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ON THE EYESPOT OF THE DINOFLAGELLATE, *NEMATODINIUM*

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INTRODUCTION

One of the most common properties of motile organisms is an ability to use the information carried by light rays to orient themselves in the world. The amount of information that can be supplied in this way is huge, as our own immensely detailed world-picture indicates.

In the simpler animals only a small part of the possible information is used. The Protozoa in particular are thought to sense only information about the direction and intensity of light rays.

Flagellated protozoa which respond to light phototactically often possess an eyespot. In *Euglena*, for example, the eyespot consists of a plate of reddish pigment granules located near the front of the cell and closely associated with a swelling in one of the two flagella (Wolken, 1958; Gibbs, 1960; Leedale, Meeuse & Pringsheim, 1965).

Such a simple structure can give only information about the presence or absence, and direction of, a source of light (Mast, 1941; Pringsheim, 1963). This means that two sources near each other are seen as one; and two widely separated sources are seen as a single source located somewhere between the two (Mast & Johnson, 1932). In order to carry information about the distribution of sources of light in the environment, an eye must contain an image-forming structure, the lens, which will transmit separately the rays from all sources to the receptive area or retina.

In one group of flagellates there is a lens-like structure. This is the eyespot of the Warnowiaceae, a family of naked dinoflagellates. Here there is a pigment mass associated with a refringent object called the lens (Figs. 1, 2). The appearance of this apparatus can be so similar to that of a mammalian eye that Pouchet, the first to describe it in detail, was certain that it acted in the same way (Pouchet, 1885, 1886, 1887). Very recently the fine structure of this organelle has been examined in two members of the family—in *Erythropsis pavillardi* by Greuet (1965), and in *Nematodinium armatum* by Mornin & Francis (1967). In both species, the lens consists of clear, concentrically arranged pieces, and is poised above a cup consisting of pigment grains. Lining the cup is a layer of fibrous material, which we termed the retinoid (Fig. 2).

This organelle is plainly much more complex than the simple eyespot of other flagellates, but whether it has a specialized function corresponding to its differentiated structure has not yet been determined. The question that I will attempt to answer in

the present paper is whether the lens is actually physically able to project an image on the retinoid. To answer this the refractive index and the shape of the lens were measured as accurately as possible.

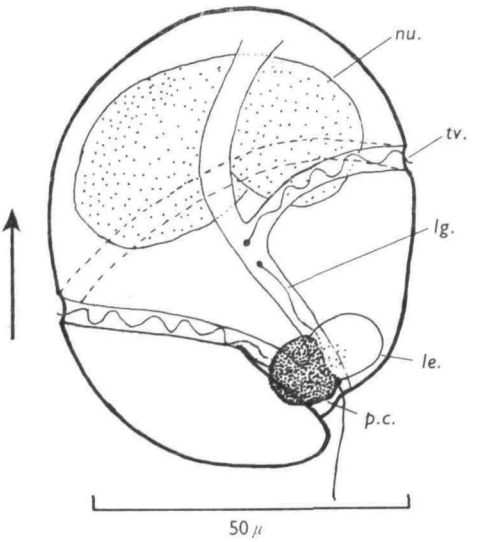

Fig. 1. *Nematodinium armatum*. *nu.*, nucleus; *tv.*, transverse, and *lg.* longitudinal flagella; *l.e*, lens; *p.c.*, pigment cup, together comprising the eyespot. The arrow indicates the direction of movement.

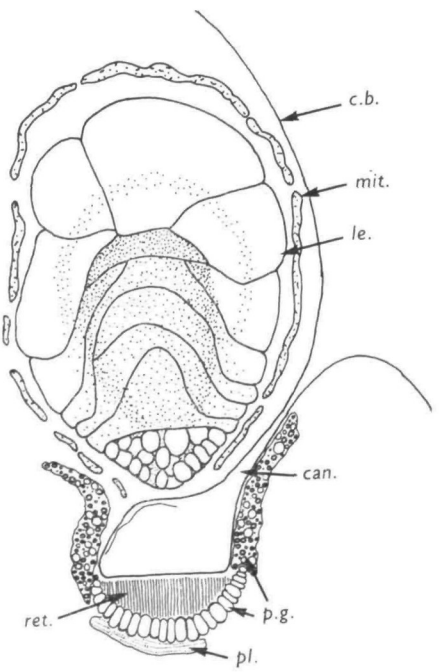

Fig. 2. A diagrammatic longitudinal section of the eyespot of *Nematodinium* as seen in the electron microscope. *le.*, lens; *ret.*, retinoid; *p.g.*, pigment granules composing the pigment cup; *mit.*, mitochondrion; *c.b.*, cell boundary; *can.*, canal connecting the space over the retinoid to the sea water outside; *pl.*, plastid.

