EFFECTS OF LIGHT AND DARKNESS ON THE EYE OF PRORHYNCHUS APPLANATUS KENNEL

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THREE TEXT FIGURES AND ONE PLATE

The eye of *Prorhynchus applanatus* Kennel consists of but two cells. One of these cells is the pigment cell and the other is the visual cell or retinula (figs. 1 and 2). The earliest investigators in this field recognized that a pigment-cup was associated with the visual elements of turbellarian eyes. In 1864 Leydig described in part the contents of this cup; but it was not until Hesse's ('97) work that we get a satisfactory account of the structure of the visual cells and pigment-cup and the relation between them. In one respect the work of Kepner and Taliaferro ('16) has carried the knowledge of the cytological details of the eye of this rhabdocoele beyond Hesse's observations, as will be later indicated.

The material and methods of fixation of the specimens for this work were on the whole similar to those used by Kepner and Taliaferro. The specimens have been collected by bringing into the laboratory pads of *Vaucheria* and other plant masses from the face of a dam over which water continually flows. These masses of algae and higher plants were placed in large glass vessels in tap water. The animals within forty-eight hours made their way to the surface in such aquaria where they were readily collected and transferred to embryological watch glasses that contained spring water. One part of each collection was placed during the day (from 9.00 a.m. until 5.00 p.m.) in the northern light of the laboratory from time to time during the months of April and May. At 5.00 p.m. each day a 40 Watt, 110 volt, Fostoria Mazda light lamp was turned on as it hung with its glass wall 21 cm. above the surface of the water

of the watch glass. No effort was made to eliminate the heat factor. In our preliminary experiments it was found necessary to isolate the ones that were to be subjected to light; for they showed a great tendency to hide beneath one another, if they were not separated. It was also found that they were killed by exposure to the open northern sky. The specimens were, therefore, placed ten feet away from a window in the laboratory. The second lot of animals of each collection was placed in a vessel of spring water and carried into a dark room. After forty-eight hours each series was fixed for two to five minutes in chrome-aceto-formalin. A given lot of fixing fluid was divided and one-half of it was used on the animals of the light series and the other portion of the fixing fluid was used for fixing the animals that had been kept in the dark. Of course the fixing of each series was done in the light or the dark as determined by the condition under which the members of the series had been kept. After the fixing all other steps were carried on in the light. The specimens from the fixing fluid were washed quickly in four changes of tap water and quickly carried through the alcohols, xylol and paraffin; so that the animals were in a paraffin block within one half hour after they had been fixed. The sections were stained in iron hematoxylin in a few cases, but Mallory's connective tissue stain was for the most part employed.

The relative position of the visual element and accessory cell in material so handled can readily be made out when immersion lenses are employed. It can thus be seen that, as Hesse has observed, the rhabdomes of the visual cells were not directed towards the light as it entered the eye, but away from the light and towards the cells of the pigment cup, the light passing through a great part of the visual cell before encountering the visual rod or rhabdome. It is interesting to note that this inverted position of the retinal elements is encountered in vertebrates, some molluscs and turbellaria. In these three groups we must, therefore, have eyes that represent either (a), a suppression of this type of eye through many intervening phyla to appear atavistically in these three groups; or (b), a retention of this type in certain mollusca and all vertebrates

from their direct ancestral stock, thus making the inference that these forms arose from a much lower level on the phylogentic tree than is usually suggested—lower even than Hubrecht's nemertean ancestry for the vertebrates; or (c), we must have in these three groups three cases of parallel evolution. We have suggested these alternatives by way of indicating the fields of general interest into which this study has led us, without committing ourselves to any of them. The analogy however, between these eyes and those of vertebrates is so striking that we have become interested in trying to find an analogy of function between the visual elements of the vertebrate and *Prorhynchus*.

The pigment cells of vertebrates are influenced by light and its absence. "In general it may be said that light induces an expansion and the absence of light a contraction, of pigment cells;" Arey ('16 b, p. 360).

