THE VISUAL CELLS AND VISUAL PIGMENT OF THE MUDPUPPY, *NECTURUS*

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ABSTRACT

Electron microscopy of the visual cells of the mudpuppy *Necturus* have revealed several new or hitherto neglected features of organization: (a) A system of deeply staining micelles in virtually crystalline array, probably located in the lamellae of the rod outer segments. These particles may contain the visual pigment, porphyropsin. Counts of the micelles, and microspectrophotometric measurements of porphyropsin in the retina and single rods yield the estimate that each lamellar micelle may contain about 50 molecules of porphyropsin. (b) Systems of about 30 cytoplasmic filaments (here called *dendrites*), continuous with the cytoplasm of the inner segment, and standing like a palisade about the outer segments of the rods and cones. In the rods, one such filament stands in the mouth of each of the approximately 30 deep fissures that carve the outer segment into a radial array of lobules. (c) A system of deeply staining particles in the membranes of the dendrites, and another in the membranes of the pigment epithelial processes. It is suggested that these may have a part in interchanges of material with the outer segments. The ciliary process is found to penetrate more deeply than is commonly supposed into the outer segments of the rods and cones. The edge of each double-membrane disc in rods forms a differentiated rim structure, both around the disc circumference and bordering the fissures. These anatomical arrangements are summarized in Figs. 13 and 14, and the relevant measurements in Table I. The dilution of visual pigment in *Necturus* rods and cones and a general consideration of their microstructures make it seem unlikely that such typically solid state processes as exciton migration or photoconduction can transport the effects of light far from the site of absorption. Excitation must, therefore, be conveyed to the receptor as a whole by some axial structure. Among axial structures, the plasma membrane is most likely to be the site of nervous excitation. The ciliary process probably plays its main role in the embryogenesis and regeneration of outer segments; and the dendrites and pigment epithelial processes in exchanges of material with the outer segments and perhaps with one another.

This paper reports electron microscope observations upon rods, cones, and double cones of *Necturus*, which, taken together with measurements of the visual pigment *in situ*, have consequences for the theory of visual excitation. Among the major problems of visual excitation are the relation of the visual pigment to the ultrastructure of the outer segment, and the means by which the action of light on this pigment induces a nervous response (34–38). Our observations bear upon both these problems.

They include also a number of significant structural features: (1) A palisade of cytoplasmic filaments ("dendrites") projects from the inner segments of the rods and cones so as to surround the outer segments. (2) A quasi-crystalline system of deeply staining micelles in the rod outer segments may contain the visual pigment. (3) A

system of deeply staining particles in the dendrites, and another in the processes from the pigment epithelium may play a role in functional interchanges with the outer segment. These are the main points; but, in addition, our observations extend the present view of the anatomy of the ciliary process in rods and cones; and of the special rim structure that forms the edges of the double-membrane discs in rods, both around their outer circumference, and bordering the deep fissures which in *Necturus* carve the discs into corollae of radially arranged lobules.

METHODS

ELECTRON MICROSCOPY: Whole eyes were removed from animals which were slightly light adapted by exposure to ordinary room light. They were fixed for 1 hour at room temperature in 2 per cent osmium tetroxide, buffered to pH 7.8 with veronal acetate, and containing 45 mg per ml of sucrose and 0.002 M calcium chloride. After fixation, the eyes were dehydrated in graded acetone-water mixtures, and then embedded in Araldite epoxy resin (21).

Thin sections were cut with a Porter-Blum microtome, and stained with saturated uranyl acetate in 50 per cent ethyl alcohol. They were examined in an RCA EMU-3D electron microscope operated at 100 kv.

GENERAL ORGANIZATION OF VISUAL CELLS

The general features of rod and cone structure are by now sufficiently familiar so that we can go rapidly to the special properties that most concern us. It will be helpful beforehand however, to summarize the general organization of these cells and the numbers and dimensions of their component structures.[1]

[1] The ultrastructure of visual cells has been reviewed recently by Sjöstrand (29–31), De Robertis and Lasansky (10), and Fernández-Morán (17).

The light microscopy of the visual cells of *Necturus maculosus* has been described by Howard (23), with a fidelity that can be judged by comparing the photomicrographs in Fig. 1 with his drawings. Three types of visual cells—rods, cones, and double cones—are distributed fairly evenly over the retinal surface, except that double cones seem to be absent from the extreme periphery. Palmer (28) found the *Necturus* retina to contain a total of 110,000 receptor cells in the proportions 35 rods: 28 cones: 10 double cones.

In what follows we shall state all dimensions in terms of osmium-fixed, dehydrated, and embedded preparations, in which comparison with fresh material reveals about 20 per cent shrinkage, so that the fresh dimensions are about 25 per cent larger.

The visual cells of *Necturus* are among the broadest known, being equaled in this regard only by those of a number of other salamanders.[2] The rod outer segments are roughly cylindrical in shape, averaging about 12 μ in diameter and 30 μ long. The cone outer segments are on the average about 24 μ long, tapering from about 9 μ at the base to 5 μ at the tip; and the outer segments of double cones are of about the same size.

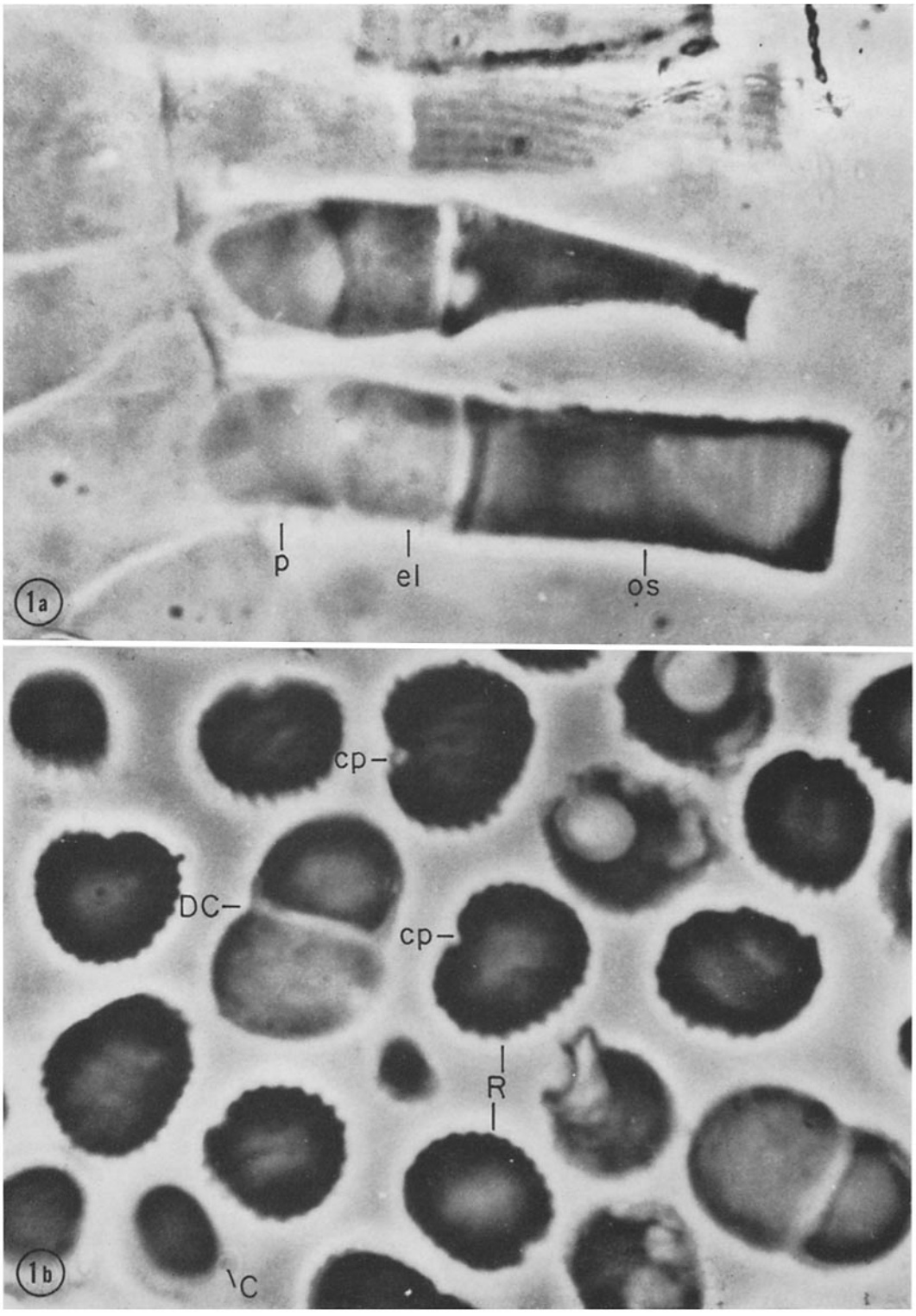
A ciliary process springing from a more-or-less typical basal body in the distal portion of the inner segment, forms the stalk or backbone structure of the outer segments (Fig. 2 *a*). Some evidence of

[2] Howard (21) cites the following dimensions of rod outer segments in amphibia: frog, 61 × 6 μ; Triton (probably a *Triturus* sp.), 24 to 28 × 12; toad, 40 to 45 × 8; *Bufo viridis*, 76 × 8; and *Salamandra*, 44 × 12 μ.

FIGURE 1 Visual cells of *Necturus* in the light microscope. Sections of retina fixed in osmium tetroxide and stained with uranyl acetate × 950.

a. Sagittal sections of 2 rods and a cone. The lowermost cone and rod display, each at the same level, outer segments (*os*); ellipsoids (*el*) (tightly packed clumps of mitochondria); and paraboloids (*p*). The uppermost rod shows the gently spiraling fissures that carve the outer segment longitudinally into radially arranged lobules.

b. Tangential section of retina at the level of the outer segments. Rods (*R*) can be recognized by their scalloped edges, the indentations indicating the mouths of the fissures. A wider cleft marks the ciliary process (*cp*). Cones (*C*) are narrower and have smooth outlines. The section contains two double cones (*DC*); one of each such pair lies more distally than the other, so that the section cuts the proximal member through its outer segment, the distal member through the less deeply staining ellipsoid.


p
el
os
1a
cp–
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R
1b
C

this appears also in cross-sections seen under the light microscope (Fig. 1 *b*).

Just below the outer segments of the rods and cones lie two prominent structures, the ellipsoids and the paraboloids (Fig. 1 *a*, *el*, *p*). The ellipsoids, as shown by Sjöstrand, are clumps of mitochondria, packed membrane to membrane in *Necturus* (Fig. 2 *b*), as in a number of other amphibia and fishes. They probably represent the densest aggregate of mitochondria to be found in these organisms. In *Necturus* they are ovoidal in long section, and tend to lie in groups oriented parallel to one another, so that the section cuts blocks of them in either cross- or long section (Fig. 14). The mitochondria of human ellipsoids display similar orientations (45). The paraboloids, when stained as here, appear relatively structureless; Yamada (43) has shown, however, that they possess a complex structure and contain large deposits of glycogen.

