described observations on the liver vitamin A in the larval lamprey and in the migrating adult. In both cases only vitamin A₁ was found. Crescitelli (1955-56) also has shown the antimony chloride test of an extract of the livers of downstream migrants, which again is characteristic of vitamin A₁. Apparently the liver of the sea lamprey contains vitamin A₁ alone throughout its life history.

Fig. 6 shows direct absorption spectra of an extract of the livers of downstream migrants. About 1 gm., fresh weight, of livers taken from 14 animals was ground with anhydrous sodium sulfate to a dry powder, and was extracted by gently stirring into several portions of petroleum ether. The extract was deep yellow in color. It contained vitamin A₁ (λ_{\max} 325 m μ) and a xanthophyll or hydroxy-carotenoid with λ_{\max} (423), 448, and 476 m μ (leaf xanthophyll: 420, 448, 478 m μ in petroleum ether).

Fig. 6 shows the result of partitioning the whole extract between petroleum ether and 90 per cent methanol. The vitamin A enters primarily the petroleum ether layer (epiphasic), showing it to exist in the liver primarily as esters; whereas the xanthophyll enters primarily the methanol layer (hypophasic), showing it to be present mainly as the free alcohol. Lamprey xanthophyll is more strongly hypophasic than leaf xanthophyll, and has not yet been finally identified. One gm. of fresh liver contains about 80 μ g. of xanthophyll and about 125 μ g. of vitamin A.

DISCUSSION

In the course of its life cycle the sea lamprey undergoes two metamorphoses. It begins as an ammocoete larva, living buried in the sand or mud of its natal stream; and blind, its rudimentary eyes buried in the tissues of the head. After 4 to 5 years in the larval condition, it metamorphoses. This involves a fundamental anatomical reconstruction, which prepares the animal for a predatory life in which it is parasitic upon fish, and which includes the appearance of well formed eyes. This metamorphosis is completed with the animal still living as did the larva, though no longer feeding. Then the lamprey mi-

grates downstream to the ocean or a lake for the growth phase, which lasts $1\frac{1}{2}$ to $3\frac{1}{2}$ years.

At the end of this phase the sea lamprey undergoes a second metamorphosis to the sexually mature adult. This is not usually spoken of as a metamorphosis, yet it has that essential character. In the growth phase the sexes can hardly be told apart without a dissection; even the gonads look alike. Now the sexes differentiate visibly. The gonads mature, and special external structures are formed for depositing eggs and sperm. The males develop a ropelike ridge along the back. At this time also either sex or both may assume golden mating tints. I have noticed that the livers of the mature animals are bright green, though those of larvae and downstream migrants are the usual reddish brown. These changes are completed in the environment of the growth phase, whether lake or sea. Then the animals migrate upstream to spawn. They have stopped feeding, and indeed may be unable to digest and absorb food. They have before them only the act of reproduction; shortly afterward both sexes die.⁴

The lampreys with which we have been concerned were examined on their migrations downstream and upstream, having shortly before completed the associated metamorphoses. It is clear that the alterations which constitute metamorphosis include profound changes in the chemistry of visual excitation.⁵

Rhodopsin and the use of vitamin A₁ in vision are characteristic of marine and land vertebrates, whereas porphyropsin and vitamin A₂ are characteristic of fresh water forms (Wald, 1938-39; 1945-46; 1952). The downstream migrant lamprey, which under ordinary conditions is about to enter the ocean, appears to anticipate this change biochemically by assuming, while still in fresh water, the marine type of visual system. Conversely the upstream migrants, even toward the beginning of their migration, already have the fresh water form of visual system. That is, the biochemical changes in the eye anticipate the migrations. In this they mimic the anatomical changes of metamorphosis. Indeed with regard to more adaptive aspects of metamorphosis, such anticipation of the environment to come is essential, for the animal cannot move to the new environment until metamorphosis has made it ready.

How do these observations reflect upon the phylogeny of vertebrate vision? There is reasonable support for the view that the vertebrate stock originated in fresh water (Smith, 1932; Romer and Grove, 1935). Coupled with the observation that porphyropsin is characteristically associated in vertebrates with fresh water existence—even with temporary phases of fresh water ex-

⁴ For information concerning the life history and metamorphoses of the lamprey I am indebted principally to the work of Gage (1927) and Young (1950).

⁵ I have tacitly assumed in this discussion that the lamprey retains the rhodopsin of the downstream migrants throughout the growth phase, changing to porphyropsin only as it approaches sexual maturity. The precise time of this change is still to be determined.

istence—this site of origin raises the possibility that porphyropsin rather than rhodopsin was the primitive pigment of vertebrate vision. The discovery of porphyropsin in lampreys some years ago seemed to support this possibility. The present observation that in the sea lamprey the visual system metamorphoses between rhodopsin and porphyropsin does not alter this position materially. The significant point is that in these most primitive of living vertebrates porphyropsin occupies much the same position as in fishes and amphibia. As yet it has not been found at all among higher vertebrates.

