

AN EXPERIMENTAL STUDY OF THE RETINA OF
THE BROOK LAMPREY, ENTOSPHENUS
APPENDIX (DEKAY)

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ONE HELIOTYPE PLATE (THREE FIGURES)

INTRODUCTORY

The aim of the present investigation was to obtain a picture of the photomechanical changes in the retina of a cyclostome to lay alongside the results of a number of workers on the other vertebrate groups.

The question as to whether the cyclostomes are to be considered a primitive group or as degenerate offshoots from another stock appears to be still open. The interest of investigators on both sides of the argument seems in recent years to have centered upon the eye more than on any one other anatomical feature. In a recent paper Keibel ('28) has gathered together the various arguments of the exponents of the 'primitive' theory, and upon the basis of his own observations upon the European brook lamprey, *Petromyzon planeri*, has disposed of them one by one and concluded that the eye of this form at least is specialized rather than primitive. Without going into the merits of the purely anatomical controversy, I wish to offer the results of this investigation as having a possible bearing on this question.

MATERIAL AND METHODS

At about the middle of April, 1928, Dr. P. O. Okkelberg and Mr. A. H. Stockard, both of this laboratory, collected a number of specimens of *Entosphenus appendix* in Fleming

Creek, a small stream at a few miles' distance from Ann Arbor. This lamprey swims free in the water during the short breeding season, and is very active and wary. It probably has excellent photopic vision, if one can judge from the difficulty with which it is approached by a wader.

Doctor Okkelberg very kindly placed some of the animals at the writer's disposal for experimental purposes. The animals live for only a few days as free adults, so only a limited number of experiments were possible at this time.

The animals were placed in a small glass tank with forced aeration overnight, receiving strong diffuse northwest light on the following day until early afternoon, when the experiments were started.

In exposing an animal to light or to darkness, it was isolated in a battery jar six inches in diameter and eight inches tall, two-thirds filled with water under forced aeration. For light adaptation four forty-watt electric lamps with parabolic reflectors were grouped about the jar, three in a circle about the sides, the fourth brought down over the top. The jar rested on a white towel so that the animal could see no dark area in any direction. For dark adaptation the jar was fitted with a snug, light-proof cylinder of cardboard with one end closed by a disc of the same material, all joints being carefully sealed with black gummed paper.

The temperature of the water was recorded at the beginning and end of each exposure. In light adaptations the water was heated considerably by the lamps in spite of a strong breeze passed through the room in an effort to prevent this.

After exposure, the animal was caught in the hand and quickly beheaded, the head split longitudinally, and the two parts thrown into separate bottles of fixative, which were left in light or in darkness, depending on the treatment of the animal.

Perenyi's fluid, Kolmer's modification of Held's fluid, and Birch-Hirschfeld's modification of Zenker's fluid were used as fixatives. The Perenyi material showed the excellent preservation which this fluid always yields on retinae, but

the characteristic poor-staining qualities rendered it unsatisfactory for this work, where the necessary measurements can be made easily only if the cytological details are well differentiated. The modified Held's fluid gave good results, but the modified Zenker's was best. One eye from each animal was fixed in Zenker's.

The lenses were removed in absolute alcohol, through corneal incisions, and the half-heads, with the eyes in situ, were embedded in parlodion by the hot method and cut 7.5 μ . Three adjacent sections through the optic nerve were taken from each eye; one was stained with Mallory's triple connective-tissue stain, one with Heidenhain's hematoxylin and phloxine, and the third was left unstained for clear observation of the retinal pigment.

Measurements were made with the aid of an ocular micrometer and expressed in micra.

EXPERIMENTAL

One animal was taken from the aquarium and the eyes fixed immediately. Other animals, eight in all, were taken in pairs and exposed simultaneously, one to light and the other to darkness, for periods of one-half hour, one hour, one and one-half hours, and two hours.

The pigment situation is shown in table 1. The thickness of the retina, including the pigment epithelium, was measured. The thickness of the pigment layer was then measured in the unstained section and divided by the thickness of the retina. It was thought that this quotient might better express the degree of pigmentation than would the thickness of the pigment layer alone.

It will be seen from the table that the average extent of the pigment in the dark-adapted retinae does not differ significantly from the average of the light-adapted retinae.

The pigment epithelium of this form is strikingly mammalian in appearance. The pigment granules are pale brown and sparse; they are evenly distributed throughout the portion of the cell vitread to the nucleus.

The rods of all the retinae examined were found to have a myoid length of about $8\ \mu$. This surprising uniformity in the length of the rod myoid led to a suspicion that perhaps it had been wrong to take the thickness of the retina into consideration in determining the extent of pigmentation, but it will be seen from the table that the absolute thickness of the pigment layer shows no correlation with exposure to light or darkness.

