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# OBSERVATIONS ON THE FINE STRUCTURE OF THE EYESPOT AND ASSOCIATED ORGANELLES IN THE DINOFLAGELLATE *GLENODINIUM FOLIACEUM*

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## SUMMARY

The eyespot of the marine dinoflagellate *Glenodinium foliaceum* is a flattened orange structure, more or less trapezoid in shape with an anterior hook-like projection. It is situated on the ventral side of the organism in the vicinity of the flagellar bases at the anterior end of the sulcus. In the electron microscope the eyespot is seen to contain two layers of osmiophilic granules 80–200 nm in diameter which usually show hexagonal close-packing. The eyespot is surrounded by a triple-membraned envelope and is not connected to any other organelle. Adjacent to the eyespot is a distinctive organelle termed the 'lamellar body'. This consists of a stack of up to 50 flattened vesicles or disks, each 16 nm thick and about 750 nm wide, the whole being orientated in an antero-posterior direction. The lamellae are continuous, at the ends of the stack, with rough endoplasmic reticulum and are joined together by occasional bridges at their edges. The bases of the two flagella lie just ventral to the lamellar body and from them roots arise which pass by the eyespot and join the subthecal microtubular system.

The eyespot of *Glenodinium* is unique both in structure and the presence of the associated lamellar body. It differs from eyespots which have been described from other algal groups and also from the more complex ocellus found in certain dinoflagellates belonging to the order Warnowiaceae. The method by which the eyespot functions is discussed and it is suggested that unidirectional stimuli could be perceived by shading of the lamellar body.

## INTRODUCTION

Eyespots or stigmata are found in some members of almost all algal classes which contain flagellated organisms. These organelles have long been thought to act as photoreceptors in spite of the fact that many organisms which do not possess eyespots are definitely photosensitive. However, when a mutant of *Chlamydomonas* which lacked an eyespot was compared with the normal type it was found that possession of the eyespot resulted in a much more uniform and precise phototaxis (Hartshorne, 1953). The eyespot does therefore appear to perform some useful function.

Structurally, the eyespots which have been investigated are found to fall into four categories. In the Chlorophyceae (Lang, 1963; Walne & Arnott, 1967) and Prasinophyceae (Ettl & Manton, 1964) the eyespot constitutes a portion of the chloroplast and is usually laterally placed in the cell, at some distance from the flagella. In the Chrysophyceae (Rouiller & Fauré-Fremiet, 1958; Belcher & Swale, 1967), in the Xanthophyceae (D. Hibberd, personal communication) and in antherozoids of the Phaeophyceae (Manton & Clarke, 1956) the eyespot is still part of the chloroplast but it is situated adjacent to the flagella, one of which may be specially modified. In the Euglenophyceae (Leedale, 1967; Walne & Arnott, 1967) the eyespot is quite independent of any chloroplast and in fact consists of only a loose aggregation of granules not surrounded by an envelope. However, this is situated near to the flagellar bases and apparently functions in association with a flagellar swelling. The final type of eyespot, the ocellus of dinoflagellates belonging to the family Warnowiaceae, is much more complex than any of the other types (Greuet, 1965; Mornin & Francis, 1967). The ocellus is situated near the posterior end of the cell and is a large structure, approximately 25 μm long and 15 μm wide. It consists of a domed lens, a chamber connected by a canal to the exterior, and a paracrystalline structure termed the retinoid with a hemispherical outer layer consisting of a single row of pigment granules.

Other dinoflagellates have long been known to possess much more simple types of eyespot than those of the Warnowiaceae and there are many which do not have any type of eyespot. In the present paper we describe in detail for the first time the structure of a simple dinoflagellate eyespot from the marine and brackish-water species *Glenodinium foliaceum* Stein. It will be seen that this eyespot does not fit into any of the four categories described above. It is not only associated with the flagellar bases but is always adjacent to a distinctive structure which we term the lamellar body.

## MATERIAL AND METHODS

The culture used for the present investigation was supplied by Dr K. Gold (New York Zoological Society) and was originally isolated from Salina Parguera, Puerto Rico. There is currently some confusion over the taxonomy of the dinoflagellates and the organism used here has been placed in several different genera. In 1878 it was first described by Stein and called *Glenodinium*; it has also been named *Kryptoperidinium* (Lebour, 1925), *Phyllodinium* (Conrad, 1926) and *Peridinium* (Biecheler, 1952). Taxonomically this clearly has proved to be a difficult organism so until the distinctions between the genera are more clearly established we prefer, with Prager (1963), to use the original name *Glenodinium foliaceum* Stein.

