THE VISUAL SYSTEM AND VITAMINS A OF THE SEA LAMPREY

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What is known at present of the distribution of visual systems suggests that the rhodopsin-vitamin A_1 cycle is the primitive vertebrate type. The marine elasmobranchs, all marine teleosts except the Labridae, and all terrestrial vertebrates so far examined possess this system alone (Wald, 1938–39). The eyes of squid and of crabs also contain exclusively components of this system (Wald, 1941). The porphyropsin-vitamin A_2 cycle seems to be a comparatively recent development, associated with the evolutionary migration of teleost fishes into fresh water (Wald, 1938–39; 1941–42).

This appraisal of the situation can be tested critically in the cyclostomes, for it implies that these animals, though all freshwater in origin, possess only the rhodopsin-vitamin A_1 system. The cyclostomes—with the closely related myxinoids—are the most primitive living vertebrates, surviving members of the ancient class or subphylum Agnatha, believed to have pursued an independent evolution since earliest vertebrate origins. All of them are fresh-water or anadromous, but this property does not distinguish them from the supposedly freshwater vertebrate ancestors (Smith, 1932; Romer and Grove, 1935). If it is true that the porphyropsin-vitamin A_2 cycle is a specifically teleost innovation, the visual systems of cyclostomes should contrast sharply with those of freshwater and anadromous bony fishes.

In the present experiments this matter is examined in the sea lamprey, *Petromyzon marinus*, an anadromous cyclostome. The life history of this animal has been described in detail by Gage (1927). It spawns in freshwater streams in the late Spring, and dies shortly thereafter. The so called ammocete larvae are blind, and differ profoundly in structure from the adults. They spend 4–5 years in the sand or mud bottoms of the parent stream, feeding upon microorganisms. At a final larval length of 5–7½ inches they metamorphose to the adult form and usually migrate to the sea. Here they appear to feed primarily upon the blood of fishes to which they attach by suction through their disc-shaped mouths. The marine phase lasts 1½–3½ years, and is terminated by the return to fresh water to spawn. Like anadromous teleosts, sea lampreys can spend their entire life cycle in fresh water without detriment.

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The lampreys used in the present experiments were sexually mature animals, about 2 feet in length, taken from brackish tidal waters of the Exeter River in New Hampshire during the spawning run. The tissues were dissected out on the river bank and transported in dry ice to the laboratory. Through the kindness of Dr. E. J. W. Barrington I have been able also to examine the livers of late larvae of land-locked *P. marinus* taken from streams about Lake Oneida in New York State.

Eye Tissues

The retinas dissected from highly light adapted animals in broad daylight were completely colorless. Under similar circumstances fish and amphibian retinas usually adhere firmly to the underlying pigment epithelium. The lamprey retina, however, readily comes away entirely free of pigmented tissue. This peculiarity is probably associated with the reported absence of pigment migration and photomechanical changes in *P. marinus* (Walls, 1935).

Batches of 40–60 retinas were extracted by shaking exhaustively with chloroform. The extract was saponified in 6 per cent KOH in methanol under a stream of nitrogen. The non-saponifiable fraction was taken up in benzine, transferred to 0.3 cc. of chloroform, and tested by mixing with 3.2 cc. of antimony chloride reagent. A spectrum of the resultant blue test solution is shown in Fig. 1.[1]

Such spectra are dominated by the high band at 692–696 mμ which characterizes vitamin A_2. A broad inflection at 620–640 mμ includes the 645 mμ "hump" which always accompanies the vitamin A_2 band, and a low absorption due to vitamin A_1. The ratio of the vitamin A_2 absorption at 696 mμ to that of vitamin A_1 at 618 mμ, computed as described elsewhere, is 89:11 (*cf.* Wald, 1938–39).

The remaining tissues of the fundus, including the pigment epithelium, choroid and sclera, also contain a great predominance of vitamin A_2. The antimony chloride reaction with an extract of these tissues, prepared exactly like that of the retinas, is shown in Fig. 2. The ratio of vitamin A_2 to A_1 absorption in this preparation is 82:18. In another experiment it was 77:23. The amount of vitamins A in these tissues is about twice that in the retina.

The lamprey retina contains, therefore, not the vitamin A_1 expected by virtue of its primitive and isolated phylogenetic position, but predominantly vitamin A_2 like an anadromous teleost. The force of this comparison was emphasized by a curious circumstance. Our lampreys were accompanied

[1] All spectra shown in this paper are original recordings drawn with Hardy's photoelectric spectrophotometer at the Massachusetts Institute of Technology. The results are expressed as per cent absorption, $1 - I/I_0$, in which I_0 is the incident and I the transmitted intensity. Before they can be used to compute relative concentrations they must be converted to extinction or optical density, $\log I_0/I$.

