The Fine Structure of Some Retinal Photoreceptors*

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PLATES 27 TO 29

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ABSTRACT

An electron microscope study has been made of octopus and amphibian photoreceptors, after fixing with $KMnO_4$ and embedding in araldite. What has previously been seen as a single dense stratum bounding the tubular compartments (octopus) or the double membrane discs (rods and cones), now shows a double structure. We interpret this as showing that these tubules and discs have similar bounding surfaces, which are probably directly related to the cell membrane. This is confirmed by the finding that the tubules and discs are (at least occasionally) continuous with the cell membrane.

The structures in the vertebrate eye that contain the visual pigments, and which consequently play an initial role in the conversion of light energy into nerve impulses, are the outer segments of the rods and cones (Figs. 1, 2). Sjöstrand (14) has shown that they contain piles of so called "double membrane discs," having the appearance of flattened sacs, $\sim$ 150 A thick and 200 to 500 A apart, which are arranged perpendicular to the light path. Throughout this paper the more general term "lamella" is used, instead of "double membrane disc." In OsO_4-fixed preparations, each lamella appears to be bounded by a single dense surface stratum, and appears to contain only a homogeneous material, about as dense as the cytoplasmic matrix (Text-fig. 1 *a*).

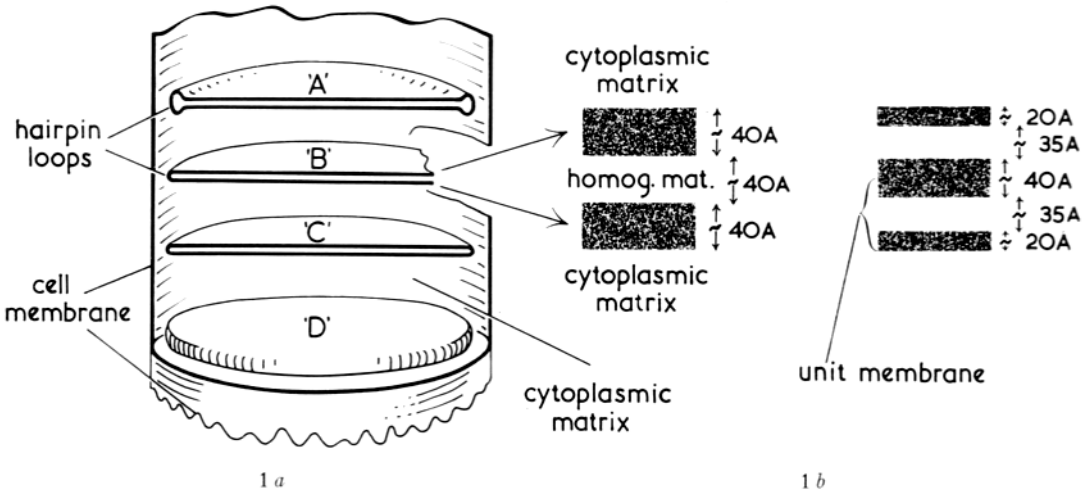
The corresponding photoreceptor structure in arthropods has been identified (16) as the rhabdome, a refractile structure formed at the borders of the pigmented retinula cells. Electron microscope studies (6, 8, 9, 19) of various arthropod rhabdomes have shown them to consist of close packed hexagonal arrays of "tubular compartments" or "tubules," $\sim$ 300 to 1000 A in diameter, arranged approximately perpendicular to the light path (Text-fig. 2 *a*). Miller (9) and Fernández-Morán (6) have provided evidence for believing that these tubular compartments are microvilliform extensions of the retinula cells. Fernández-Morán (6) has shown that the contents of each of the tubules is bounded by an $\sim$ 20 to 30 A thick dense stratum, separated from the corresponding strata of adjacent tubules by an $\sim$ 60 A wide gap (Text-fig. 2 *a*). The receptor layer of the retina of the squid and octopus contains structures whose arrangement and dimensions have, in the past, caused them to be thought of sometimes as rods, and sometimes as rhabdomes. However, their fine structure has recently been shown to be much more analogous to that of the arthropod rhabdome: they contain a somewhat similar arrangement of tubules (of about the same dmensions), and they are adjacent to cells which are similar to arthropod retinula cells (17, 18).