Perhaps associated with their great breadth, as a means of providing increased surface for exchanges of material, the rod outer segments in *Necturus* are cut into columns, radially arranged about a more or less solid center, by a series of 22 to 31 (av. 27) deep longitudinal grooves or fissures. For this reason, the rod outer segment in cross-section has a scalloped edge (Figs. 1 *b*, 3 *a*). In a surface view the fissures appear as parallel lines running the whole length of the outer segment and frequently, though not always, spiraling gently to the right or left (Fig. 1 *a*; *cf.* also Howard, 23). The outer segments of the cones and double cones lack such fissures, and their cross-sections are smooth in outline (Figs. 1 *b*, 3 *b*).

The plasma membrane of the inner segment extends so as to envelope the entire outer segment, including the ciliary process. What begins embryologically or in the regeneration of outer segments as the plasma membrane of the cilium ends as the plasma membrane of the entire outer segment.

As in other vertebrates, the bulk of the outer segment after fixation in osmium tetroxide appears to be made up of a pile of transverse, paired membranes. These are arranged differently in rods and cones. In rods, each pair of membranes is sealed around the edge by a special, bulging, deeply staining rim-structure, so as to form a closed "double-membrane disc" (Sjöstrand). A rod outer segment in *Necturus* contains about 37 such double-membrane discs per micron of length, making a total of about 1100 discs.

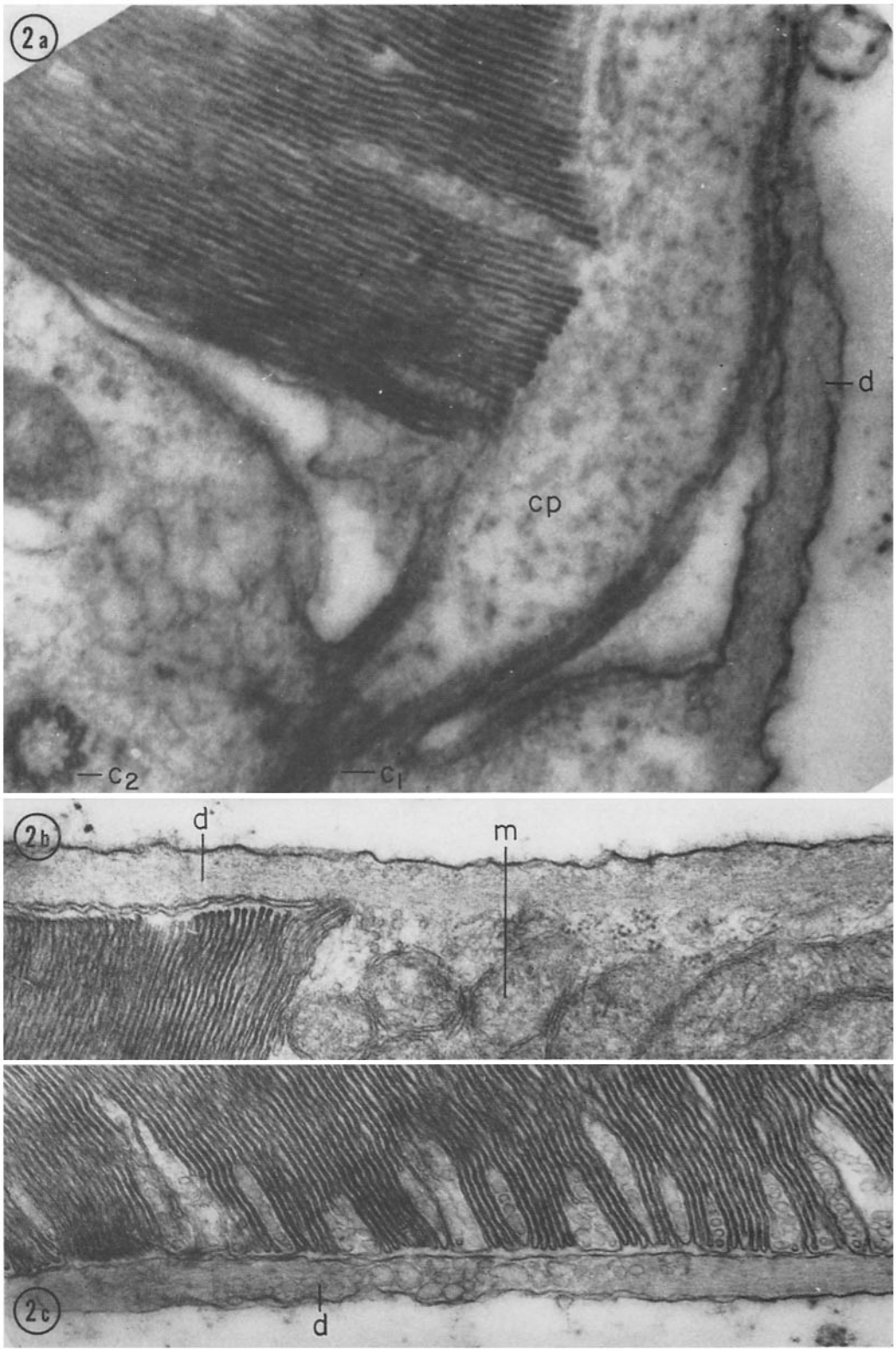
The transverse membranes of a cone or double cone have a quite different arrangement in which the plasma membrane at the side across from the ciliary process is repeatedly infolded to form the transverse double layers (Figs. 2 *c*, 3 *b*, 4). This structure is essentially the same as that first described by Sjöstrand in perch cones (33–35), and

FIGURE 2 Portions of two rods and a cone in sagittal section.

a. Rod, showing the base of the outer segment and its connections with the inner segment. The latter contains the basal body (c_1), a modified centriole from which springs the ciliary process (*cp*); the second centriole (c_2), oriented typically at right angles to the basal body; and a dendrite (*d*), continuous with the cytoplasm of the inner segment, and running up outside the ciliary process. The ciliary process bears the outer segment as on a stalk, and is its only direct connection with the inner segment. One plasma membrane envelopes the inner and outer segments, including the ciliary process, and is reflected also over the dendrites. × 61,200.

b. Rod showing mitochondria (*m*) which form the ellipsoid, and a dendrite (*d*) springing from this region. The dendrite is separated from the outer segment by two thicknesses of the common plasma membrane that envelopes both organelles. × 39,000.

c. Cone showing a portion of outer segment with a dendrite beside it. In cones the double lamellae arise by repeated infoldings of the plasma membrane on the side across from the ciliary process. On this side, therefore, the double membranes separate, instead of joining at the edge, each membrane coming around to form one of the adjoining pair. For this reason also this side of the cone lacks a separate, external plasma membrane. At various points in this section an external loop of membrane is seen to enclose one or several inner loops. At all these places, the double membranes which end in inner loops have broken into vesicles toward the edge. The formation of such loops-within-loops by vesiculation is discussed in the text. × 39,000.


2a
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cp
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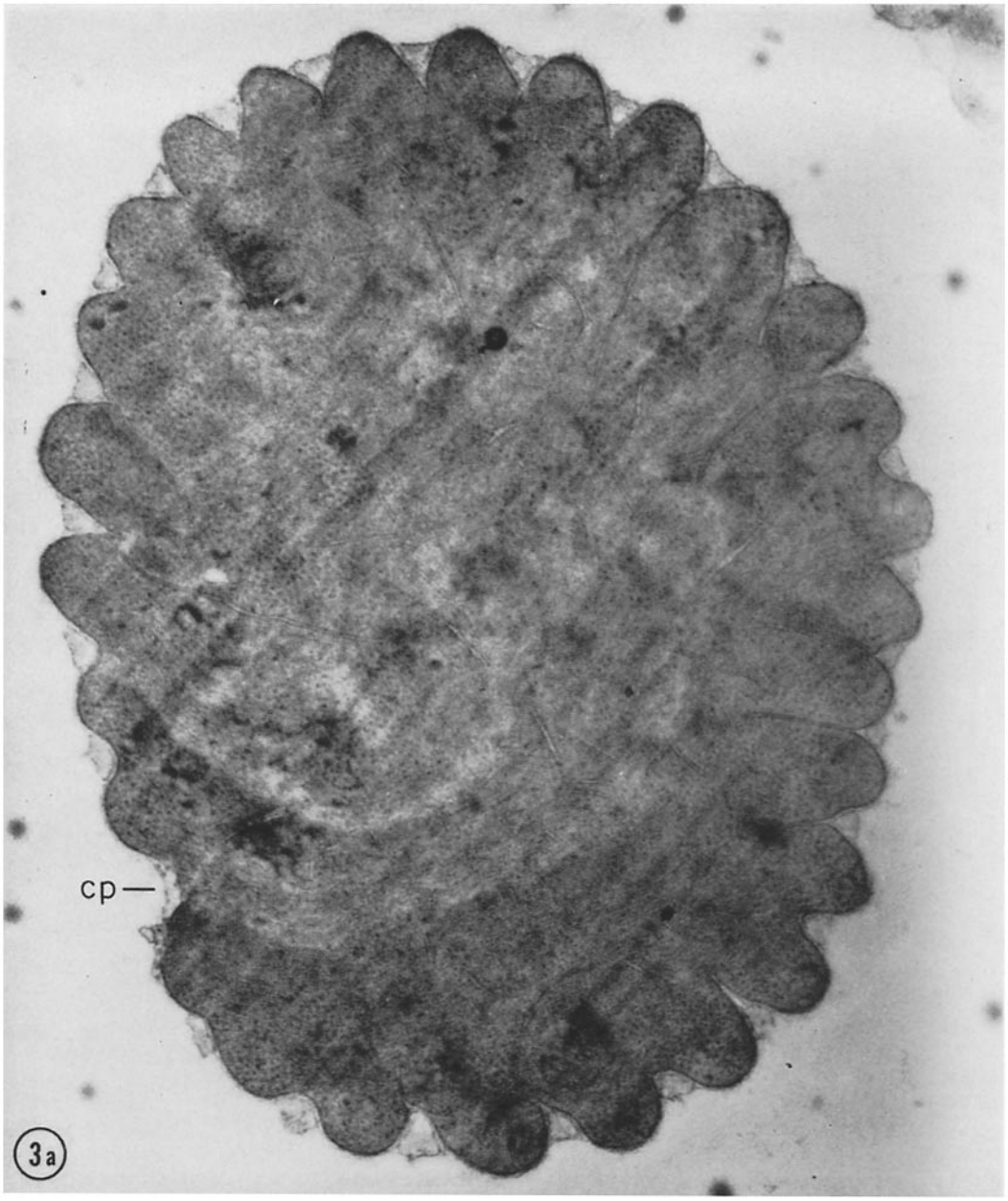


FIGURE 3 Cross-sections of *a*, a rod, and *b*, a cone outer segment. × 12,400.

a. The rod outer segment is cut into lobules by 27 fissures, a dendrite fitting closely into the mouth of each fissure. The ciliary process (*cp*) is flattened, and displays the cut ends of several ciliary filaments. On close examination, a regular pattern of deeply staining lamellar micelles can be seen to cover most of this section.

b. (*See opposite page*) The cone outer segment is smooth in outline and is surrounded by 30 dendrites. In the flattened ciliary process (*cp*) the filaments have entirely lost the radial pattern they display more proximally. No lamellar micelles can be identified reliably. A plasma membrane covers the ciliary process and extends about halfway around the outer segment at either side.

since found also in the cones of the toad (12), frog (17) and monkey (4). In cones the essential structure of the outer segment seems to be that of a single, repeatedly infolded plasma membrane.