The organism was cultured in Erdschreiber enriched sea water at 16–20 °C under 16 h light, of approximately 1000 lx, per day.

For electron microscopy cells were sedimented by centrifugation and fixed at room temperature in 3 % glutaraldehyde at pH 7·8 in phosphate buffer and with the addition of sucrose to 0·2 or 0·3 M. After rinsing in buffer the material was postfixed with cold 1 % osmium tetroxide at pH 7·8. Dehydration was by means of an alcohol series and embedding in Araldite. Sections were cut with glass or diamond knives, stained in uranyl acetate and lead citrate and examined in a Zeiss EM 9A electron microscope.

## OBSERVATIONS

### *The eyespot*

With the light microscope the eyespot of *Glenodinium foliaceum* is seen to be situated in a ventral position at the anterior end of the sulcus. It is irregular in outline but usually is more or less rectangular or trapezoid, with a hook-shaped projection at the anterior end (Fig. 1). When viewed from the side the eyespot is seen to be thin and flattened and occasionally more than one layer is visible. In some cells the eyespot appears granular and it always has a bright orange colour. Its over-all dimensions do not normally exceed 6 μm long by 3 μm wide (the cell is approximately 30 × 25 μm).

In the electron microscope the eyespot is seen as a flattened sac with rounded edges which contains two rows of large osmiophilic granules (Figs. 3–7). Sometimes the sac lies flat but more often it is folded back on itself to give a U- or J-shaped profile in section (Figs. 3, 5). Occasionally it is branched (Fig. 4). It would appear that there is only one eyespot sac and when two appear to lie together in a section this is due to the plane of the section passing through a folded or branched portion of the eyespot.

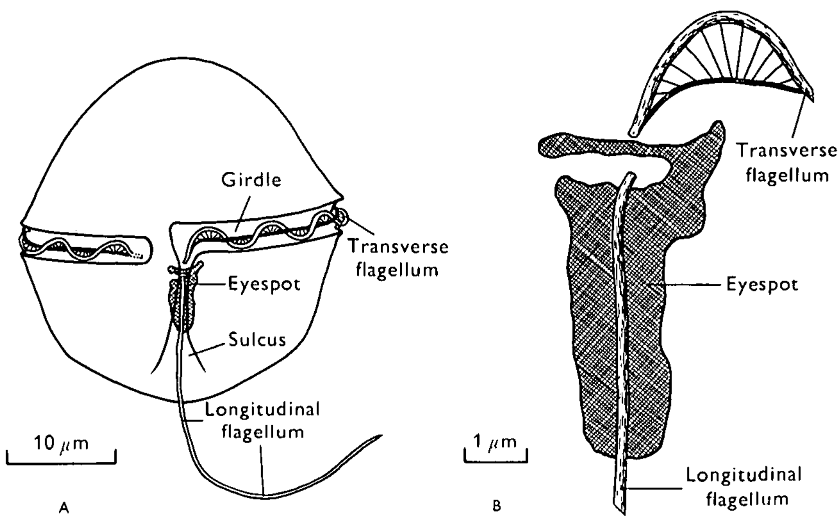


Fig. 1. The eyespot as seen in the light microscope. A, ventral view of a cell to show the location of the eyespot with regard to the transverse flagellum lying in the girdle and the longitudinal flagellum lying in the sulcus. B, enlarged view to show the mode of insertion of flagella in relation to the hook-shaped anterior projection of the eyespot.

Surrounding the eyespot is a triple-membraned envelope (Fig. 5) identical in appearance to that which surrounds the chloroplasts of dinoflagellates (Dodge, 1968). The envelope has no pores and has not been found to be connected with the membranes of any other organelles such as chloroplasts or nucleus. Occasionally, in what are probably old organisms, the envelope appears to become rather broken down (Fig. 13) and although the granules remain together they are then less regularly arranged.

Within the eyespot there are normally two rows of osmiophilic granules (or globules), one layer adjacent to the upper part of the envelope and one layer adjacent to the lower part of the envelope. At bends and branches the pattern may become slightly distorted. Normally the two rows are separated by a central space, containing finely granular material, up to 100 nm wide. The osmiophilic granules are generally packed close together and thus in tangential section (or face view) appear hexagonal (Fig. 6). Where they are less tightly packed they appear circular in outline (Fig. 7) and in such eyespots small tubular membraneous structures are often found between the granules. The eyespot granules range from 80 to 200 nm in diameter, the larger granules usually being found at the edges or bends in the eyespot. The normal thickness of the eyespot is therefore in the region of 350 nm (central space 100 nm, two rows of 100 nm diameter granules and two triple envelopes) but at the bends the thickness may increase to nearly twice this figure.