We have studied $KMnO_4$-fixed and araldite-embedded preparations of octopus "rhabdomes" and amphibian (frog and toad) rods and cones. In this note, we are concerned with (*a*) the fine structure of the membranes of the tubules of the octopus rhabdome, (*b*) the nature of the lamellae in amphibian rods and cones, (*c*) the relationship of the tubules and lamellae to cell surfaces.

OBSERVATIONS AND COMMENTS

1. Figs. 5 and 6 show that the closely packed tubular compartments of the octopus "rhabdome" are each bounded by separate cylindrical dense layers. The general arrangement, disregarding details of membrane structure, is like that found

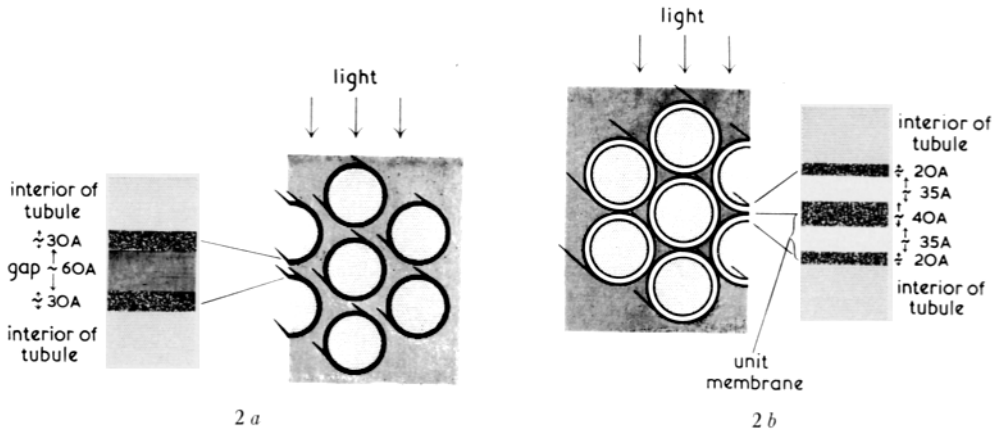
* The substance of this communication was presented as a Demonstration at the Symposium on Electron Microscopy held at University College, London on April 16–17, 1959.



1 *a* 1 *b*

TEXT-FIGS. 1 *a* and 1 *b*. Cut-away view of a rod outer segment, summarising the main findings of previous workers in 1 *a* and including our new findings in 1 *b*. Four lamellae are shown, '*A*', '*B*', and '*C*' bisected, and '*D*' intact. The lamellae are completely surrounded by the cytoplasmic matrix of the outer segment (though some authors have found them to be touching the cell surface membrane along part of their rim) (*homog. mat.*, homogeneous material.)

'*B*' is enlarged on the right (Text-fig. 1 *b*) to show the layers within each lamella, as described by previous workers. In *b* the more detailed structure of the lamella, as derived from the present work, is included.

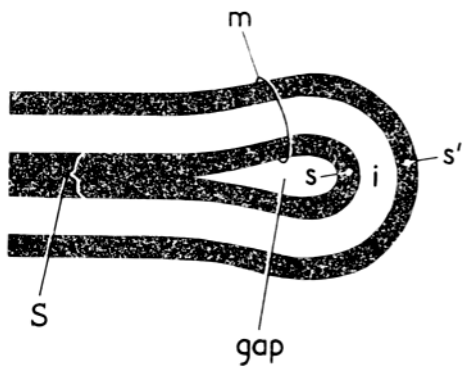


2 *a* 2 *b*

TEXT-FIG. 2 *a*. Diagram of the close packed tubules of the insect rhabdome, constructed from a description by Fernández-Morán (6) of OsO_4-fixed preparations. The details of the membranes of two neighbouring tubules are shown enlarged on the left. The 20 to 30 A thick dense surface strata of neighbouring tubules are separated by an $\sim$ 60 A gap.