An apparent complication in this pattern requires further attention. Occasionally in the course of longitudinal sections of *Necturus* cones one sees that on the naked side of the outer segment one of the transverse membranes makes a wide loop closed off by a new edge-loop lying within the larger loop formed by the membrane above and below. If this is their true explanation, such loops-within-loops do not interrupt significantly the over-all pattern of a single, repeatedly infolded membrane.[3]

In spite of their differences in arrangement, the paired membranes of the cone outer segment have nearly the same spacing as those of the rods. We

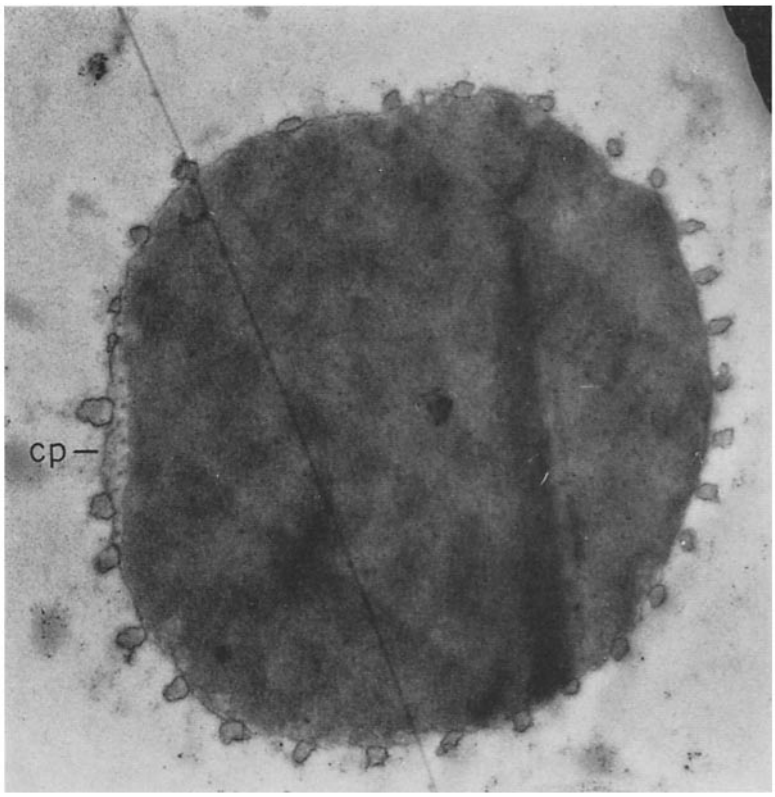


FIGURE 3 *b*

around 1 to 3 other double membranes, so yielding the appearance of 1 to 3 small loops within a larger loop (Fig. 2 *c*). Cohen (4) has noted similar formations in monkey cones.

Such formations may arise as follows. The double membranes of both rods and cones in this and other animals are fragmented at points into groups of vesicles, which for the most part remain lined up in the plane of the double membrane. Wherever a double membrane or short succession of them breaks into vesicles toward the edge, that leaves the remainder of the double membrane find on the average 31 pairs of membranes per micron, or about 750 pairs in the whole outer segment.

The single membranes that form a pair in a

[3] There is good reason to believe that the vesicles are something more than fixation artifacts. In the present material they tend to be found near the edge, where fixation is probably best, rather than in the interior of the outer segments. Also in vitamin A–deficient rats, one can follow an orderly and progressive disintegration of the rod outer segment membranes by such vesicle formation (14, 12).

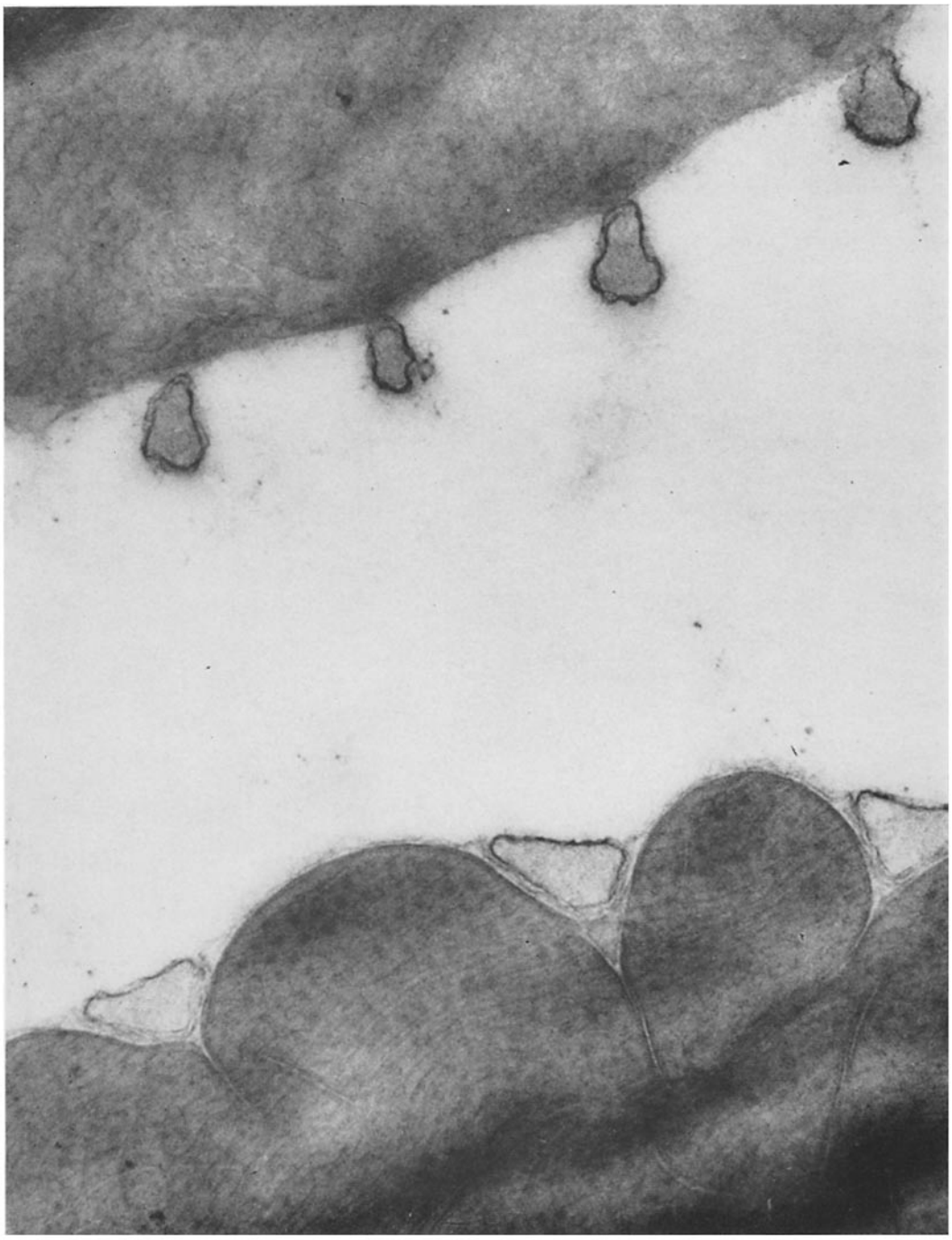


FIGURE 4 Portions of cross-sections of a rod and a cone outer segment. The rod outer segment is cut by deep fissures, the mouth of each of which contains a dendrite, roughly triangular in cross-section. Each dendrite is enclosed in a plasma membrane, a second thickness of plasma membrane surrounding the outer segment. Dendrites stand also around the cone, but here only the plasma membrane around the dendrites is evident, since the cone lamellae are themselves infoldings of the plasma membrane. The rod cross-section shows clearly the regularly spaced lamellar micelles, not evident in the cone.

rod or cone are about 50 A thick. At many points, the two membranes of a pair seem to be in contact; at others they are separated, though rarely more than 30 to 40 A. Sjöstrand (33) found the membranes of double-membrane discs in rods of the toad, *Bufo bufo*, to be similarly spaced. In *Necturus* the space between the single membranes of a pair stains about as lightly as the space between pairs, and displays no evidence of special organization.

the ciliary process, and the double-membrane discs. Each disc encloses a narrow internal space, which seems to be as structureless and lightly staining as the ground substance outside the disc. We have, therefore, three spaces to consider: extracellular, intracellular, and intradiscal; and three types of organized structure: the plasma membrane, the ciliary filaments, and the disc membranes.

TABLE I

Structural Features of Outer Segments of Necturus Rods and Cones

All dimensions refer to fixed and dehydrated sections. Dimensions in the fresh condition are about 25 per cent greater. The characteristics of double cones are like those given here for cones.

	Rods	Cones
Dimensions	12 × 30 μ	5–9 × 24 μ
Longitudinal fissures	22–31 (av. 26)	none
Double membrane discs or paired membranes:		
Per micron of length	37	31
Total in outer segment	1100	750
Thickness of unit membranes	50 A	50 A
Space between membranes of a pair	0–40 A	0–40 A
Space between pairs of membranes	150 A	200 A
Repeating unit (membrane pair + interspace)	270 A	320 A
Dendrites	25–30 (av. 27)	25–33 (av. 29)

Particles:
in rod lamellae (micelles): 30 mμ wide × 5 mμ thick; 140 per μ²; 35 million per rod
in dendrite membranes: 17 to 25 mμ diameter.
in pigment epithelial processes: 25 mμ diameter.
Porphyropsin (rods): extinction at 525 mμ: 0.07; 15 per cent absorption; 1.74×10^9 molecules per rod.