*The lamellar body*

Adjacent to the eyespot is a distinctive structure which we have called the lamellar body (Figs. 3, 4, 11, 13). It has not been possible to identify this structure by light microscopy as it is presumably colourless and is partly hidden by other organelles.

It consists of a stack of flattened vesicles which are arranged more or less parallel to each other (Fig. 8) and which are oriented in the cell so that the stack lies in an antero-posterior direction. The shape of the lamellar body is clearly variable, as the micrographs show. The most common shape seen in the sections is a long rectangle, suggesting that the body may often be more or less cylindrical. At times it has wider portions (Fig. 11) and it may be bent (Fig. 13) or have a small portion which is separate from the main body and at an angle to it.

The number of lamellae which have been seen in sections varies from 50 (in Fig. 8) to 5, which small number was probably not a fair sample of the whole lamellar body of that cell but of only a small offshoot. The average for 23 sections is 25 vesicles per body. The longest lamellar body was 3 $\mu$m long and the widest 1·2 $\mu$m wide, but the average dimensions are 2 $\mu$m long and 0·75 $\mu$m wide.

The lamellae are regularly spaced with gaps of about 40 nm between adjacent vesicles. The space is occupied mainly by small granules, which appear to be similar to the fine granular component of the adjacent cytoplasm. In some sections there is an indication of regularly arranged 7·5 nm granules between the lamellae, although these are not always observed. At the edges and ends of the lamellar body a few ribosomes are found between the vesicles. Ribosomes are very clearly seen in the glancing section of the edge of a lamellar body shown in Fig. 9, where the ribosomes can readily be distinguished from the other particulate matter by their larger size (15 nm). The flattened vesicles or disks which make up the lamellar body are about 16 nm thick, most of this being accounted for by the two membranes each of approximately 7 nm. The space in the vesicles is very small and the membranes often appear to be pressed together, although this appearance is in part due to oblique sectioning (as in Fig. 10). The edges of the vesicles are slightly inflated and there are occasional connexions between adjacent or next-but-one vesicles. From serial sections it is clear that the connexions are short but they are probably staggered, so that there is continuity from one end of the lamellar body to the other. One puzzling feature is the way in which the connexions are often, but not always, found at only one side of the lamellar body, as for example in the long lamellar body shown in Fig. 8 where 12 connexions are seen on one side and none on the other.

There is no membrane or envelope surrounding the lamellar body although at times a loose array of endoplasmic reticulum (ER) vesicles is seen adjacent to the body (Fig. 4). At the edges and ends of the lamellar body there are direct connexions between the vesicles and the ER. In glancing sections (Fig. 9) it is impossible to tell where ER ends and lamellar body starts. However, in many sections no connexions with the ER are seen along the edges of the lamellar body and this suggests that such connexions may be mainly localized at the ends. The lamellar body is frequently situated so that one end almost touches the eyespot (Figs. 3, 4, 11), but no organic connexion has been found between the two organelles.

*Flagellar bases, roots and the pusule*

The relationship between the eyespot, lamellar body and flagella is illustrated in diagrammatic form in Fig. 2. The basal bodies of the two flagella lie beneath the eyespot and usually above the lamellar body and they appear to be almost surrounded