TEXT-FIG. 2 *b*. The close packed tubules of the octopus "rhabdome," as found by us (Fig. 5) in $KMnO_4$-fixed preparations. The details of the membranes of two neighbouring tubules are shown enlarged on the right. Additional structural detail (described in the text) is seen between the tubules: instead of three strata, five are now distinguished.

by Fernández-Morán (6, Text-fig. 2 *a*) in the insect rhabdome. The surface membrane of each tubule is not, however, a single dense line $\sim$ 20 to 30 A thick as described by Fernández-Morán (6), but rather two parallel dense lines, each $\sim$ 20 A thick, separated by a narrow gap of $\sim$ 35 A, the whole structure forming a "unit membrane" (see p. 89). Where the outer dense lines of adjacent



TEXT-FIG. 3. Diagram of the hairpin loop at the edge of a lamella. Compare with Fig. 4 (inset), and refer to text for lettering.

tubules touch, they adhere to give a dark, thickened layer (Text-fig. 2 *b*). This layer, which occurs in the middle of the "gap" between neighbouring tubules, has not, to our knowledge, been described by previous investigators. It has also been seen by us in some OsO_4-fixed preparations.

2. Previous observations on the lamellae of vertebrate rods and cones have shown them to contain, inside their single dark bounding layer, only a homogeneous material about as dense as cytoplasmic matrix (Text-fig. 1 *a*). Figs. 3 *b*, 4, 7, and 8 show that there is a dark layer or stratum in the middle of this homogeneous material (Text-fig. 1 *b*).[1] This is shown as *S* in Fig. 4 (inset). At the edges of a lamella the outer ~20 A dense strata (*s'* in Text-fig. 3) are continuous in a hairpin loop. The central dense stratum (*S*) is seen to be formed by contact of two continuous ~ 20 A dense strata (*s*) running parallel to the outer stratum (*s'*) and separated from it by a light interzone (*i*) ~ 35 A across. These relations are shown in Fig. 4 (inset). Occasionally the two central dense strata (*s*) are seen separated in other places, *e.g.*, *g* in Fig. 4. It appears that the ~ 150 A thick lamellae are formed by the adhesion of two ~ 75 A thick "membranes" (*m* in Text-fig. 3), each consisting of two parallel dense lines separated by a narrow gap.

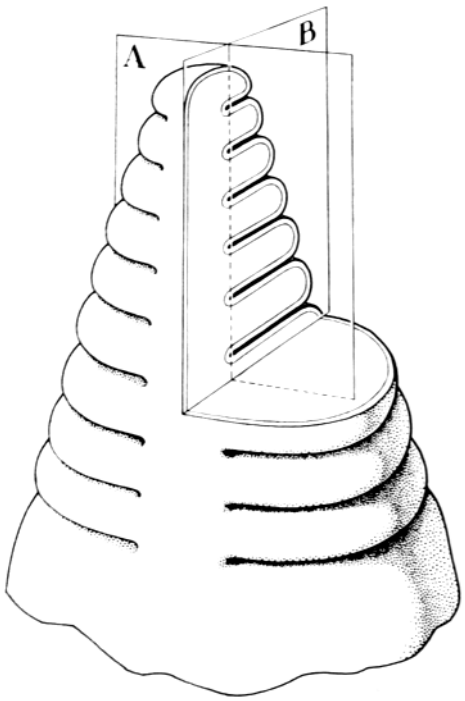
Recently some new criteria have been set up for defining cell or plasma membranes (10–13). A structure consisting of two parallel dark lines ~ 20 A thick separated by a light zone ~ 35 A across has been found to be characteristic of all cell membranes and their derivatives after $KMnO_4$ fixation and (though less frequently) OsO_4 fixation. This structure, the "unit membrane," is clearly identical with the "membranes" (described above) bounding photoreceptor lamellae or tubules. This alone suggests that these structures might be derivatives of the cell membrane. We have found that the surfaces of the lamellae and tubules are often *continuous* with the cell membrane. Therefore, all these structures are considered to be derivatives of the cell membrane.