In *Necturus* rods the double-membrane discs are about 140 A apart, making a repeating unit (disc plus space) of about 270 A. In cones the pairs of membranes are about 200 A apart, so that the repeating unit is about 320 A; and the same dimensions seem to hold in double cones.

The numbers and dimensions of the outer segments of the rods and cones and their component structures are summarized in Table I.

As the result of these dispositions, a rod outer segment possesses the following over-all arrangement: The plasma membrane, continuous with that of the inner segment, is filled with an apparently homogeneous, lightly staining ground substance, in which are embedded the filaments of

Cones present a simpler construction. Here there are only two organized structures: the ciliary filaments, and the multiply folded plasma membrane; and two spaces, extracellular and intracellular. It should be noted that here the narrow space between the membranes of each pair—what is in a rod the intradiscal space—is extracellular, and presumably filled with the extracellular fluid.

SPECIAL STRUCTURES

CILIARY PROCESS: This springs from a basal body in the distal portion of the inner segment, a modification of one of its centrioles. The second centriole lies nearby, typically oriented at right angles to the first (Fig. 2 *a*). Both bodies are com-

posed of 9 triple filaments, having the typical form and arrangement found in the basal bodies of all cilia (Gibbons and Grimstone, 20; Gibbons, 19).

The proximal portion of the cilium contains 9 doublet filaments, arranged peripherally; it lacks the additional 9 secondary and 2 central filaments present in motile cilia. Distally the outer filaments become single, then decline in number, and eventually peter out. Distally also the filaments lose their peripheral arrangement, the ciliary process flattening out along the edge of the outer segment (Figs. 3 *a*, *b*).

The ciliary process extends further into the outer segments of the rods and cones than is commonly supposed. It is conventionally described as penetrating only about a micron into the outer segments, probably because the usual longitudinal sections do not follow it further. We find vestiges of it in about half our rod cross-sections, and in almost all our cone cross-sections. From this and collateral evidence we conclude that it extends about halfway up the rod outer segments and considerably further in the cones.

ROD FISSURES: An average of 27 deep fissures, spaced more or less regularly around the circumference of the rod outer segment and running its entire length, carve it peripherally into a radial array of vertical columns (Fig. 3 *a*). The fissures are parallel-sided and about 10 mμ across. They penetrate as deeply as 2.5 to 3.5 μ into the interior of the outer segment, and frequently fork at their inner ends. Also the interior of the outer segment appears to be criss-crossed by further fissures, oriented randomly, and running for various distances axially (Fig. 5). As a result, the outer segment is sliced longitudinally in all directions, yet probably never so as to isolate a segment of the rod from the remainder. That is, each double-membrane disc, though elaborately channeled, is probably continuous internally.

RIM STRUCTURE OF ROD DOUBLE-MEMBRANE DISCS: The rod fissures are bordered on both sides by the same special rim-structure as bounds the outer edges of the double-membrane discs (Fig. 5). In cross-sections of rods, almost exactly parallel with each disc edge, whether along the fissures or around the circumference, a fine line is visible, more deeply staining than the rest of the membrane, yet less deeply staining than the edge itself (Figs. 5 *b*, *c*). This follows the edge wherever it goes, at a constant distance of about 20 mμ. Since this is also the width of the edge-loops seen in longitudinal sections (Fig. 5 *a*), the fine line may mark the inner boundary of the differentiated rim structure. Certainly it seems to have some connection with this structure, for the paired membranes of cones, which lack such differentiated rims, lack also this fine line bordering their edges.

Surface views of the rim structure of the rod double-membrane discs often display an appearance of periodicity (Figs. 5 *b*, *c*), owing either to the presence of deeply staining, regularly spaced granules or cross-striations, it is hard to say which. This periodicity emphasizes the special character of the rim structure, which clearly possesses a fundamentally different organization than the remainder of the disc membrane.

ROD AND CONE DENDRITES: Standing about the outer segments of the rods and cones is a palisade of long, narrow cytoplasmic processes, each continuous at its base with the cytoplasm of the inner segment. These structures appear in longitudinal section in Fig. 2, and their arrangement about the outer segment is shown in the cross-sections of Figs. 3, 4 and 6. Since they are neuronal processes extending from the cell body on the side from which excitation is received, we will speak of them as rod and cone *dendrites*, without however wishing to imply that they are concerned with conducting excitation.

Once we had become aware of these structures, they could be recognized in the descriptions and figures of other workers. It is clear that Sjöstrand and Elfvin (36) saw them in toad rods, though in a subsequent discussion Sjöstrand (33) seems to have identified them with cilia.[4] They apparently

[4] Sjöstrand (29; *cf.* also 32) must have been referring to these structures in the following description: "The connection between outer and inner segments is maintained through one or several thin, cilia-like stalks (reference is made here to a figure showing a true connecting cilium). In the guinea pig and cat retinas, only one such stalk is present in each receptor. In the rods of the toad retina, there are several stalks arranged in a ring at the junction of the outer and inner segments." Later in the same paper Sjöstrand says, "At the base of the outer segments, the peripheral filaments (*i.e.*, of "connecting stalks") disappear, and the free filaments continue for some distance along the incisions of the double membrane discs of the outer segments. In the toad rods, there are as many connecting stalks as there are incisions in these discs." And again, "The connecting stalks show some similarities to the vibratile cilia of, for

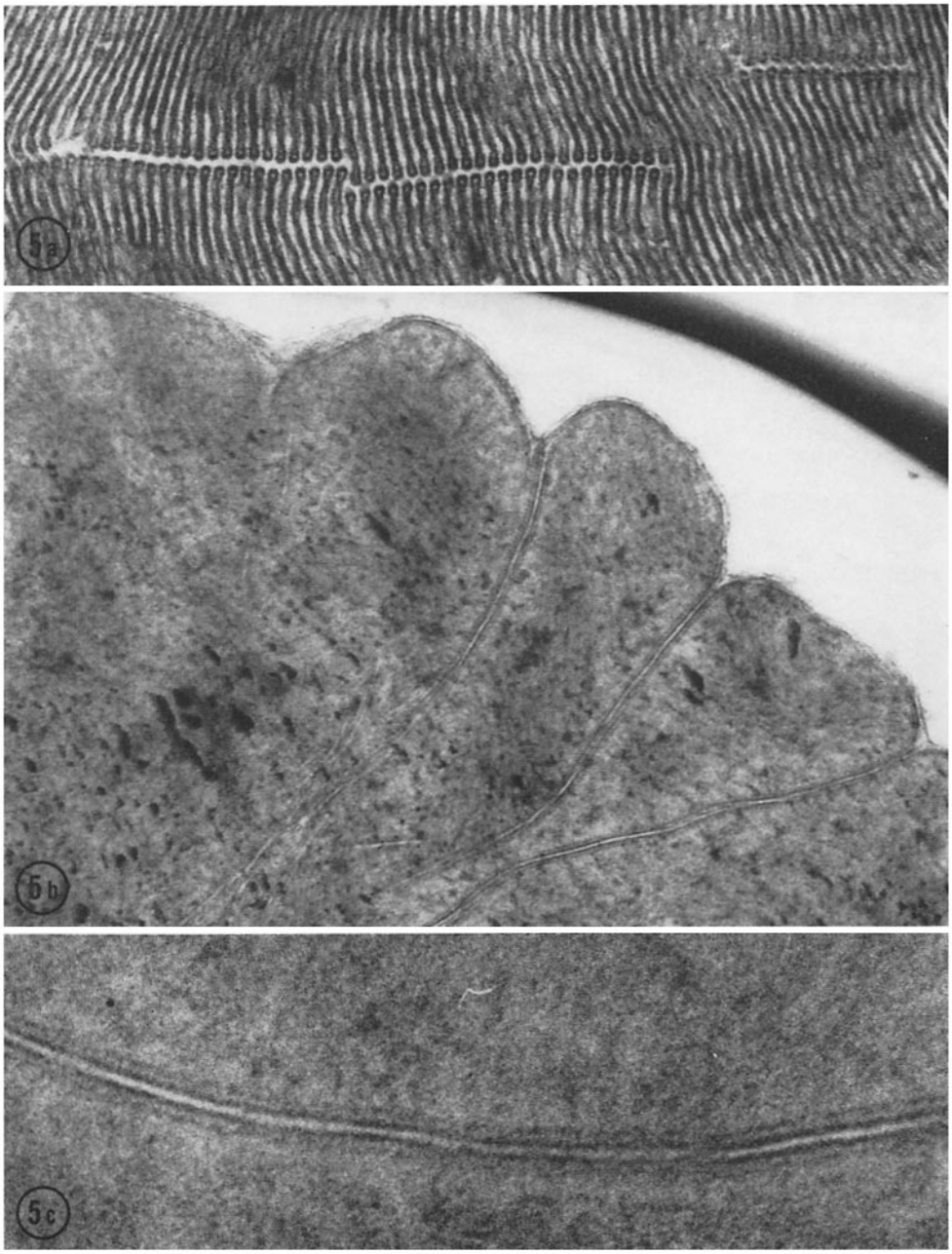


FIGURE 5 Special structure of the edges of rod discs.

a. Sagittal section of an outer segment, showing fissures. Each double membrane is closed at the border of a fissure, just as at the outer edge, by a special rim structure, which shows in such a section as this as a broad, thickened loop. × 61,200.

b. Tangential section, showing the special rim structure that borders the rod lamellae at their outer edges and along the fissures. This stains more deeply than the interior of the lamellae and possesses a distinct fine structure, marked off by an inner border that runs parallel with each outer edge at a distance of about 20 mμ. The absence of dendrites shows this to be a distal portion of the outer segment. × 39,000.

c. Detailed structure of the rim bordering a rod fissure showing the deeply staining inner border, and marked evidence of some periodic structure (granules?) striations? along each rim. × 97,000.

correspond also with the "cytoplasmic prolongations" that Carasso (3) found in visual cells of frog tadpoles; though it is not clear how this author believed them to be related to the "cytoplasmic prolongations" she described earlier (2), lying along the inner segment and perinuclear zone in gecko rods. They have been observed, arranged much as in *Necturus*, in the rods of adult *Rana pipiens* (Wald and Philpott, unpublished observations). The structure characterized by Cohen (4) as forming a "calyx or cup" around the bases of the outer segments of rods and cones in the rhesus monkey also may be of this nature. Cohen shows this structure only in longitudinal sections, in which one cannot decide whether it is a cup or—as is more probable—such a palisade of narrow processes as in *Necturus*. Sjöstrand's figure of a perch cone (33: Fig. 6) shows a narrow extension of the cytoplasm of the inner segment running up beside the outer segment, probably also to be identified with what we here call a dendrite.