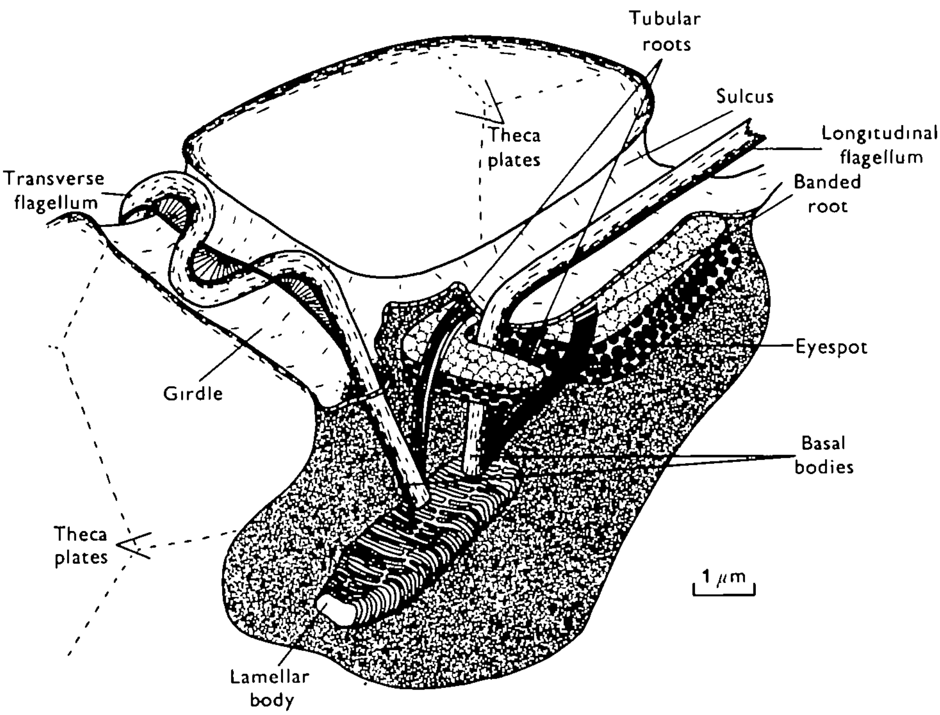


Fig. 2. Three-dimensional diagram illustrating the origin of the two flagella just above (ventral to) the lamellar body. The tubular roots originate from the lower part of the basal bodies and terminate beneath the theca or join the subthecal microtubular system. The banded root curves up from the basal body of the longitudinal flagellum and ends at the theca. The eyespot lies beneath the sulcus and contains osmiophilic granules. Some of the plates of the theca which covers the cell are shown.

by these organelles (Fig. 13). The two bases are at a slight angle to one another and the longitudinal flagellum passes through a gap in the eyespot (Figs. 1, 2). The flagella enter separate canals situated at the junction of the sulcus and girdle.

Attached to the flagellar bases are roots, of which each flagellum appears to have a different and separate provision (Fig. 2). Both flagella have a single band of 10–30 microtubules, oriented at an angle to the flagellum, which run from the base of the basal body to terminate under the theca or to join the subthecal microtubular system, which in *Glenodinium* consists of rows of 4–6 microtubules. In Fig. 12 one of these roots is shown in longitudinal section and in Fig. 13 both are shown in roughly transverse section. These roots pass very close to the eyespot and may even pass through it.

The longitudinal flagellum also possesses a banded root which commences at one side of the foot of the basal body (Fig. 13) and passes, at a different angle from that of the tubular root, towards the theca. This root passes very close to the eyespot and may connect with it (Figs. 2, 12). In section it is seen to consist of two areas of rather amorphous looking dark-stained material separated by three light bands, two of them being narrow and one wider (Fig. 12). In structure this root is very similar to the root system of *Amphidinium carteri* (Dodge & Crawford, 1968) but its orientation is rather different.

In the flagellar base region we also find parts of the pusule, a typical dinoflagellate organelle of unknown function, which opens to the exterior via the flagellar canals. In *Glenodinium* it consists of a system of branched vesicles in which each branch is of more or less equal diameter. The vesicles are surrounded by three membranes (Figs. 3–5) and contain numerous vesicular structures each surrounded by two membranes. No connexion has been observed between the pusule vesicles and the lamellar body or eyespot, although they do lie adjacent to each other at times (Fig. 5).

## DISCUSSION

The literature contains very little information about the simple type of dinoflagellate eyespot. No doubt one reason for this is that perhaps less than 5% of these organisms possess eyespots. Those having eyespots are almost all freshwater species. Biecheler (1952) described rather accurately the form of the eyespot in *Glenodinium foliaceum* (as *Peridinium foliaceum*) and its position in the cell. Prager (1963) observed a strong birefringence of the eyespot granules. The remaining reports for other dinoflagellates in which eyespots have been observed simply note the red colour, the position, which is always near the head of the sulcus, and the shape, which usually has been shown as elliptical or narrowly triangular.

In its fine structure the eyespot of *Glenodinium* is not markedly different from the eyespots of other algae. Like them it consists of close-packed lipid droplets containing carotenoid pigment and, although not now associated with the chloroplasts, presumably has derived from a chloroplast. Unlike the eyespot of the Euglenophyceae, which is a loose aggregation of individual membrane-bound granules situated near the flagella, the dinophycean eyespot is a unified structure bounded by a distinct envelope.