Unit membranes may adhere along either their inner or outer surfaces, forming "internal" or "external" compound membranes, respectively (12). Our evidence indicates that the compound membranes of the vertebrate photoreceptors studied are of the external type. These external compound membranes, shown consistently and clearly after $KMnO_4$ fixation, are less often clearly visible after fixation with OsO_4, because the middle dense stratum is not always well preserved. It is interesting to compare this with a similar situation encountered in myelin. Each myelin lamella is an external compound membrane joined to its neighbours in the compact myelin structure. The intraperiod line of myelin corresponds to the central dense stratum of free external compound membranes. After $KMnO_4$ fixation the intraperiod line of myelin is always clear and distinct (7, 10–13). After OsO_4 fixation, though the period remains the same, the intraperiod line often is not seen at all, or appears as irregular granules. An anologous situation exists in photoreceptor structures.

3. Miller (9) has presented evidence for believing that the tubules seen in the rhabdome of the horseshoe crab (*Limulus*) are actually microvilli of the retinula cells. Fernández-Morán (6) has found a similar structure in some insect rhabdomes. Although the tubule system of the octopus "rhabdome" is highly complex, we have evidence that some of the tubules are microvilli of the retinula cells. This indicates that the ~ 150 A compound membranes are of the external type.

The thickness and separation of the membranes forming the lamellae in vertebrate photoreceptors is similar in rods and in cones, though Sjöstrand (14) and De Robertis (3) have found some differences. In the frog and toad retina, we have found the lamellae of the rods and cones to be identical, in so far as they are both formed by the apposition of two unit membranes. However, in agreement with others, (5, 14) we have noted that the rod

[1] Fernández-Morán (*Science*, 1959, **129**, 1285) states in an abstract that he has observed a central dense stratum of comparable thickness in this place.



TEXT-FIG. 4. Diagram of the outer segment of an amphibian cone. The lamellae are formed by intuckings of the cell membrane. Sections in plane A, *e.g.* Figs. 1, 7 "*a*," would show in-tuckings on both sides of the section; those in plane *B*, *e.g.* Figs. 3, 7, would show these only on one side.

lamellae often have deep incisions which appear to be lacking in the lamellae of the cones. Fernández-Morán (5), in studies of fragmented frog rods, showed that groups of lamellae are often oriented with their corresponding incisions directly above one another. We have found this orientation to occur over considerable distances, giving rise to long channels in longitudinal sections (Fig. 4).

There is a further important difference between the rods and cones in the relation of the discs to the rest of the outer segment. In longitudinal sections of rod outer segments, the great majority of the lamellae show no connection, in the plane of section, with the surface membrane (Fig. 2). However, part of the rim of *every* cone lamella is joined to the cell membrane of the outer segment (Figs. 1, 3 *a*, 3 *b*, and 7). In every specimen we have examined, all the lamellae of the cone are in-tuckings of the surface membrane, as shown in Text-fig. 4.[2] Every longitudinal section of the outer segment of a cone corresponds to either plane *A* (Figs. 1, 7 *a*) to the cell membrane of the outer segment (Figs. 1, or plane *B* (Figs. 3 *a*, 7) in Text-fig. 4. So the cone lamellae, being formed by the adhesion of the *outer* surfaces of two unit membranes, are *external* compound membranes.