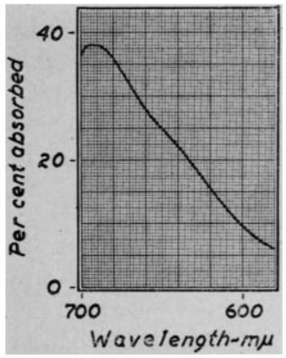

FIG. 1

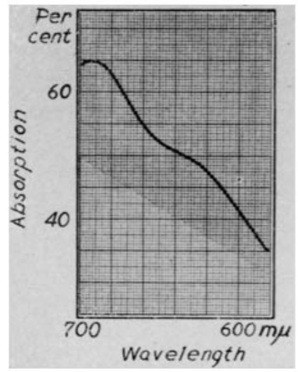

FIG. 2

FIG. 1. Spectrum of the antimony chloride reaction with an extract of lamprey retinas. A high band at 692 mμ due to vitamin A_2 and a low absorption in the region of 618 mμ due to vitamin A_1 are present in the proportion 89:11. The total non-saponifiable oil from 40 retinas, dissolved in 0.3 cc. chloroform, had been mixed with 3.2 cc. of antimony chloride reagent and the spectrum measured in a layer 1 cm. in depth.

FIG. 2. Spectrum of the antimony chloride reaction with an extract of lamprey pigment epithelia, choroids and sclera, prepared as in Fig. 1. Both vitamin A_2 and A_1 absorptions are present in the ratio 82:18.

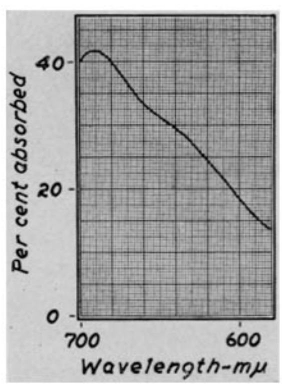

FIG. 3

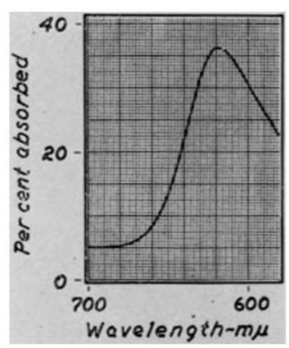

FIG. 4

FIG. 3. Spectrum of the antimony chloride reaction with an extract of retinas and pigment epithelia from the alewife, prepared as in Fig. 1. Only the band at 693 mμ due to vitamin A_2 appears in this spectrum. These alewives, anadromous teleosts, accompanied the lampreys used in the present experiments in their spawning migration.

FIG. 4. Spectrum of the antimony chloride reaction with a chloroform extract of the livers of late larvae of *P. marinus*. The spectrum shows the presence of the vitamin A_1 band at 618 mμ alone.

in their spawning migration by a large number of alewives, anadromous teleosts coming in from the sea for the same purpose. The retinas and attached pigment epithelia of a number of these fish were treated precisely like the lamprey tissues. They yielded the spectrum shown in Fig. 3, a single band at 693 mμ due to vitamin A_2. Thus the retinal vitamin A patterns of the lamprey and alewife run parallel with their salinity relations in spite of their wide phylogenetic separation.

Compared with freshwater and anadromous teleosts, however, the lamprey retina contains an extraordinarily small amount of vitamin A_2. If its content is stated as 1, that of the pickerel retina is about 5, the calico bass 14, the white perch 25, the alewife 65, and the carp 200 (Wald, 1938–39).[2]

For this reason porphyropsin must appear in very low concentration in the lamprey retina, so low as to discourage an attempt on our part to extract it. But both the presence of porphyropsin and its low concentration can be inferred from earlier observations. Kühne (1878 *a*, *b*) reported that retinas of dark adapted *P. fluviatilis* display a strikingly feeble color, which despite its low saturation could be identified clearly as purple, inclining in hue toward violet or bluish. Walls (1935) describes the color of dark adapted *P. marinus* retinas as "truly purple (rhodopsin is usually red in color)." The purple color is characteristic of porphyropsin.

Liver

The larval lamprey possesses a liver, gall bladder, and bile duct of normal appearance. During metamorphosis both the latter structures are lost, and with them all direct connection with the intestinal lumen. Yet the liver grows thereafter to a large size, and presumably retains important metabolic functions. In the migrating adult it is deep green in color, due apparently to suffusion with bile pigments.