The eyespots of the Chrysophyceae show clear association with flagella. They are always situated near to the flagella and in the case of *Chromulina* (Rouiller & Fauré-Fremiet, 1958; Belcher & Swale, 1967) there is a close spatial relationship between the eyespot and a small non-emergent flagellum. In both of the described species the eyespot is still a part of the chloroplast but in *Chromulina placentula* (Belcher & Swale, 1967) it is attached by only a narrow process.

What really makes the eyespot of *Glenodinium* unique is its association with the lamellar body. Although this association would appear to be purely spatial it is a fact that the lamellar body is not present in the twenty or so other dinoflagellates which we have examined and which also lack eyespots. No similar structure has been reported from any other algae—with or without eyespots. In trying to find analogues of this structure one cannot avoid noting a structural similarity between the lamellar body and the outer segments of the retina in the vertebrate eye. In the outer segment piles of disks make up the main part of the rods and each disk is 1–1·5 $\mu$m wide and approximately 12·5 nm thick (Nilsson, 1965). These dimensions are clearly in the same range as those of the lamellar body. Vertebrate cones differ from rods in that the disks are joined to the plasma membrane surrounding the cone and thus the structure resembles repeatedly folded plasma membrane (Nilsson, 1965). The number of disks in the retina is much larger than in the lamellar body, being up to 1900 in rods and 450 in cones.

It is rather strange that the ocellus found in dinoflagellates of the order Warnowiaceae, which has been long thought to be a primitive eye, has a structure almost entirely different from the eyespot of *Glenodinium*. There would seem to be no homology between what is found in *Glenodinium* and the lens and retinoid of *Nematodinium* and *Erythropsis* (Mornin & Francis, 1967; Greuet, 1965) except for the single layer of pigment granules which form the base of the ocellus. This could be thought similar to the pigment granules of the eyespot.

The function of eyespots is generally considered to be associated with phototaxis. However, an eyespot clearly is not essential for this as a great many algae lacking eyespots still show a marked phototactic response. In the dinoflagellates Halldal (1958) plotted the action spectra of *Peridinium trochoideum*, *Gonyaulax catenella* (which were identical, with a peak of 475 nm) and of *Prorocentrum micans* (peak at 570 nm), none of which have eyespots. Halldal suggested that the most photosensitive region is situated at or near the flagellar bases.

Considerable controversy has existed since the early experiments of Engelman (1882) over whether the eyespot is a true photoreceptor or whether it simply acts as a carefully positioned shade over a photoreceptor. The latter hypothesis, whilst being feasible in *Euglena* where the photoreceptor is thought to be the flagellar swelling (evidence reviewed by Leedale, 1967), is untenable in members of the Chlorophyceae, where the eyespot is merely a small part of the chloroplast with nothing distinctive to shade. However, in *Glenodinium* we again find a structure, the lamellar body, which is usually situated beneath the eyespot and which could conceivably be the photoreceptor. Thus, light which falls on the organism normal to the plane of insertion of the flagella could pass through the gap in the eyespot and thus exert a directional stimulus upon the photoreceptor (Fig. 2). The next problem is how this stimulus could be transmitted back to the flagella in the absence of clear connexions. However, this transmission could presumably be effected by diffusion of a chemical.

In this connexion it is of considerable interest that in the vertebrate retina a reduced flagellum (the ciliary stalk) is situated between the outer segment with its pile of disks and the inner segment with its mitochondria. This cilium presumably conveys stimuli in much the same way as the basal bodies in *Glenodinium* would pass on stimuli to the free parts of the flagella and thus elicit a rapid phototactic response.

Thus, at present it is impossible to give a complete account of the functioning of the eyespot of *Glenodinium* and its attendant organelles. It is unfortunate that the smallness of the organism makes it difficult if not impossible to carry out micro-experiments with light beams and cell surgery.