Although the great majority of rod lamellae seem to be unconnected with the cell surface, occasional ones, at the base of the outer segment, can be seen to be formed from the cell surface as in-tuckings (Fig. 8). Since it would appear rather unlikely that there are two distinct mechanisms for the formation of rod lamellae, we suspect that all the lamellae form as in-tuckings of the cell surface. It cannot yet be decided whether the area of connection disappears by a "pinching-off" process (making isolated lamellae), or whether it persists, but is confined to so small a region that the plane of section rarely passes through it. The mechanism suggested by our findings differs from that postulated by Carasso (1) and De Robertis (2). According to their hypothesis, the lamellae are formed by the progressive fusion of intracytoplasmic vesicles.

Studies of the dichroism of frog rods, *e.g.* reference 4, have indicated a high degree of parallel orientation among the rhodopsin molecules, which suggests that they are attached in or very near the lamellae. At least in the case of cones, the lamellae are continuous extensions of the limiting membrane of the visual cell. The in-tucking mechanism thus provides a direct morphological continuity between the site of light absorption (the lamellae), and the cell membrane of the visual cell. This continuity of the cell membrane may be important in the transmission of the signal after light reception.

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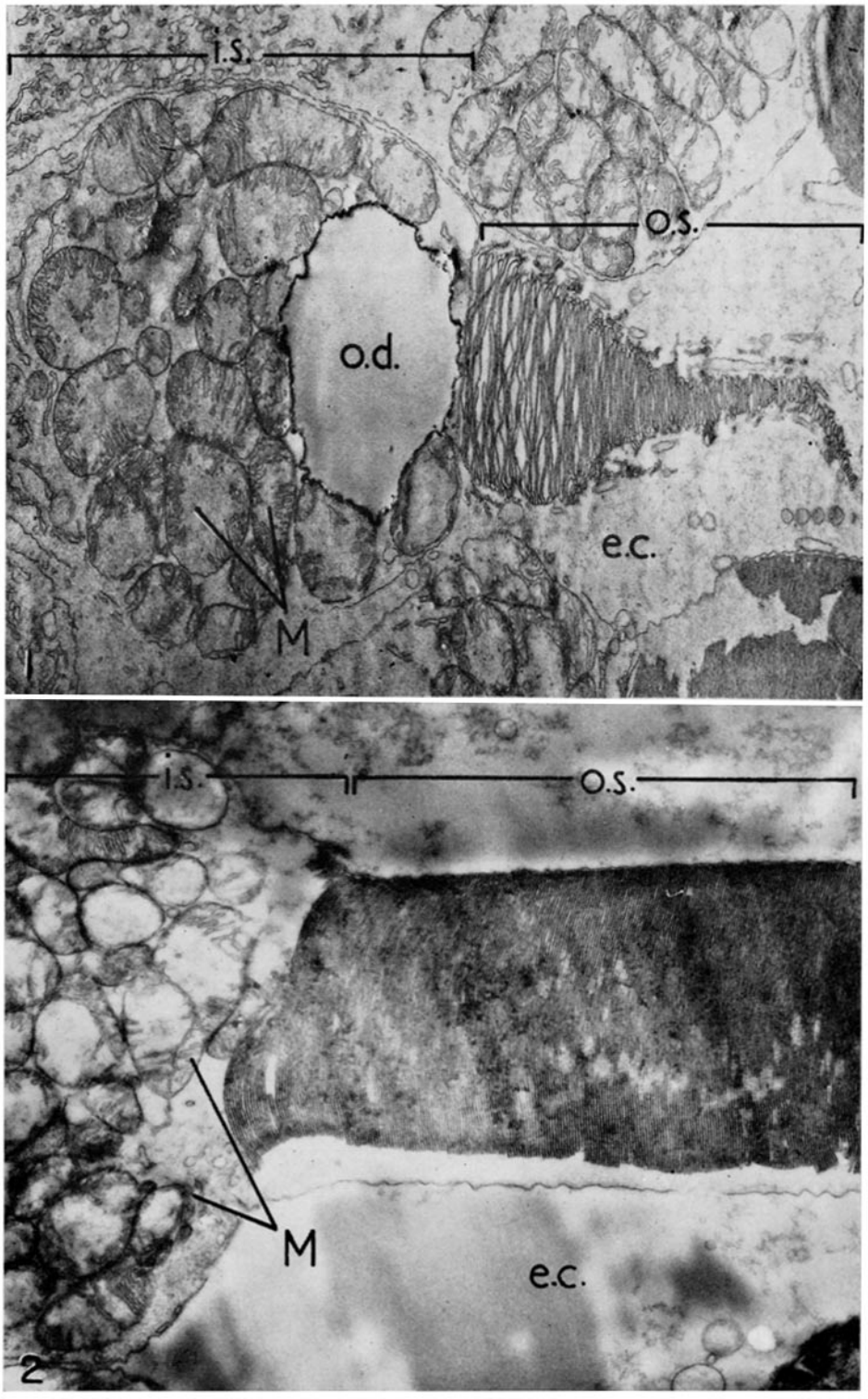
EXPLANATION OF PLATES