MATERIALS AND METHODS

Collections were made during June of 1966 at Scripps Institute of Oceanography, La Jolla, California. Towards the end of the month a species of *Nematodinium* was found in surface water at a density of 1 to 40 individuals in 10 l. The organisms were collected by slowly filtering 10 or 20 l. of surface water taken from the end of the Scripps pier through a strainer with netting of 30 μ aperture. Single individuals were picked out from the concentrated filtrate. Eight animals were fixed in 1 % osmium tetroxide and embedded in Epon 812, and another 10 were preserved frozen in sea water. The specimens were all of similar appearance and very like *N. armatum* as described by Dogiel (1906) at Naples. They were 50–70 μ in length and had many reddish pigment bodies scattered near the cell surface.

Upon returning to Vancouver I found in surface water from Howe Sound a few individuals of an organism resembling the *N. armatum* of Lebour (1925) in being uniformly pale yellow but like the La Jolla specimens in all other respects. The two collections perhaps represent two forms of a single variable species

EXPERIMENTS AND RESULTS

(1) *Refractive index of the lens*

Estimate (*a*). The refractive index (n_1) and focal length (l) of a spherical lens are related by the formula

$$n_1 = \frac{2n_2 l}{2l - r},$$

where r = radius of the sphere, n_2 = refractive index of the medium surrounding the lens. If r, l and n_2 can be measured, n_1 can be calculated. If the lens is not spherical but irregularly rounded, as may be true of *Nematodinium*, this formula is only approximately valid.

Experiment. The lenses of *Nematodinium* preserved by freezing were used for this experiment. The frozen cells were readily identified upon thawing, as each contained a shapeless pigment spot and a more or less rounded lens. The average radius of each lens was measured directly. To determine the focal length the protoplasts were mounted in sea water on glass slides on the microscope stage. The microscope condenser was removed so that the slide was illuminated by parallel light rays from the lamp. With this arrangement, a bright spot of light, the image of the lamp filament, could be seen by focusing the microscope on a plane some distance above the *Nematodinium* lens. The distance from the mid-point of the lens to this focal plane was measured by using the fine adjustment of the microscope. From this value, the radius of the lens and the refractive index of sea water (= 1·34 at 20–25° C.), the average refractive index of the lens was determined to be 1·65.

A control experiment, however, performed on polystyrene spheres 10–15 μ in diameter (kindly supplied by Dow Chemical Corp.), showed that this method gave a refractive index 0·10 units too large. The error is probably due to a consistent mistake in locating the exact centre of the lens, and to spherical aberration. Correcting the

lens refractive index for this error gives: refractive index of *Nematodinium* lens = 1·55 (number of measurements = 6; standard deviation = 0·0011).

Estimate (*b*). A second estimate of the refractive index was obtained by applying the same method to a somewhat deformed single lens which had been crudely extracted from a live *Nematodinium* at Vancouver. The corrected refractive index of this lens was 1·49.

Estimate (*c*). A third estimate resulted from examination of approximately 1 μ sections of Epon-embedded material in an interference microscope. Charles Culling kindly performed the measurements. The refractive index of the outer part of the lens was found to be just that of the mounting medium, i.e. 1·52. The coincidence may actually mean that the lens was dissolved during the embedding procedure. This involved passage from water to 100% alcohol and propylene oxide both of which are fat solvents. Fauré-Fremiet (1915) found, however, that the very similar lens of *Erythropsis agilis* does not dissolve in fat solvents.

Fauré-Fremiet also found, and I have confirmed the observation, that the lens does swell in water and aqueous fixatives. Such swollen lenses conceivably have their substance diluted, and therefore are of lower refractive index than when in the living organism.

In spite of these possible doubts, the three methods all suggest approximately the same value and I will take their average, or 1·52, as a true estimate of the refractive index.

(2) *Size and shape of lens*

To find the exact focal length of a non-spherical lens whose refractive index is known, its size and shape must be measured.

The precise shape of the *Nematodinium* lens is difficult to determine from observations on living animals, since the deep pigment cup obscures the base of the lens. For this reason, the shape was determined from longitudinal sections of fixed and embedded animals, such as that shown in Fig. 2. The outline of the lens in these sections is not smooth, but bulged. The lens does not appear bulged in life and this appearance is probably an artifact due to fixation and embedding. Fauré-Fremiet (1915) noted that all aqueous fixatives, and solutions of osmium tetroxide in particular, caused immediate swelling of the lens. Schütt (1895) and Kofoid & Swezy (1921) mentioned the same fact.

Another difficulty is that none of the sections cut is precisely longitudinal. The true shape must be reconstructed by fitting together a series of neighbouring sections.

These procedures lead to the oval shape shown in Fig. 3 as an approximation to the shape of the lens in the living organism. This will be referred to as the *ideal lens*, for convenience.