The accessory cell or pigment cell associated with the visual cell of *Prorhynchus* is closely applied to the rhabdome or receptor of the retinula (fig. 1). In eyes that have been subjected to alternation of day and night conditions, when the specimens are free to seek and find optimum conditions of illumination, this cell in each eye is found to have a uniformly stratified cytoplasm. Little change is to be observed in these cytoplasmic strata when the eyes have been illuminated continuously for forty-eight hours or longer. Figures 10, 11, 12 show the closely crowded strata of cytoplasm in the pigment cells that have been continuously illuminated for two nights and two days or longer. It is to be seen here that, if anything, these eyes show a contraction of the pigment cells. We have met with one apparent exception to this type of reaction on the part of an illuminated pigment cell. This exception is shown in figure 9. Here the cytoplasmic strata are less crowded and lie less regularly about the contour of the rhabdome. Examination shows that this is the right eye of the animal and that the left eye has a typical light adapted pigment cell. It is suggested by this that the right eye might have been shaded in some manner. When we first undertook to expose animals to the continued illumination, it was found that the specimens would frequently disgorge some

of the contents of the enteron and then bring its anterior end to lie beneath this ejected food mass. Care was taken, then, to remove such material whenever it was present, but it is possible that a small piece of shading material might have escaped our attention in this particular specimen. Unless in this or some other manner this exceptional eye had been shaded we have no way to explain its departure from the type of a light adapted eye. This one exceptional case, however, constitutes a low coefficient of variation when we consider that twenty animals or forty eyes of this series of light adapted eyes were studied.

On the other hand, all dark exposed pigment cells have shown that the lamellae of their cytoplasm move apart with the result that the cell itself becomes greatly expanded (figs. 2 to 8). The section adjacent to and belonging to the section shown in fig. 1 also shows a greatly expanded pigment cell. The reaction of the pigment cell of the eye of *Prorhynchus*, therefore, stands in sharp contrast to that of the eye of a vertebrate, in that light causes a contraction and dark an expansion of it, while the reverse is the reaction of the pigment cell of a vertebrate eye. The response of the pigment cell of *Prorhynchus*'s eye to light is similar to that of the photoreceptor's pigment cell of *Amphioxus*, as Crozier ('17) describes.

In the retinula or visual cells of some vertebrates the following cytoplasmic details have been observed: (1), an inner segment, the myoid, with which the nucleus is directly associated; (2), a middle region, designated the ellipsoid, which is to be found in the eye of fish, amphibia, reptiles and birds; and (3), the outer segment, the visual rod or rhabdome (text-fig 1). Kepner and Taliaferro ('16) found three cytoplasmic regions in the retinula of *Prorhynchus*; (1) the inner one most closely associated with the nucleus; (2) the middle segment, a lens-shaped region, that in a freshly dissected eye was highly refractive, thus strongly resembling the ellipsoid of a vertebrate visual element; and (3) an outer segment, the rhabdome. These authors recognized, at the time their paper had appeared, that they had carried the knowledge of the histological details of a turbellarian eye a step beyond where Hesse ('97) had taken it. However, it was not

Text-fig. 1 A. Shows a retinula of the frog (left) and the retinula of *Prorhynchus* (right). Three cytoplasmic regions are to be seen. Lines 1 and 1' indicate the receptors or rhabdomes; line 2 connects the ellipsoid of a frog with its analogue in *Prorhynchus*; lines 3 and 3' connect the myoid of a frog's retinula with its analogue in the retinula of *Prorhynchus*.

B. Two dark adapted retinulae of frog (left). Observe elongated myoid of cone and contracted myoid of rod and no change in other cytoplasmic segments. One dark adapted retinula of *Prorhynchus* (right). Anterior end to right. Note the relative small size of the middle cytoplasmic segment and the low, trough-like rhabdome.