I have discussed elsewhere biological parallelisms between euryhaline fishes and amphibia (1945-46; 1952). Euryhaline fishes occupy much the same position between fresh water and marine fishes that amphibia occupy between fresh water fishes and land vertebrates. Metamorphoses and migrations are characteristic of both groups. All the euryhaline fishes I have examined were in their growth phase, in middle life. All of them possessed either mixtures of the rhodopsin and porphyropsin systems, in which the type associated with the spawning environment predominated, or that type alone. On the other hand, amphibia, examined at various phases in their life cycles, exhibit distinct metamorphoses of visual systems. Thus the bullfrog tadpole enters metamorphosis with the porphyropsin system, and emerges from it with the rhodopsin system. This is the type of performance we find in the sea lamprey. It represents a distinct variation from the relatively stable patterns found in euryhaline fishes, while still preserving the general association of porphyropsin with fresh-water life, and rhodopsin with marine and terrestrial habit.⁶

The change from porphyropsin to rhodopsin in the metamorphosis of the bullfrog has the appearance of a biochemical recapitulation. It seems to recall in the act of metamorphosis the change from porphyropsin in an ancestral fresh water fish to the rhodopsin of land-living forms. How then interpret the reverse change from rhodopsin to porphyropsin in the sea lamprey? Does it imply that rhodopsin preceded porphyropsin in lamprey evolution?

I think the answer to this question lies in the recognition that this change in

⁶ As the visual system of the bullfrog changes from porphyropsin to rhodopsin when it metamorphoses from a tadpole, its hemoglobin also changes profoundly (McCutcheon, 1936; Riggs, 1951-52). It seemed worthwhile to look for comparable changes in the lamprey. The properties of lamprey hemoglobin had already been examined in upstream migrants (Wald and Riggs, 1951-52). Dr. Riggs has now also examined the hemoglobin of downstream migrants, obtained through the kindness of Dr. Vernon Applegate from the same source and at the same time as our animals (personal communication). He has found no differences from our earlier measurements in the shape of the oxygen equilibrium curve, the affinity for oxygen, or the change in affinity with pH (the Bohr effect). Apparently the downstream and upstream migrants possess the same hemoglobin. This leaves open the possibility that the larvae have a different hemoglobin; *i.e.*, that there may be a change in hemoglobin associated with the primary metamorphosis.

the sea lamprey is part of the *second metamorphosis*. I have found precisely the same phenomenon in the New England spotted newt, *Triturus viridescens* (Wald, 1946; 1952). Following a larval period in fresh water, this animal undergoes a first metamorphosis to the terrestrial red eft. Later it undergoes a second metamorphosis to the sexually mature adult, which returns to fresh water to spawn. I found that the red eft contains predominantly rhodopsin, the water phase adults predominantly porphyropsin. The *second metamorphosis* involves the transfer from rhodopsin to porphyropsin, just as in the sea lamprey.

The second metamorphosis in an amphibian or migratory fish or lamprey is not to be viewed as the recapitulation of some earlier evolutionary transition, but as the adaptation to a life cycle that involves two environments. To complete the cycle it is necessary to bring the adult back into the original environment to spawn; and this in some instances involves changes which in part reverse those of the first metamorphosis.

It should be interesting now to explore the visual systems of other types of lamprey. All lampreys begin their lives in fresh water; some species never leave this environment. Kühne (1878) reported finding the retinas of *Lampetra fluviatilis*, an anadromous form like *P. marinus*, very feebly purple in color, inclining in hue toward violet or bluish. Presumably this was a sexually mature adult, and apparently its retina contained porphyropsin. Crescitelli cites Lovern *et al.* (1939) as having observed that the eyes of *Lampetra fluviatilis* contain a great preponderance of vitamin A₁ over A₂. These animals were apparently sexually mature adults of average weight 36.6 gm. The authors, however, state only that in the antimony chloride test with an extract of the eyes they could measure a very small extinction (0.015) at 617 m μ and none at 693 m μ . They make no mention of observing a specific absorption band, and lacking this one cannot be sure that any vitamin A was present in the extracts.⁷

On the other hand, Crescitelli reports measurements with a single extract of five sexually mature Pacific coast lampreys (*Entosphenus tridentatus*, an anadromous form) which appeared to contain rhodopsin. In this, *Entosphenus* may be like the bullfrog, which goes over to rhodopsin at its first metamorphosis, and so far as we know retains it thereafter. That is, *Petromyzon marinus* and *Entosphenus tridentatus* among lampreys appear to exhibit the same relations of visual system to life cycle as do the spotted newt and bullfrog among amphibia.⁸