Actually, these structures have a much older history. Some 90 years ago Max Schultze (31) published the following remarkable description of them: "The rods in Man and the Mammalia, like the cones, present a superficial striping of the internal segment. The striae run in the form of the finest lines, generally 8 to 10, at equal intervals around the inner segment parallel to the longitudinal axis, or, as on the cones, in a lengthened spiral, as far as the division between inner and outer segment. If the latter has fallen off, and the preparation has been well preserved in perosmic acid, there may be seen, projecting a little beyond the external end of the inner segment, and continuous with its striae, a collection of exceedingly fine fibrils, forming as it were a basket-work which formerly enclosed the external segment . . . In spite of their fineness it is possible, even upon the highly refractive external segment of the rods of Man, to recognize the exquisitely fine lines which run straight or in faint spirals over the surface."[5] The dendrites should be just visible at the highest magnifications available in the light microscope.

We would gather from the foregoing that rod and cone dendrites are widely distributed among animals. They may not be universal, however, for Dowling and Gibbons (14; also personal communication) have seen no evidence of them in rods of the albino rat.

In *Necturus* rods, one dendrite lies in the mouth of each fissure, so that about 27 dendrites stand around each outer segment. About an equal number of dendrites is spaced at fairly regular intervals about the smooth surface of a cone (Fig. 3). The rod dendrites are typically triangular in cross-section, conforming to the shape of the mouths of the fissures. The cone dendrites are usually more slender, and irregularly shaped. Each is 0.2 to 0.5 μ wide. In longitudinal sections, the cytoplasm of the dendrites frequently displays a distinctly fibrillar structure (Figs. 2 *a*, 7); in other instances it may be highly vesiculated (Fig. 2 *c*).

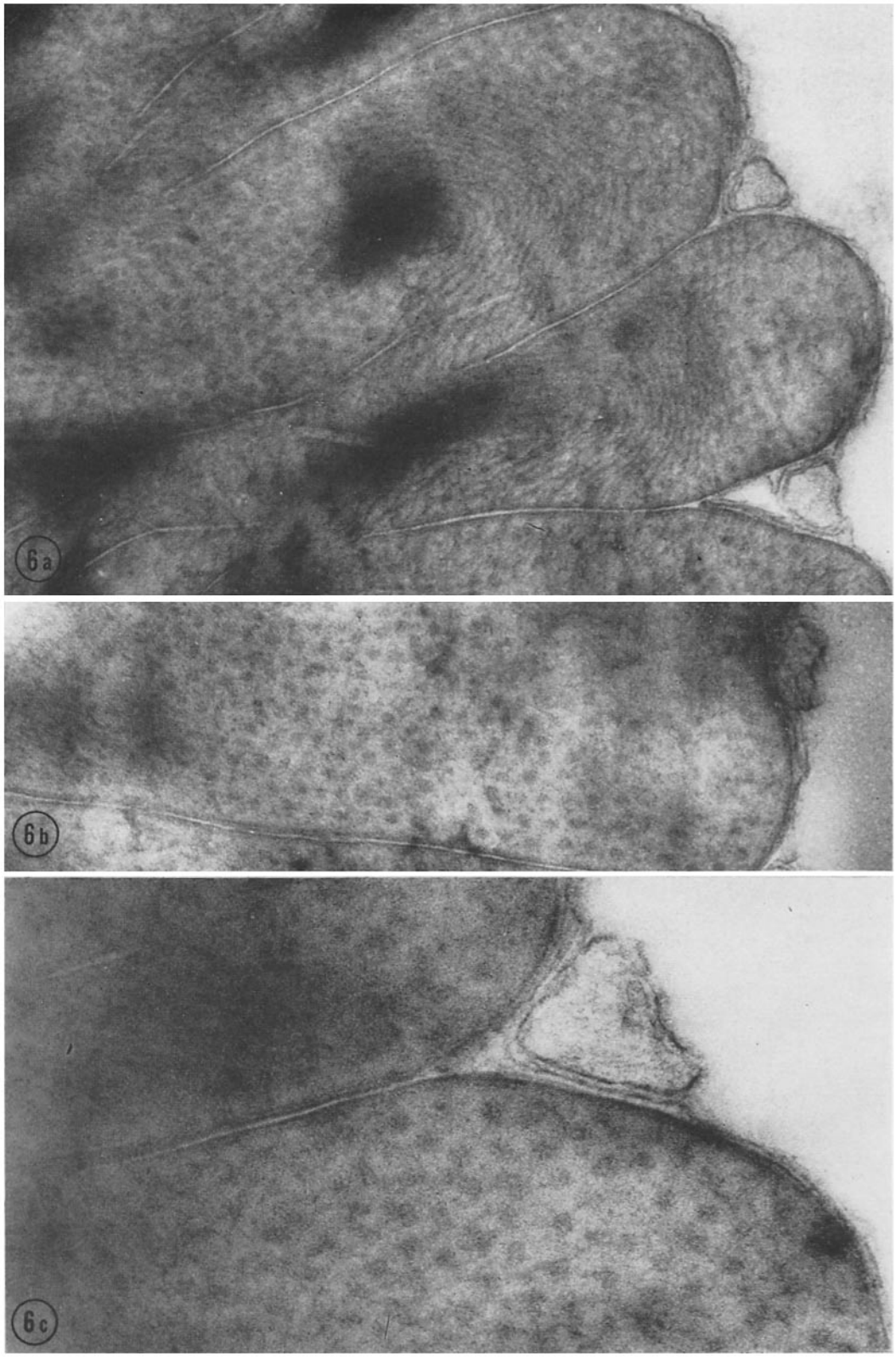
The same plasma membrane which envelopes the inner and outer segments is reflected also about the dendrites. Two thicknesses of this plasma membrane, therefore, separate the ground substance of a rod outer segment from that of the dendrites (Fig. 4). The same is true of a cone on the side that contains the ciliary process, but on the opposite side one sees only the single thickness of plasma membrane that covers the dendrites (Fig. 4, right). On the other hand, since the transverse lamellae of the cones are themselves infoldings of the plasma membrane, here again two thicknesses of plasma membrane lie everywhere between the ground substance of the outer segment and that of the dendrites.

We have already noted that the ciliary process extends about halfway up the rod outer segments, though much further into the cones. Our cross-

instance, ciliated epithelia. However they are lacking the central pair of filaments . . . ." Apparently Sjöstrand had identified these structures with ciliary processes, of which, so far as we know, there is never more than one in a rod or cone outer segment, lying within the membrane, whereas the dendrites lie just outside it.

[5] We are indebted to Prof. E. Yamada for drawing our attention to this reference.

FIGURE 6 Rod lamellar micelles. The micelles are about 30 mμ in diameter, and are spaced about this distance from one another. The striations resembling fingerprints in *a* may be owing to the section having cut across a mound of superimposed lamellae, of which the striations represent the cut edges. *a*, *b*, × 39,000; *c*, × 61,200.


6a
6b
6c

sections of rod and cone outer segments either display both cilium and dendrites or lack both (*cf.* Figs. 5, 8 *a*, *b*). It seems, therefore, that the dendrites, like the ciliary process, extend about halfway up the rod outer segments, and considerably further along the cones. Occasional cross-sections of cones show the absence of both structures, so that here, too, they eventually peter out.

DENDRITE PARTICLES: The dendrites display a system of very dense, button-shaped particles (Fig. 7), which appear to lie on the outer surface of the dendritic membrane (see especially Fig. 7 *a*). Though we cannot yet be sure of this, there is a strong suggestion in these and similar figures that on occasion the particles may be detached from the membrane. The particles are roughly hemispherical in shape, and about 17 to 25 mμ in diameter.

PIGMENT EPITHELIUM: The outer segments extend between the inner segments and the cells of the pigment epithelium. Their functional dependence upon the pigment epithelium has been recognized since Boll and Kühne, and involves among other things regular and demonstrable exchanges of material (*cf.* Wald, 38, 40; Dowling, 13). Apart from the ciliary process, which maintains direct cytoplasmic continuity between the inner and outer segments, the outer segments tend to make broader contact with the pigment epithelium than with their own inner segments. In *Necturus* the tips of the rod and cone outer segments are usually found embedded in the cell bodies of the pigment epithelium (Fig. 11).

Both the inner segments and the pigment epithelial cells maintain contact also with the sides of the outer segments, the inner segments through the dendrites, the pigment epithelium through a comparable system of fine cytoplasmic processes (Fig. 14). At the proximal end of the outer segments, the dendrites are prominent and close, the pigment epithelial processes sparse and remote (Figs. 3, 4); at the distal end these positions are reversed, the dendrites thinning and eventually petering out entirely, while the pigment epithelium processes grow increasingly dense and make more and more intimate contact (Fig. 10). In this sense these two systems of cytoplasmic filaments complement each other, the one system taking over from the other along the shaft of each outer segment.

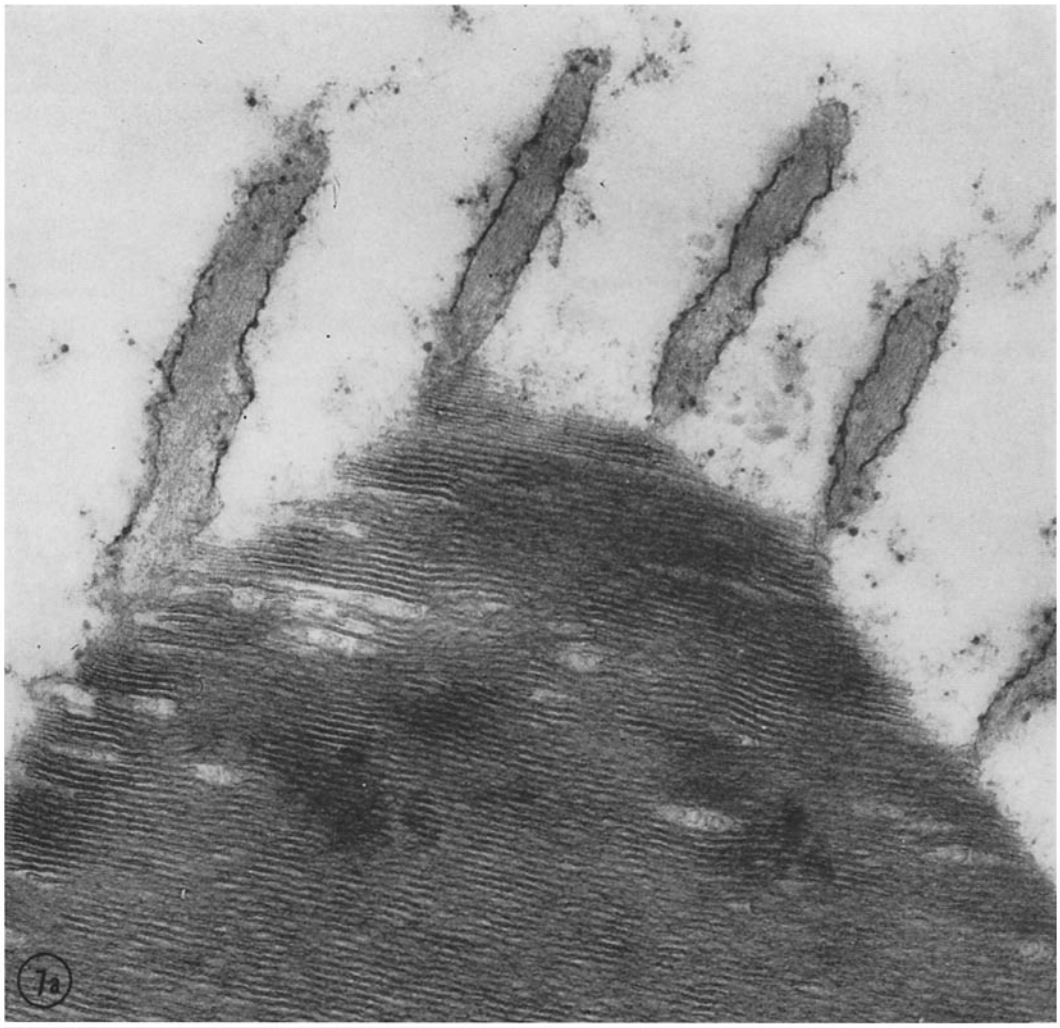
The large pigment granules from which the pigment epithelium takes its name are ellipsoidal in shape, the two axes measuring on the average about 0.4 × 1.2 μ. Each granule is surrounded by a membrane (Figs. 9 *a*, *b*; *cf.* Yamada, 44).