C. Two light adapted retinulae of frog (left). Observe contracted myoid of cone and elongated myoid of rod; no change in other segments of the cytoplasm of these cells. One light adapted eye of *Prorhynchus* (right). Observe absence of middle segment of cytoplasm and the high, rounded rhabdome. The drawings of rods and cones of frog taken from Arey ('16b).

until the appearance of Arey's ('16) work that the authors of this paper realized that the analogy, if not the homology, between a visual element of a vertebrate and that of *Prorhynchus* was so evident. Text figure 1 was made at the suggestion of Mr. W. H. Taliaferro.

This structural analogy suggested the problem of learning whether or not there was to be found a functional analogy.

The functional changes of visual cells in certain vertebrates have recently been well described and the literature of the subject reviewed by Detwiler ('16) and Arey ('16).

Englemann (85, p. 500) found that in the eye of the snake *Tropidonotus* matrix, which contains no rods in the retina, the cones contracted but little; also that in *Testudo graeca* it is doubtful whether any contraction takes place. Angelucci ('94) however, claims "that in *Testudo marina* contraction of the cones does take place, though less in extent than in the frog. . . ." Chiarni ('06) also reports that in the eye of *L. agilis* the cones shorten when the eye is brightly illuminated, but only slightly, for the cones measure in dark eyes 25 to 35 μ , in light eyes 23 to 30 μ . Finally Garten ('07) found also a very slight contraction (not more than 1.2 μ) in the eye of *Chamelion*. (Detwiler (16) p. 166-7.)

In speaking of his own work, Detwiler says:

Light causes a migration of the pigment and a contraction of the cones in both the tortoise and lizard retina, the extent of migration in the tortoise averaging 3.6 μ , and in the lizard 3.1 μ . The extent of the contraction of the cones in the tortoise averages 2.3 μ (p. 186). "To the contractile portion of the cone's inner member Englemann applied the significant term 'myoid'. . . . The contractility of the myoid is extraordinary, since in some fishes light produces a shortening of this part to 10% of the length which it assumes in darkness. If effective at all, light always causes a shortening and darkness an elongation of the cone cell" while the "myoid of the rod-visual cell elongates in light and shortens in darkness" in all investigated vertebrates.

Arey ('16-b), p. 441. The latest observations, therefore, indicate that a shortening or an elongation of the retinulae takes place by means of a modification of the myoid. The change in the size and shape of the rhabdome has nothing to do with the total length of the visual cell. No change in the ellipsoid of the visual cell of the vertebrate has been recorded.

The functional changes to light as judged from our study of the fixed condition in the eye of *Prorhynchus*, stand in sharp contrast to the changes in the retinulae of vertebrates. The reaction of the visual cells to light and darkness is more pronounced than that of the pigment cells of the eyes.

No nuclear change has been observed in the retinula of *Prorhynchus* by us. The change in the cytoplasmic segment, that is

associated with the nucleus and hence the analogue of the vertebrate visual cell, seems to be passive and conforms to that of the other segments of the cytoplasm. Continuous illumination causes the middle refractive segment, which is analogous to the ellipsoid of the vertebrate eye, to disappear in the fixed material. No trace of it is to be seen in the eyes shown in figures 11 and 12. The striation to be seen at the base of the rhabdome in figure 10 may represent the vestiges of it.

Figure 8 shows the most conspicuously dark adapted eye that we have found. Here there is a trace of the middle, cytoplasmic segment to be seen. This middle region of cytoplasm is depicted as the lightest region of the rhabdome in figure 8. It is to be observed in this frontal section that the middle region is thickest at its posterior end; the relative thickness of the middle segment is also shown in the figures 6 and 7, which too are frontal sections. Figures 2, 3, and 4 show transverse sections taken at the level of the posterior end of this middle segment of the retinula or visual cell. In figure 1 we have a section shown that is exceptional. This section was only somewhat transverse. The adjacent section of this eye does not show the middle segment so conspicuously. This particular eye is exceptional as a dark adapted one in that it has shown no departure in its middle segment from that of an eye that has been subjected tonight and day alternation of light and dark. The rhabdome in this section also departs little from that of an illuminated eye; the adjacent section, however, gives us a typically dark adapted rhabdome.