⁷ Steven (1950) has measured the spectral sensitivity of the photoreceptors in the tail of the ammocoete larva of the brook lamprey, *Lampetra planeri*, a permanently fresh water form. He found the sensitivity to be maximal at 520 to 530 m μ , and suggests that this may be associated with the presence of porphyropsin. He has measured also the light responses of another cyclostome, the marine hag, *Myxine glutinosa*, a blind animal whose skin photoreceptors appear to be maximally sensitive at 500 to 520 m μ (1955).

⁸ Still another variable may enter this situation. Lamprey retinas possess two types

In discussing his discovery of rhodopsin in the sea lamprey, Crescitelli remarks that it would be logical to suppose this to be the primitive vertebrate visual pigment, since all the invertebrate eyes so far examined contain components of the rhodopsin system (vitamin A₁, retinene₁; Wald, 1941; 1945-46). Hence porphyropsin as the primitive vertebrate pigment would represent a gratuitous discontinuity in the otherwise smooth transition from invertebrate to vertebrate rhodopsins.

This argument involves a common confusion of contemporary animals with ancestral forms, which I once shared, and have discussed elsewhere (Wald, 1952). Vertebrate eyes were not derived from invertebrate eyes. The only invertebrate phyla which have developed proper image-forming eyes—the Arthropods and Molluscs—were probably not in the line of vertebrate ancestry, nor are their eyes in any way homologous with vertebrate eyes. Each of these phyla developed eyes in complete independence. The Echinoderms, from which we suppose the vertebrates to have sprung, have never developed eyes. No discontinuity is implied therefore in the notion that when vertebrates did develop eyes, they began with porphyropsin.

The alternative view is that, having started with rhodopsin, the vertebrates developed porphyropsin secondarily, in close genetic association with fresh water habit, and particularly with the habit of spawning in fresh water. This was my original thought (1938-39). I think the other view—that porphyropsin was the primitive vertebrate pigment—accords better with the belief that vertebrates originated in fresh water, and with the appearance of porphyropsin among the lampreys.

In any case it is now clear that lampreys toy with the distribution of rhodopsin and porphyropsin in close association with environmental habit, much as do the fishes and amphibia. I say "toy" because it is difficult to attach any special advantage to the use of either pigment in any of the environments in question. The genetic characters which decide the environment seem also to determine the choice of visual system as a gratuitous by-product. Why, particularly if this distribution is so trivial, it follows the same complex patterns in cyclostomes, fishes, and amphibia, is an extraordinarily interesting problem. It may possibly mean that an identical hereditary mechanism—perhaps an identical array of genes—is at work throughout this great span of organisms.

of visual cell, long and short, which have variously been described as rods or cones. Walls (1935) defends with his usual clarity and passion the view that the short cells are rods, the long ones cones. Among a wide sample of lampreys, he found *Entosphenus tridentatus* to have the highest proportion of short to long cells (8:1), whereas in *P. marinus* this ratio was 3:1, and in the *Lampetras* about 1:1. It should be interesting to know whether these ratios change during the life cycle.

SUMMARY

The life cycle of the sea lamprey, *Petromyzon marinus*, includes two metamorphoses. At the end of a period spent as a blind larva, buried in the mud of streams, a first metamorphosis prepares it to migrate downstream to the sea or a lake for its growth phase. Then, following a second metamorphosis, it migrates upstream as a sexually mature adult to spawn and die.

The downstream migrants have a visual system based upon rhodopsin and vitamin A₁, whereas that of the upstream migrants is based upon porphyropsin and vitamin A₂. The livers contain vitamin A₁ at all stages.

The sea lamprey therefore exhibits a metamorphosis of visual systems, like those observed earlier among amphibia. The presence of porphyropsin in this member of the most primitive living group of vertebrates, as in fishes and amphibia, supports the notion that porphyropsin may have been the primitive vertebrate visual pigment. Its association with fresh water existence throughout this range of organisms also is consistent with the view that the vertebrate stock originated in fresh water. The observation that in the life cycle of the lamprey rhodopsin precedes porphyropsin is not at variance with the idea that porphyropsin is the more primitive pigment, since this change is part of the *second metamorphosis*, marking the return to the original environment. The observation that in lampreys, fishes, and amphibia, porphyropsin maintains the same general association with fresh water, and rhodopsin with marine and terrestrial habit, suggests that a single genetic mechanism may govern this association throughout this wide span of organisms.

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