The pigment epithelial processes are so narrow that the pigment granules must lie lengthwise in them. For this reason longitudinal sections of the processes cut almost all the granules lengthwise (Fig. 8), whereas tangential sections show almost all the granules in cross-section, hence more or less circular (Figs. 10 *a*, *b*). In the cell bodies of the pigment epithelium, as expected, the granules are randomly oriented (Fig. 11).

The tight fit of the granules in the pigment epithelium processes probably accounts for the old observation that on dissection of the dark-adapted eye (*e.g.*, frog) the retina comes away readily from the underlying pigment epithelium, whereas in the light-adapted eye these tissues cling tightly to each other. Apparently in the dark-adapted eye the pigment epithelial processes fit loosely among the rods and cones and are readily withdrawn; whereas the large numbers of pigment granules that migrate into them during light adaptation apparently swell them, producing a tight fit.

PIGMENT EPITHELIAL PARTICLES: The pigment epithelial processes contain a system of small, densely staining particles about the same size as those in the dendrites, but differing in shape and location (Fig. 8). They appear as lens-shaped thickenings in the plasma membrane, giving it a beaded appearance. Occasionally, where a section cuts a membrane tangentially, one sees the particles face-on and roughly circular. The high concentration of these particles and their arrange-

FIGURE 7 The dendrite particles. *a*. Oblique sagittal section through a cone outer segment showing portions of 6 dendrites. Deeply staining, roughly hemispherical particles, 17 to 25 mμ in diameter, appear as though attached to the external surface of the plasma membranes of the dendrites. The cytoplasm of the dendrites displays a distinctly fibrillar structure. × 39,000. *b*. Sagittal section of a cone showing a dendrite with particles, some on the membrane, others apparently in surface view, the latter roughly circular in outline. The dendrite cytoplasm again appears distinctly fibrillar. × 61,200.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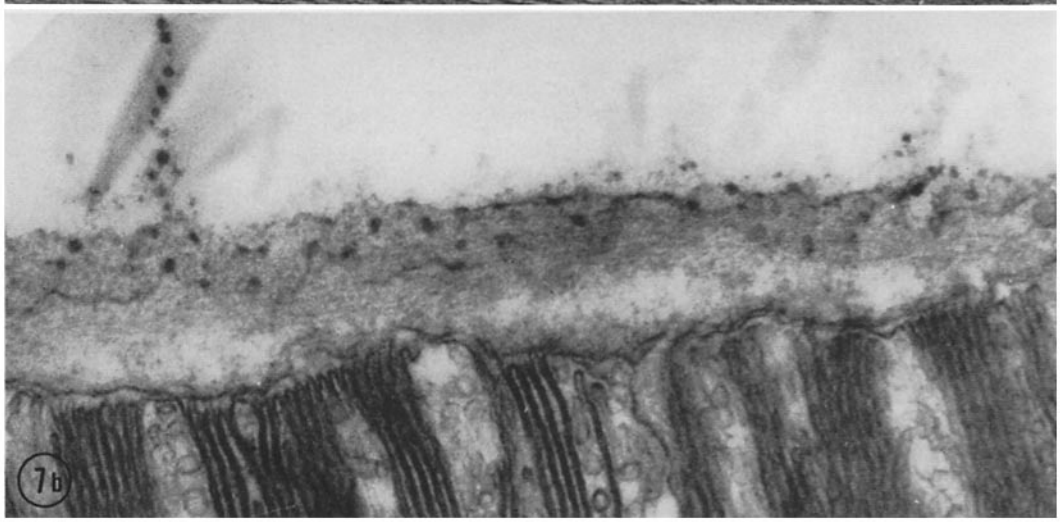
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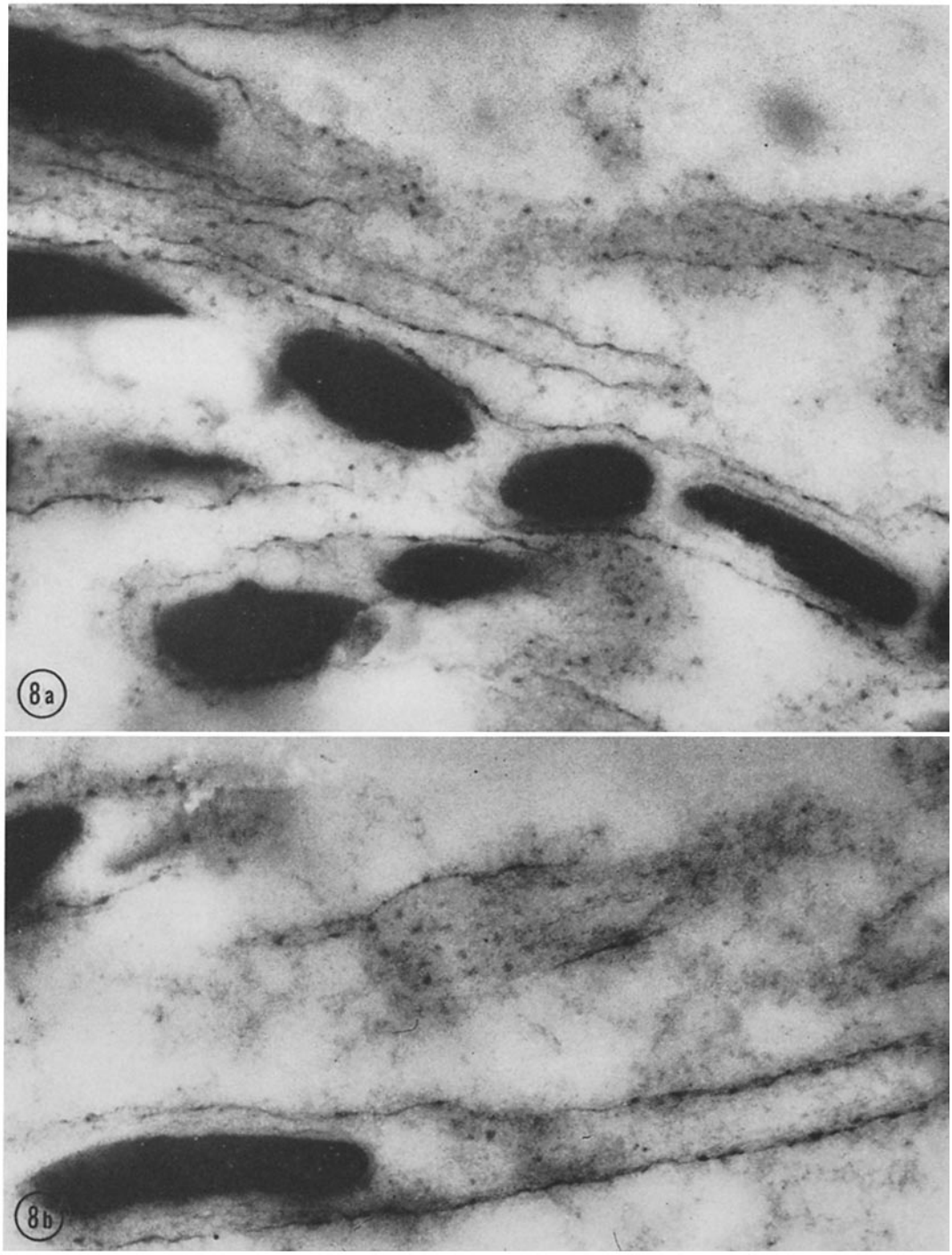


FIGURE 8 Pigment epithelial particles. Sagittal sections of pigment epithelial processes showing the deeply staining particles, which appear as lens-shaped thickenings in the plasma membrane. The particles are about 25 mμ in diameter. The large pigment granules are almost all seen in long section, since the processes are so narrow that the granules must lie in them lengthwise. × 39,000.

ment in the membranes make them easy to identify in the processes. We cannot yet say whether they occur also in the cell bodies of the pigment epithelium.

Such particles may be widely distributed among vertebrates, for several electron micrographs of the rat retina prepared by Dowling and Gibbons show them prominently (Fig. 9). These were albino animals, and so of course display no pigment granules.