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Figs. 3, 4. Two of a series of oblique longitudinal sections (some distance from each other) through the eyespot (*es*) and lamellar body (*lb*). Note the position of the flagellar bases (*b*) and banded flagellar root (*br*) and sections of the pusule (*p*). Part of the nucleus (*n*) can be seen in the bottom left-hand corner and the cell covering or theca can be seen at the top. ×21000.


b
br
b
lb
es
p
3

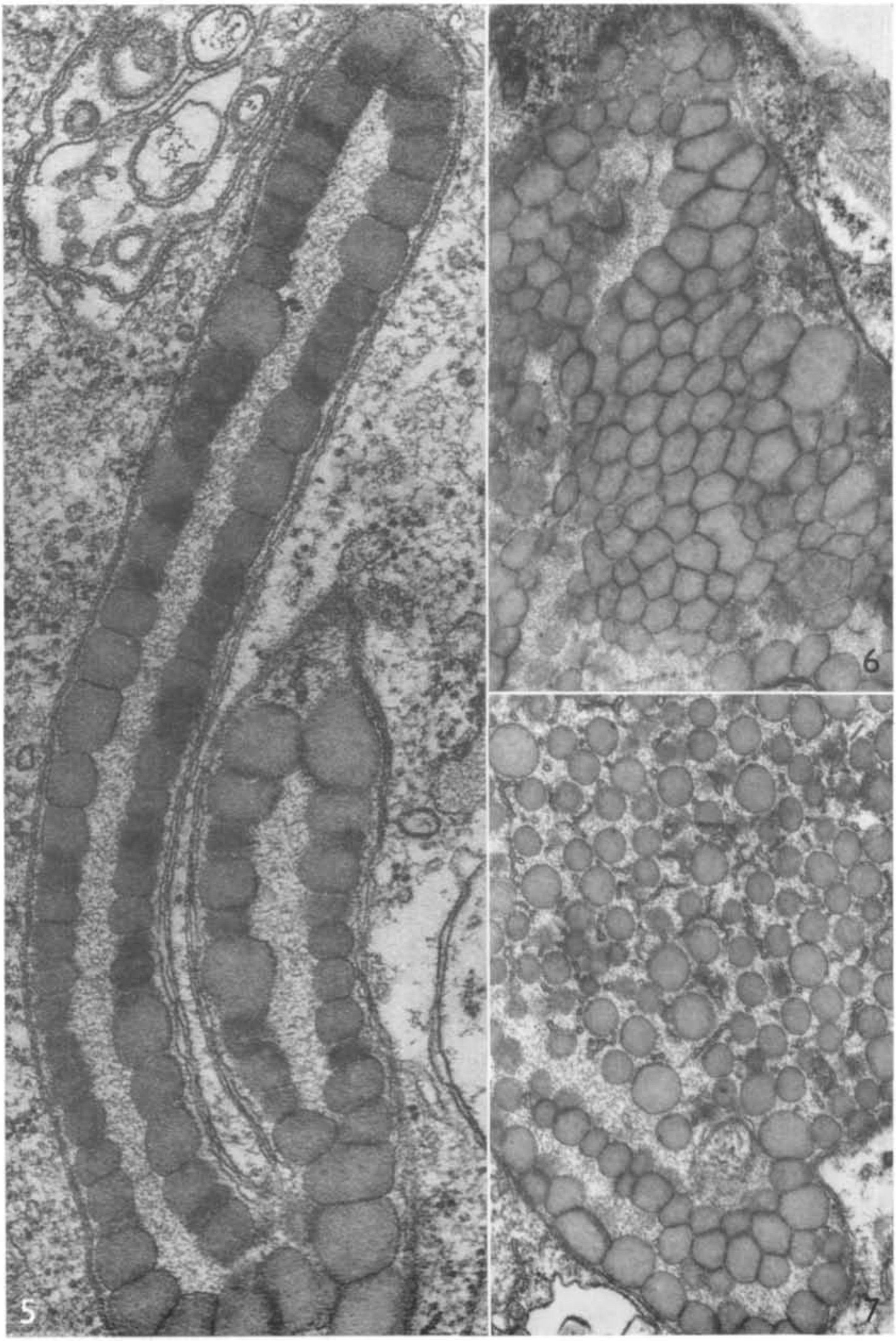

br
lb
p
es
n
4

Figs. 5–7. The eyespot.

Fig. 5. A transverse section through a folded eyespot showing the 2 rows of pigment-containing granules and the 3 membranes which surround the structure. × 70000.

Fig. 6. A tangential section through a pigment layer of an eyespot, showing hexagonal close-packing of the granules. × 36000.

Fig. 7. A similar section to Fig. 6 but through a less closely packed portion of the eyespot. Small tubular structures can be seen between many of the granules. × 40000.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Figs. 8–10. The lamellar body.

Fig. 8. A median longitudinal section of a large lamellar body consisting of 50 flattened vesicles. Note the occasional connexions along the left-hand side of the lamellar body. × 62 500.