PLATE 27

FIG. 1. General view of part of the inner segment (*i.s.*), oil drop (*o.d.*), and outer segment (*o.s.*) of a frog cone. The light travels from left to right. The outer segment contains piles of lamellae, arranged perpendicular to the light path. On either side of the cone are two rod cells. (*M*, mitochondria; *e.c.*, extracellular space.) All figures are of $KMnO_4$-fixed preparations. × 14,000.

FIG. 2. Part of the inner segment (*i.s.*) and outer segment (*o.s.*) of a frog rod. The light travels from left to right. The outer segment contains piles of lamellae arranged perpendicular to the light path. × 13,000.



(Moody and Robertson: Fine structure of retinal photoreceptors)

PLATE 28

FIG. 3 *a*. The outer segment of a frog cone, showing an area of continuous cone cytoplasmic matrix (*cyt.*) on the periphery of which many lamellae (*l.*) are formed from in-tuckings (*i*) of the cell membrane. The cone is completely surrounded by an extracellular region (*e.c.*). The dotted rectangular region is enlarged in Fig. 3 *b*. × 37,000.

FIG. 3 *b*. Enlarged portion of Fig. 3 *a*, showing the unit membrane structure of the lamellae and their formation as in-tuckings (*i*) of the cell surface. The arrow indicates the hairpin loop (*h.l.*) in which the unfused central layers can be most clearly seen. × 120,000.

FIG. 4. Part of the outer segment of a frog rod (the direction of the light path being from left to right). The plane of section includes an incision passing through a whole series of lamellae. The lamellae bordering the cytoplasmic matrix of the incision show hairpin loops (arrows and inset enlargement). The alignment of the discontinuities forms a continuous channel (*c*), showing that the lamellae are piled with the incisions above one another. × 130,000. The details in the inset enlargement (× 310,000) are discussed in the text.