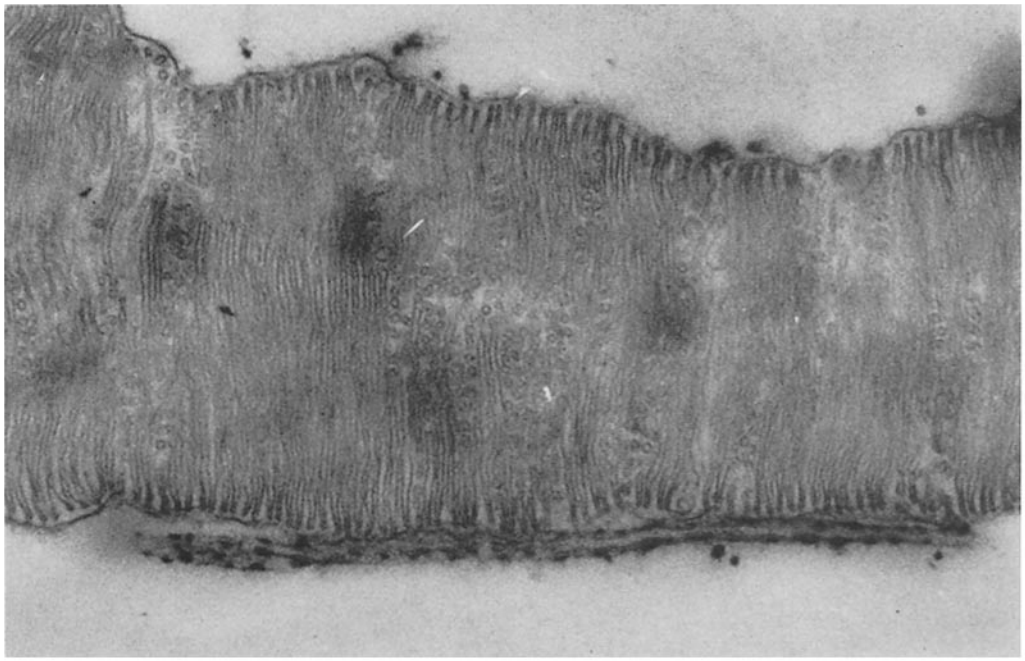


FIGURE 9 Rod outer segment from the rat, with a portion of a pigment epithelial process containing deeply staining particles in its plasma membrane. The particles are roughly circular in outline, and about 25 to 40 mμ in diameter. Pigment granules are absent, since this was an albino animal. Unpublished micrograph from Dowling and Gibbons (15). × 44,000.

In the majority of our micrographs the pigment epithelial processes do not appear to make direct contact with the outer segments (*e.g.*, Figs. 8, 10 *a*). However, occasionally one observes a much closer association (Figs. 10 *b*, *c*), such as might be expected if the processes and their particles were concerned in the transfer of substances between outer segment and pigment epithelium. This possibility will be considered further in the Discussion.

ROD LAMELLAR MICELLES: The rod lamellae, as seen in cross-sections of the outer segments, display a system of deeply staining micelles, spaced so regularly as to represent virtually crystalline array (Figs. 3 *a*, 4, and 6). The micelles appear to be distributed evenly over the entire area of the disc. Most parts of the rod cross-section shown in Fig. 3 *a* display them; where they are absent it is probably because the section is cutting through an interdiscal space. The micelles are approximately circular, and about 30 mμ in diameter, somewhat larger, therefore, than the particles of the dendrites and pigment epithelial processes. The best evidence that the micelles lie in the transverse membranes is that they are the most deeply staining structures we see in the cross-sections of rods, and if they were located instead in the inter- or intradiscal spaces, one should see them there in longitudinal sections.

One might ask why in longitudinal sections they do not appear as more deeply staining segments of the membranes themselves. The answer may be that since the sections are about 100 μ thick, they should include at least two rows of overlapping micelles, giving the appearance of a continuous structure. For this reason also the micelles may be assumed to be about as thick as a lamellar membrane, about 5 mμ. They are probably, therefore,

roughly disc-shaped, about 30 mμ wide and 5 mμ thick. One mole of such particles should occupy a volume of 2.1 million cm^3, and, assuming a typical protein density of 1.3, should weigh about 2.7 million gm.

There are about 140 such particles per square micron of lamellar surface. Since the area of a single rod lamella is about 113 μ^2, there should be about 15,800 micelles per single membrane, or 31,600 per double-membrane disc. If, as we suppose, the average outer segment possesses about 1100 discs, it contains about 35 million micelles.

We have not been able to identify such micelles in cones. Assurance that they are not merely fixation or staining artifacts in rods is offered not only by their great regularity of size and spacing, but by such a section as shown in Fig. 4, in which a portion of a rod displaying the micelles faces a portion of a cone which lacks them.

In addition to the micelles, rod and cone cross-sections occasionally display more or less parallel striations, looking in the aggregate somewhat like fingerprints (*cf.* Fig. 4, 6 *c*). These probably represent places where the section has cut tangentially into a mound of double-membrane discs, of which the parallel striae are the cut edges.

VISUAL PIGMENT

The visual pigment of *Necturus* is primarily porphyropsin, as was to be expected from the demonstration that the retina contains primarily vitamin A_2 (37). In the present experiments this pigment has been measured *in situ*, both in patches of retina and in single rods, with the recording microspectrophotometer described elsewhere (1).

For these measurements, animals were kept dark overnight, then beheaded, and the retinas removed in Ringer solution under deep red light. The whole retina or a portion of it was mounted on a slide with the receptor cells facing upward, immersed in suspension media of various refractive indices. The media contained also 0.1 M hydroxylamine, which combines rapidly with the retinene released on bleaching the visual pigment, to form the corresponding retinene oxime.

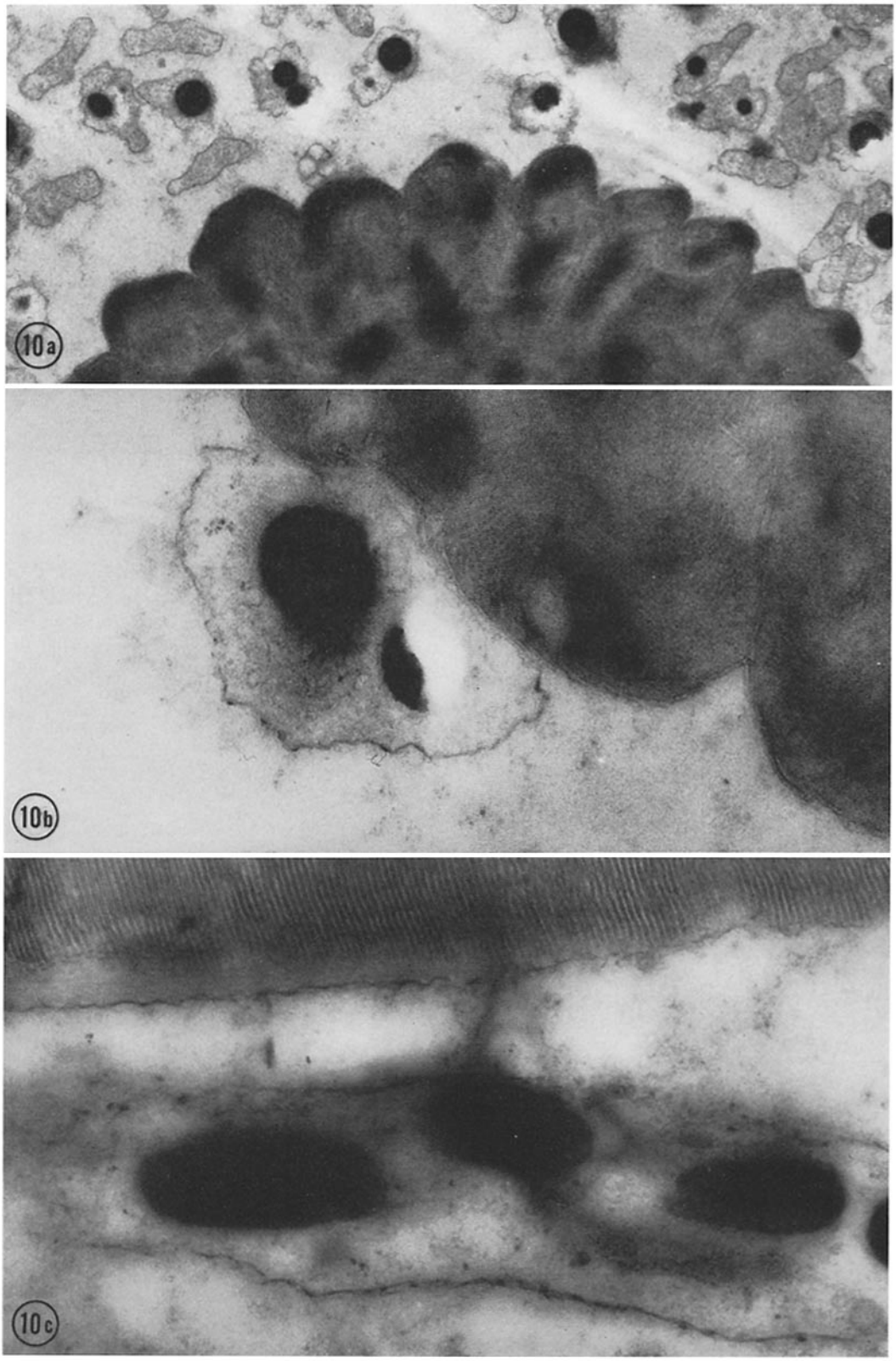
The difference spectrum of porphyropsin, measured in a patch of *Necturus* retina, is shown in Fig. 12. The absorption spectrum was recorded, first in the dark-adapted condition, then again after completely bleaching the retina by exposure to bright light. The product of bleaching porphyropsin under these conditions is $retinene_2$, which promptly combines with hydroxylamine to yield $retinene_2$ oxime (λ_{max} 383 mμ). This has no appreciable absorption at wave lengths longer than about 460 mμ. The difference in extinction before and after bleaching, shown in Fig. 12, is, therefore, identical with the true absorption spectrum of the visual pigment above 460 mμ, and accurately measures its concentration.

The absorption maximum of porphyropsin in the retina lies at 525 mμ. The average extinction at this wave length (E_{max}), measured in a number of preparations, is 0.07, equivalent to a maximum absorption of about 15 per cent. This is a rather dilute pigmentation, as is apparent also from the light purple color of dark-adapted retinas. (Compare the frog, with a rhodopsin extinction of about 0.50 (8), and *Salamandra maculosa*, with a retinal extinction of 0.57 (7).)

The visual pigment measured in such a patch of retina is almost entirely the porphyropsin of the rods, as shown by the fact that single rods yield very nearly the same spectrum. The cones presumably contain another pigment, in too low concentration to affect these measurements appreciably.

A special series of spectrophotometric measurements in plane-polarized light passing through the sides of the rods has shown that in the outer segments the molecules of porphyropsin have a specific net orientation. As with the rhodopsin of frog rods, their chromophores lie predominantly in the transverse plane, perpendicular to the rod

FIGURE 10 Sections showing the close approximation of pigment epithelial processes to outer segments at their distal ends. The absence of dendrites from the rod cross-sections in *a* and *b* shows them to involve the distal ends of the rods. In *b* the plasma membrane of a pigment epithelial process is directly apposed to that of a rod. In *c* there is some indication of a possible protoplasmic bridge between a pigment epithelial process and an outer segment, with several pigment epithelial particles on the bridge. This was the only such observation, and its significance is problematical.