Fresh livers of late larvae were extracted by shaking exhaustively with chloroform. The extract, tested with antimony chloride, yields the single band at 618 mμ which characterizes vitamin A_1 (Fig. 4). The fresh liver weighs about 20 mg., and each liver contains about 1.3 μgm. of vitamin A, or about 61 μgm. per gram of fresh tissue. This should be a low but not unusual concentration in the livers of teleost fishes (Sakamoto, 1941).

The identification of vitamin A in adult livers proved to be troublesome.

The tissues were ground fresh with an equal weight of anhydrous sodium sulfate, and were extracted with *n*-pentane in a Soxhlet apparatus. About 10 per cent of the tissue weight of deep brown or greenish brown oil was obtained. A portion of this tested with antimony chloride yielded a highly turbid, impenetrable mixture. The

[2] The very low concentrations of vitamin A_2 in the eye tissues and A_1 in the liver (see below) of the migrating lamprey suggest that it is in a state of severe nutritional deficiency. Probably the lamprey, like a number of teleost fishes, stops feeding toward the beginning of its spawning migration.

oil was saponified in 6 per cent KOH in methanol at 50°C. under nitrogen. The non-saponifiable fraction, extracted with benzine, was surprisingly large, and when tested with antimony chloride yielded brown mixtures with rapidly changing spectra in which the bands of vitamins A could not be identified. Finally a portion of the non-saponifiable oil dissolved in benzine was run through an adsorption column of equal parts magnesium oxide and celite. An orange band was adsorbed high on the column, and below this a broad colorless zone which fluoresced strongly greenish-white in ultraviolet light. Such fluorescence is characteristic of vitamin A_1. The fluorescent zone was cut away from the rest of the column, eluted with 1 per cent methanol in benzine, and the extract tested with antimony chloride.

The resultant spectrum is shown in Fig. 5. It displays a single band at 612 mμ, due to vitamin A_1. In the course of this procedure too much material

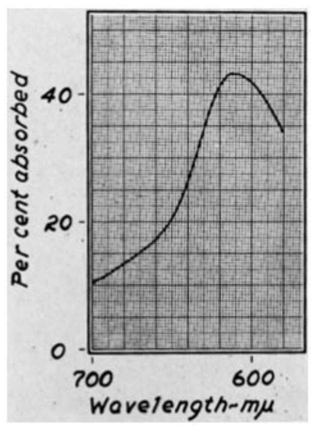

FIG. 5. Spectrum of the antimony chloride reaction with an adsorbed fraction of the non-saponifiable oil from adult lamprey livers. The spectrum shows the presence of vitamin A_1 alone.

was lost to permit an estimate of the vitamin A content of the adult liver, but this was clearly very low.

The lamprey liver therefore reverses the vitamin A pattern of the eye tissues, containing primarily if not exclusively vitamin A_1. Such reversals of vitamin A configuration in eyes and livers have previously been encountered in fresh-water and anadromous teleosts: the carp, chinook salmon, and brook trout (Wald, 1938–39).

DISCUSSION

Earlier experiments have demonstrated a genetic correlation between the salinity relations of a wide variety of fishes and the composition of their visual systems. The present observations extend this relation smoothly into the group of cyclostomes. The anadromous sea lamprey possesses a great predominance of the porphyropsin-vitamin A_2 system, precisely like an anadromous teleost.

This observation enormously broadens the evolutionary status of vitamin A_2, heretofore believed to be associated exclusively with the entry of teleost fishes into fresh water. It shows that the capacity to use and probably to synthesize vitamin A_2 is a deep and recurrent vertebrate property. Since lampreys possess vitamin A_2, freshwater elasmobranchs may be expected to do so also; and the same may be true even of freshwater invertebrates. In any case the lamprey result calls into immediate question the concept that A_1 is the primitive and fundamental vertebrate vitamin A.

SUMMARY

The porphyropsin-vitamin A_2 cycle has been found heretofore only in the retinas of bony fishes capable of existence in fresh water. Cyclostomes, due to their primitive and isolated phylogenetic position, might be expected to possess the rhodopsin-vitamin A_1 cycle common to marine elasmobranchs, almost all marine teleosts, and all terrestrial vertebrates so far examined. Yet the anadromous sea lamprey, *Petromyzon marinus*, possesses primarily the porphyropsin system, like an anadromous teleost. This observation greatly extends the phylogenetic association of vitamin A_2 with the capacity for freshwater existence.

Compared with freshwater and anadromous teleosts, the lamprey retina contains the porphyropsin system in extremely low concentration.

The remaining eye tissues, like the retina, contain both vitamins A_1 and A_2, the latter greatly predominant. The livers of larval and adult lampreys, however, appear to contain vitamin A_1 alone. This situation also is not without teleost precedent, since the carp and certain anadromous salmonids display similar reversals of vitamin A pattern in the liver and eye tissues.

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