(3) *Location of the focal plane*

The focal plane of the ideal lens was found by tracing the paths of rays entering the lens parallel to its longitudinal axis, using the estimated value for refractive index with Snell's law of refraction. The rays focus on a zone centered 4·4 μ below the pointed end of the lens (Fig. 3). This point is just within the base of the pigment cup, in the area called the retinoid. The result is just that expected if the function of the lens involves formation of an image and its reception by a photosensitive substance in the retinoid.

(4) *Characters of the image*

Even if the lens does form an image, the image may be so poor that it contains no more information than a mere spot of light. The resolving power of the lens was estimated by direct measurement of the resolution in the images formed by plastic spheres of the same size as the lens.

Plastic spheres were mounted in a beam of parallel rays, as described above. One of a set of metal discs with two tiny holes punched through the centre was placed in the light beam near the lamp. The image of the two holes which was formed by a plastic sphere was observed. It was found that when the two holes were separated by a distance which made their angular separation 2° to 3° they were just resolved as two. This experiment was repeated with a single lens extracted from a living Vancouver

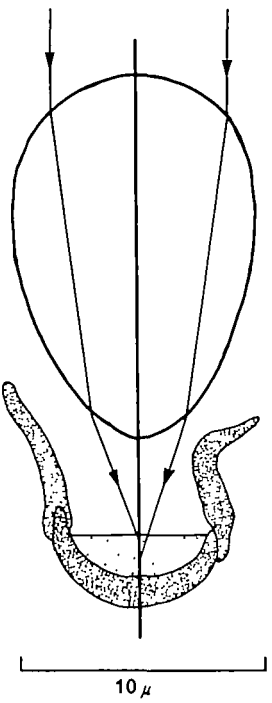

Fig. 3. The 'ideal lens' described in the text. The two arrows show two light rays originating from a source at infinity and coming to focus in the retinoid. Since the lens is not symmetrical about its long axis, there is no single point of focus but rather a zone of focus.

Nematodinium and gave the same result in that case. An angular separation of 2° to 3° corresponds to 0·4–0·5 μ separation of images in the image plane. These values compare closely with the 0·6 μ resolution expected from diffraction effects, as given by the formula $d = \lambda l/b$, where d = the separation in the image of two points just resolvably distinct, b = diameter of the aperture, λ = wavelength of light used, l = focal length of the lens system.

The effect of the mitochondria and other particles in the layer of cytoplasm surrounding the lens will be to decrease the resolution by scattering light. The focal

length of the lens will not be changed since the refraction index of cytoplasm is almost exactly that of seawater (e.g. Allen & Roslansky, 1958).

(5) *Field of view*

The field of view of the ideal lens of Fig. 3 is found by ray tracing to be about 30°.

(6) *Depth of focus*

Because the focal length of the lens is so short, all objects more than a fraction of a millimetre distant will form images on the same focal plane. For objects closer than about 100 μ the image plane will be located significantly deeper in the pigment cup. An object 26 μ distant from the front of the lens will be imaged on a plane 2 μ below the pigment cup, and the pattern of light at the level of the true focal plane in the retinoid will be a rather poor image of the object. Objects closer than this will be badly out of focus.

DISCUSSION

Variations in shape and refractive index

Our experiments have shown that the *Nematodinium* lens is physically capable of focusing light on the inner part of the pigment cup, and therefore able to form images there of objects outside. The focal length was calculated for the ideal lens, however, and it is important to know how closely the focal length depends on the exact shape and refractive index of the lens.

The effect of variations in refractive index is not very great. An increase of 0·10 in the refractive index would only shorten the focal length of the ideal lens by about 1·3 μ. On the other hand, the shape of the lens, and especially of the end of the lens within the pigment cup, affects the focal length very strongly. A lens which tapers to a sharp point at the inner end has a much shorter focal length than one which is more nearly spherical. This effect is difficult to express quantitatively, but ray tracing shows the effect of different shapes. For example, a lens having both ends with the radius of curvature of the outer end of the ideal lens, and which is the same length as the ideal lens, will have a focal length 3·6 μ longer. All this implies that the shape of the lens must be precisely controlled if a sharp image is to be formed in the sensitive area.

These measurements do not determine the precise function for which the eyespot is used, but they do imply that the function involves image formation. Whether *Nematodinium* does use any of the potential information in the image formed by its eye will have to be determined by experiments relating its behaviour to visible changes in the world of outside objects. I hope to pursue these investigations shortly.

SUMMARY

1. *Nematodinium* possesses an eyespot which consists of a lens closely apposed to a pigment cup containing a supposedly light-sensitive retinoid.

2. The shape of the lens is described and its refractive index measured. From these data it is concluded that light from sources outside the cell will be brought to focus on the retinoid. The quality of the image formed is assessed.

3. A variety of functions are possible to the organelle. It is suggested that functions

involving image formation may be realized, even in the absence of a true retina and nervous system.

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