The reactions of the rhabdome to light on the one hand and darkness on the other, as seen in the fixed condition, are the most conspicuous changes of all those observed in this study. In eyes that have been subjected to day and night alternation in laboratory light the rhabdome has a low cone-shaped contour both in transverse and longitudinal sections (Kepner and Taliaferro, '16). The rhabdomes of eyes that have been illuminated continuously for forty-eight hours have relatively high axes and relatively small diameters. The free margin of such rhabdome is uniformly curved and directed laterally (figs. 10, 11, 12). The

frontal section of a dark adapted eye shows a rhabdome that is relatively low and greatly elongated;—the posterior end being the thickest (figs. 5, 6, 7, 8 and 9). Transverse sections show that these rhabdomes are concave (figs. 2, 3, and 4). Thus we have the visual rod of this eye becoming low and elongating in the dark and becoming high and rounding up in the light.

Pigment cell and visual cell, therefore, of *Prorhynchus* in the dark seem to make an effort to locate light by increasing their receptive surfaces.

Text-fig. 2 Outline drawing of dorsal ganglia and eyes of light adapted eyes. Note the rounded contour of eyes and their forwardly directed axes. Taken from living specimen.

In addition to this reaction of the part of the elements of the eyes, the eyes themselves change their axes with reference to that of the body as a response to light and darkness. A living specimen under strong illumination has the axis of its eye anteriorly directed at an angle of about 45 degrees from the axis of the body; while the eye of a dark adapted animal has its axis laterally directed at right angles to the axis of the body of the animal (text-figs. 2 and 3). The dark adapted eye shifts its position as if in this way also light were being sought.

Text-fig. 3 Reconstruction of dorsal ganglia and eyes of dark adapted eyes. Note the elongation of eyes and their laterally directed axes.

CONCLUSIONS

1. Stimulation by light results in a contraction of the accessory cell or pigment cell.

In sustained darkness the cytoplasmic lamellae of the pigment cell open up or move apart, resulting in the expansion of the cell.

2. The three cytoplasmic regions of the retinula or visual cell show more or less marked changes in response to light and darkness. The nucleus-bearing part of the visual cells somewhat widened in the dark. The refractive, middle segment (analogous to an ellipsoid of a vertebrate retinula) disappears in continuous illumination and is most conspicuous in eyes that have been subjected to optimum illumination. The rhabdome in light adapted eyes is a rounded cone-shaped body, while in dark adapted material it is an elongated trough-shaped structure with its long dimension directed parallel to the axis of the body of the animal.

3. Despite the analogy that is apparent between the structure of the retinula of a vertebrate and that of a *Prorhynchus*, there is no analogy in functional changes shown. In the former it is

the myoid that most markedly changes form, in the latter it is the rhabdome that is most conspicuously modified in response to light and darkness.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

- 1 Approximately transverse section of right eye. Dorsal side up. Dark adapted (?). $\times 1500$
- 2 Nearly transverse section of left eye. Dorsal side up. The mate of eye shown in figure one. Dark adapted. $\times 1500$
- 3 Transverse section of left eye. Dorsal side up. Dark adapted. $\times 1500$
- 4 Transverse section of right eye. Dorsal side up. Dark adapted. $\times 1500$
- 5 Frontal section of left eye. Anterior end up. Dark adapted. $\times 1500$
- 6 Nearly frontal section of right eye. Anterior end up. Dark adapted. $\times 1500$
- 7 Nearly frontal section of left eye. Anterior end up. Dark adapted eye. $\times 1500$
- 8 Nearly frontal section of left eye. Anterior end up. Dark adapted eye. $\times 1500$
- 9 Nearly frontal section of right eye. Anterior end up. Light adapted eye. $\times 1500$
- 10 Approximately frontal section of right eye. Anterior end up. Light adapted eye. $\times 1500$
- 11 Nearly frontal section of left eye. Anterior end up. Light adapted eye. $\times 1500$
- 12 Nearly frontal section of right eye. Anterior end up. Light adapted eye. $\times 1500$