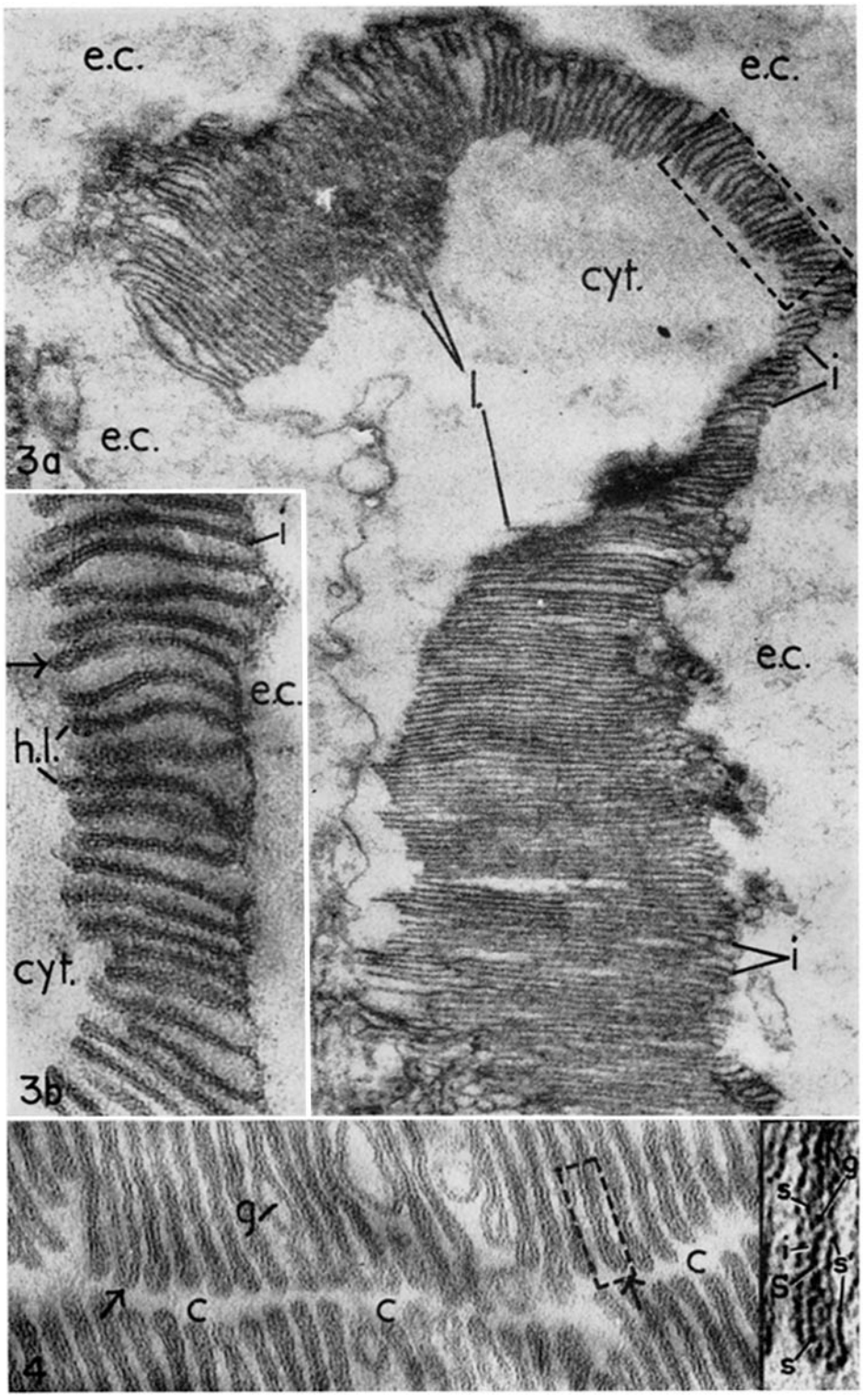


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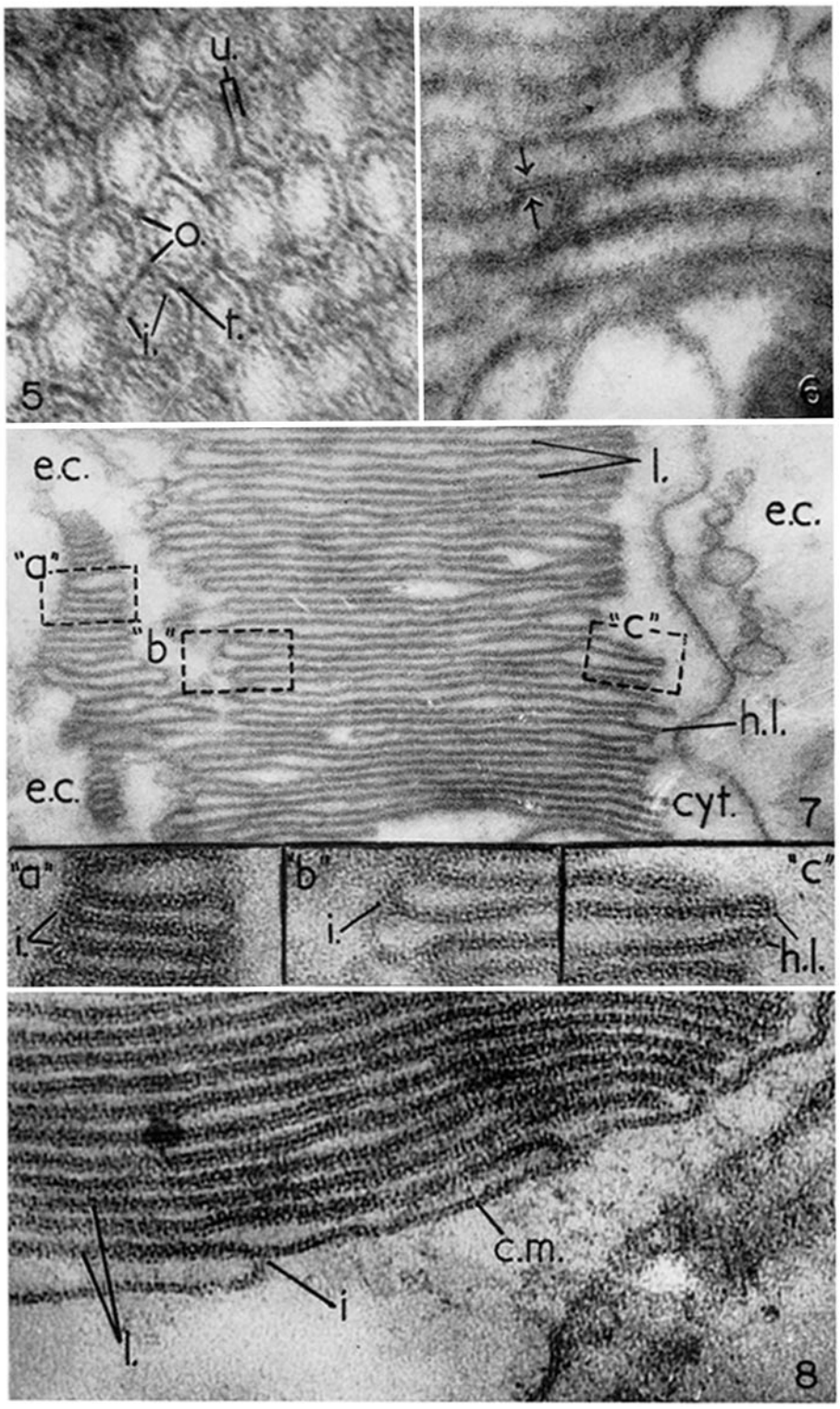
PLATE 29

FIG. 5. Cross-section of the octopus "rhabdome" showing tubules $\sim$ 500 A in diameter close packed in hexagonal array. Each tubule is bounded by a "membrane" (*u.*), composed of two parallel dense strata, an outer (*o.*), and an inner one (*i.*). The outer strata of contiguous tubules adhere to give a dark, thickened layer (*l.*). $\times$ 260,000.

FIG. 6. Tubules of the octopus "rhabdome" seen in longitudinal section. The compound membranes at the junction of the tubules can be seen between the aligned arrow tips. They are usually absent because the highly curved surface membranes of the tubules lie only occasionally at right angles to the plane of the section. $\times$ 210,000.

FIG. 7. A frog cone, showing the formation of the lamellae (*l.*) by in-tuckings (*i.*) of the cell membrane from the left. ("*a*", "*b*", and "*c*" are the corresponding inset enlargements below). "Hairpin loops" (*h.l.*) are formed at the cytoplasmic borders of the lamellae (note inset enlargement of "*c*"). The group of lamellae to the right has a continuous layer of cytoplasmic matrix (*cyt.*), and corresponds to plane "*B*" of Text-fig. 4. $\times$ 69,000.

FIG. 8. The outer segment of a rod (frog), showing detailed structure of the lamellae (*l.*). The cell membrane (*c.m.*) forms a lamella at an in-tucking (*i*). $\times$ 160,000.



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