The cone myoid was found to vary in length from about $15\ \mu$ to about $25\ \mu$, with the average at $20\ \mu$. There was no

TABLE 1

EXPOSURE	TEMPERATURE	THICKNESS OF RETINA	THICKNESS OF PIGMENT LAYER	THICKNESS OF PIGMENT LAYER EXPRESSED AS PERCENTAGE OF THICKNESS OF RETINA
<i>hours</i>	<i>degrees C.</i>	μ	μ	<i>per cent</i>
Dark, 2	18.5 to 19.0	168	8	4.8
Dark, $1\frac{1}{2}$	19.5 to 19.0	192	10	5.2
Dark, 1	19.0 to 18.5	216	12	5.5
Dark, $\frac{1}{2}$	17.0 to 17.0	184	9	4.9
Diffuse daylight, 7	16.0 to 16.0	180	8	4.4
Light, $\frac{1}{2}$	17.0 to 22.5	168	9	5.3
Light, 1	18.5 to 23.0	168	9	5.3
Light, $1\frac{1}{2}$	18.5 to 27.5	176	10	5.7
Light, 2	18.5 to 24.5	168	9	5.3
Average per cent pigmentation of four dark-adapted retinae,				5.1 per cent
Average per cent pigmentation of five light-adapted retinae,				5.2 per cent

correlation with location in the retina or with exposure to light or darkness.

Although the number of animals studied is not large, the writer feels safe in concluding that the photomechanical changes so typical of the retinae of all other vertebrate groups, with the exception of the mammals, are entirely lacking in *Entosphenus* appendix.

Certain histological and cytological observations may be of sufficient interest to warrant mention. Most of the retinae showed large numbers of cones with their nuclei lying completely outside the external limiting membrane. Others were

pulled partly through, and showed a constriction at the level of the membrane. The nuclei outside the membrane and the parts of the others which lay in intermediate positions did not stain so intensely as did those on the inside of the membrane. The cones having such nuclei were otherwise normal and did not seem to constitute a special type. No double nor twin cones were observed. The cones are much larger in all respects than the rods (figs. 1 and 2). The ratio of cones to rods is about 1:1 in all parts of the retina.

DISCUSSION

The absence of photomechanical changes in this form may be looked on as an indication of specialization of the eye, as suggested in the introductory paragraphs. This group of phenomena is at a low ebb in nocturnal animals in general. *Entosphenus appendix* spends several years in the mud as a larva, then metamorphoses and lives for several months more as an adult, still buried in the mud. With the inception of sexual maturity, the animal leaves the mud and swims free for a few days to breed, then presumably dies (Okkelberg, '21). If this preponderant period of darkness in the life-cycle can be considered nocturnal life, one might expect the retina to undergo specialization in a 'nocturnal' direction.

When the retina of *Entosphenus* is examined with this point in mind, it discloses some evidence of adaptation to nocturnal habits. The rods in nocturnal teleost fishes are usually very small and very numerous, and are congregated especially densely in the region dorsal to the optic nerve, thus causing a thickening of the retina at this point. The retina of *Entosphenus* lacks this thickening, presumably because of the even distribution of the rods, and the rods are comparatively large and few in number. Nevertheless, there is a strong resemblance to the retina of *Amieurus nebulosus*, which is a dim-light fish with poorly developed eyes (Arey, '28, fig. 449). The absence of twin cones agrees with Wunder's ('25) findings on the small eyes of nocturnal teleost fishes.

The lake and marine lampreys, with their exposure to light for a period of several months as adults, might be expected to show a different situation. In a recent letter to the writer, Prof. S. H. Gage states that in 1913 he examined the retinæ of light- and dark-adapted lake lampreys (the Cayuga Lake lamprey is believed to be a land-locked *Petromyzon marinus* (Gage, '27)) and could see no evidence of pigment migration. This form seems to be more or less nocturnal in habits, for in ascending small streams to breed it rests during the day and travels only at night. Professor Gage's experiments were not exhaustive, and he made no measurements of rods and cones. Further investigation of these forms would seem to be indicated.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

- 1 Photograph of a portion of the retina of *Entosphenus* appendix near the ora serrata. $\times 500$. (The intense coloration of the pigment epithelium is due to the photographic technique, not to pigment.)
- 2 Rod and cone from upper region of retina. $\times 1300$.
- 3 Cone from fundus of retina, showing nucleus pulled partly through the external limiting membrane.

ABBREVIATIONS

P.E., pigment epithelium
C.O.S., cone outer segment
R.O.S., rod outer segment
C.E., cone ellipsoid
R.E., rod ellipsoid
C.M., cone myoid

R.M., rod myoid
C.I.P., intermediate plate of cone
R.I.P., intermediate plate of rod
N.,N., nuclei of rods and cones
E.L.M., external limiting membrane