Figs. 9, 10. Two more of the same series of sections as is shown in Figs. 3, 4, through a single lamellar body. Fig. 9 is a glancing section through the edge of the lamellar body (*lb*) to show the many ribosomes associated with the membranes, the connexions between the vesicles and the clear connexion, at the top, with the rough endoplasmic reticulum (*er*). × 60 000. Fig. 10 (at lower magnification) shows the more normal appearance of the same lamellar body in a median section. In this position it is almost wedged between two parts of the eyespot (*es*). × 35 000.

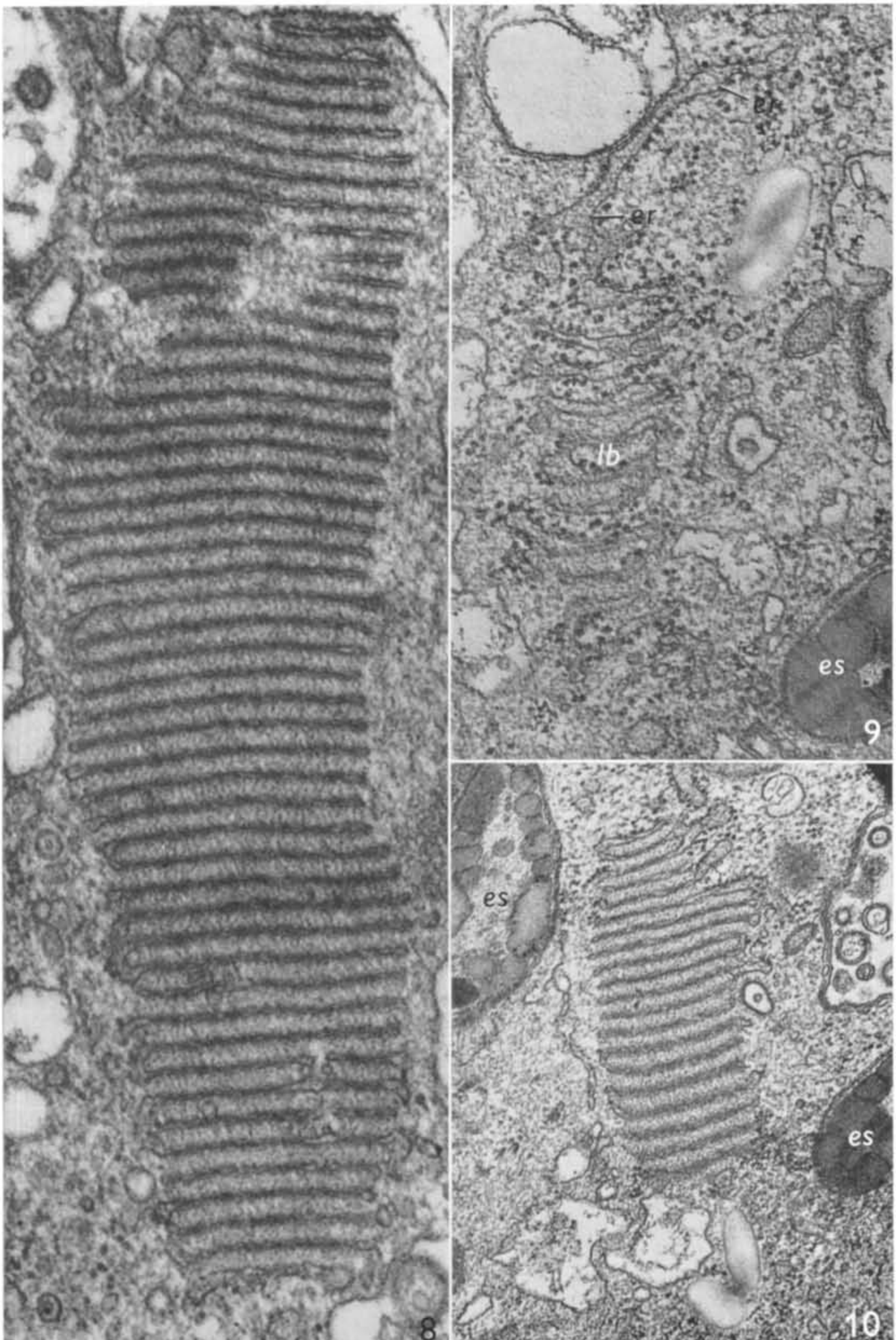
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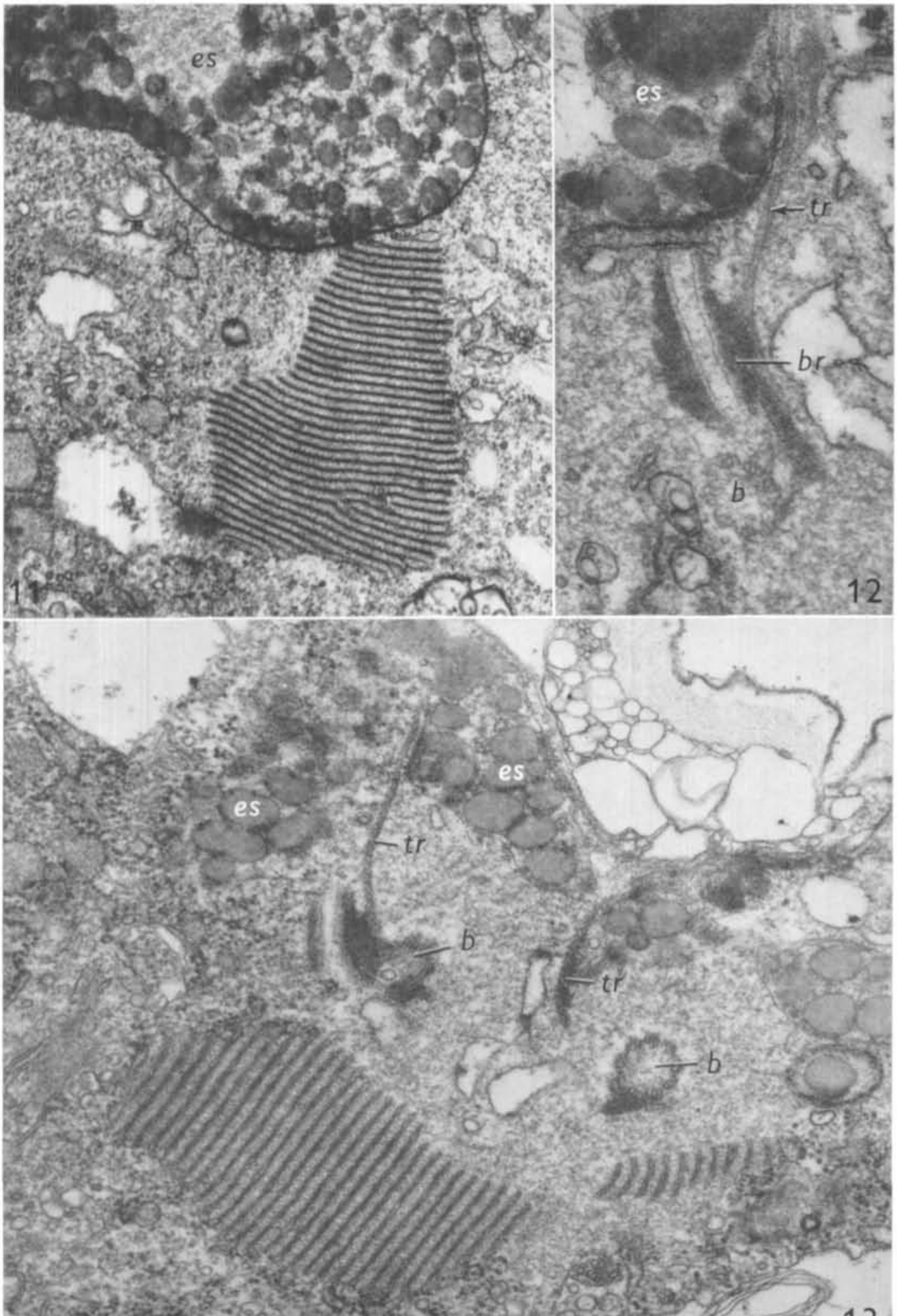
Fig. 11. A close association between a somewhat degenerate eyespot (*es*) and a very wide lamellar body. × 30000.

Fig. 12. A transverse section of a flagellar base (*b*) a little above its foot, showing the tubular root (*tr*) and banded root (*br*) now a little distance from the flagellum but in close proximity to the eyespot (*es*). × 60000.

Fig. 13. An oblique longitudinal section which cuts the flagellar bases (*b*) almost transversely and shows the tubular roots (*tr*) passing through the eyespot (*es*) towards the theca. No connexion is seen between the flagellar bases and the lamellar body. × 36000.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