10a
10b
10c

axis. This is the most effective position for the absorption of light passing down the rod axis, the normal direction in the intact eye.[6] We will assume tentatively that because of this orientation, porphyropsin in *Necturus* rods—like rhodopsin in frog rods (Wald, Brown, and Gibbons, 41, 42)—has 1.35 times as great an extinction for *unpolarized* light passing down the rod axis as if it were oriented randomly, as in solution.

The extinction measured in such a patch of retina as shown in Fig. 12 might be expected to be somewhat smaller than that measured in a single rod, because of light passing between the rods in the former instance. In fact, however, we have not found an appreciable difference between both types of measurement. The reason for this appears to be that, particularly when the retina is suspended in Ringer, relatively little light passes between the rods. The rod outer segments act effectively as light pipes, capturing and transmitting the great bulk of the light incident upon the retina (Fig. 12 *b*; *cf.* also 8). For these reasons we have not attempted a correction for this factor.

The molar extinction of porphyropsin is about 30,000 (Wald, Brown, and Brown, unpublished observations). If we take the average E_{max} of porphyropsin in *Necturus* rods to be 0.07, and assume an orientation factor of 1.35 as explained above, the average rod should contain about 1.8×10^9 molecules of porphyropsin.

We shall discuss below reasons for assuming that the porphyropsin is located in the membranes of the double-membrane discs. Since a rod outer segment contains on the average about 1100 such discs, each disc contains about 1.6 million molecules, and each component membrane, therefore, about 800,000 molecules of porphyropsin.

The details of this calculation follow.

The molar extinction of porphyropsin at about 525 mμ, randomly oriented as in true solution, is about 30,000. This is, therefore, the extinction of a (hypothetical) solution containing 1 mole of porphyropsin per liter, and measured in a layer 1 cm in depth. Such a liter of solution would present an absorbing layer of area 1000 cm^2. To the degree that Beer's and Lambert's Laws hold, one can say, therefore, that 1 mole of porphyropsin, spread in a layer of area 1000 cm^2, *in any concentration or depth*, should have an E_{max} of 30,000. Then the retinal E_{max} of 0.07, divided by 1.35 to bring the extinction to what it would have been were the retinal porphyropsin oriented randomly—hence E_{max} 0.052—is equivalent to what would be obtained if $0.052/30{,}000 = 1.73 \times 10^{-6}$ moles of porphyropsin were spread over an area of 1000 cm^2. Since a *Necturus* rod outer segment has an average diameter in the fresh condition of about 15 μ, hence a cross-sectional area of about 177 μ^2 (177×10^{-8} cm^2) a rod should contain $(1.73 \times 10^{-6}) \times (177 \times 10^{-11}) = 306 \times 10^{-17}$ moles of porphyropsin. Since one mole contains 6.02×10^{23} molecules, this is equivalent to 1.84×10^9 molecules of porphyropsin per rod.

[6] A pigment molecule—more accurately, its chromophore—absorbs light maximally when its axis coincides with the electric vector of the incident light, and hence when it lies perpendicularly to the light ray.

DISCUSSION

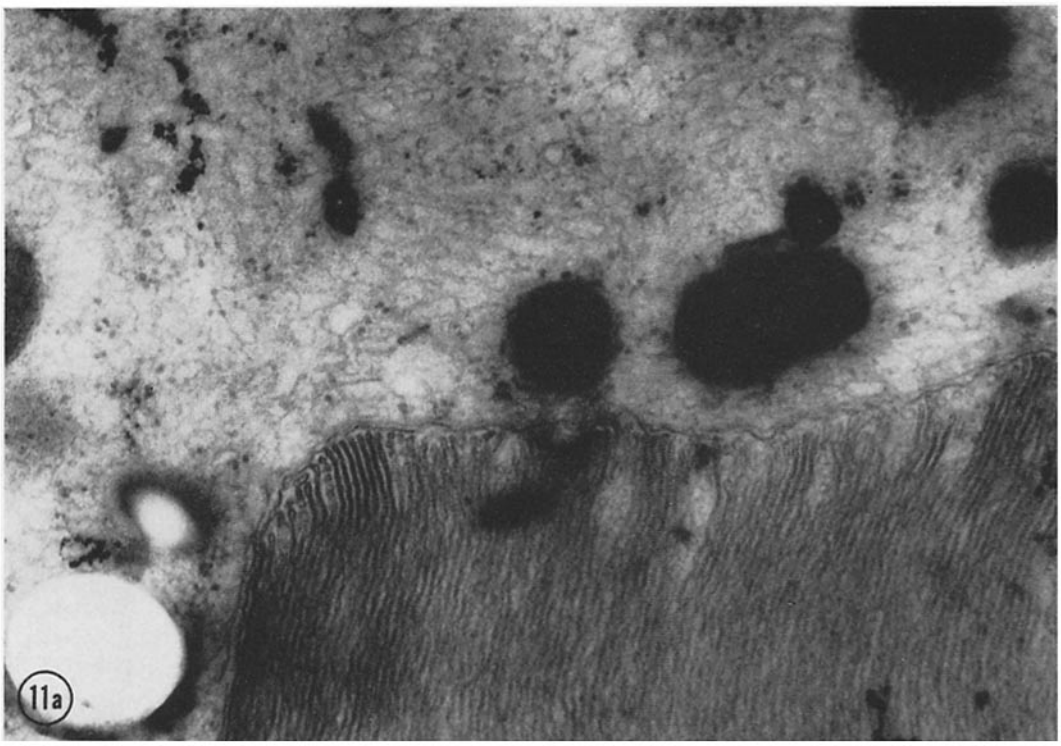
The essential structures of the outer segments and their relations with the inner segments and pigment epithelium processes are summarized in Figs. 13 and 14.

Significance of Rod Lamellar Micelles

Though no reliable basis yet exists for localizing porphyropsin in the structure of the outer segment or for identifying the material of the lamellar micelles, we should like to consider the possibility that the porphyropsin is located in the micelles.

We have assumed, for reasons already stated, that the micelles are located in the membranes of the double-membrane discs (*cf.* Fig. 13). Something may be said also of the likelihood that the porphyropsin is located in the micelles. The molecules of porphyropsin, as already noted, are oriented in the rod structures, their chromophores lying predominantly in planes parallel to the lamellae. It is difficult to see how this organization could be achieved outside the membranes, and reasonable to associate it with the micelles, the only visibly organized structures within the membranes. One may add that porphyropsin, a lipo-

FIGURE 11 Intimacy of contact between the distal ends of *a*, a cone, and *b*, a rod outer segment, and cell bodies of the pigment epithelium. At this level the plasma membranes of the pigment epithelial cells are directly apposed to those of the outer segments. × 39,000.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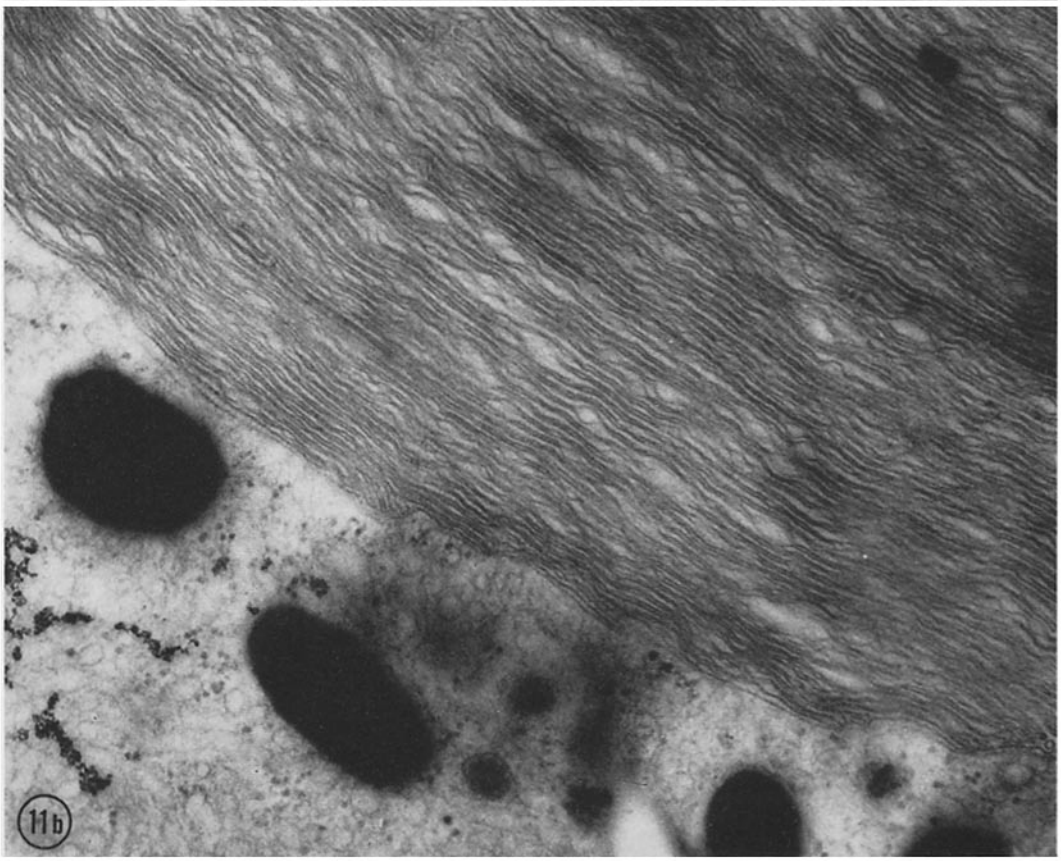
11b

protein containing sulfhydryl groups and a highly unsaturated chromophore, can be expected to bind heavy-metal electron stains strongly, and might well be associated, therefore, with the deeply staining micelles.

If this is indeed its location, since we have computed that the average rod outer segment contains about 35 million particles, and about 1.8×10^9 molecules of porphyropsin, each particle may contain about 50 molecules of porphyropsin.

We have estimated the weight of a mole of rod particles to be about 2.7 million gm. If the particles were composed entirely of porphyropsin, the latter would have a maximum molecular weight of about 54,000. Cattle rhodopsin as usually prepared has an apparent molecular weight of about 40,000 (24). Frog rhodopsin seems to be of comparable size. Our rough computation, therefore, yields a reasonable value for the molecular weight of porphyropsin, and suggests the possibility that this pigment, if located in the lamellar particles, may account for virtually their entire composition.

Energy Migration in the Outer Segments

The outer segments of rods and cones are quasi-crystalline structures in the sense that many of their component molecules display a high degree of mutual orientation. Their universal lamellation, to which in *Necturus* rods one can add the regularity of spacing of the lamellar micelles and the dichroism of porphyropsin, are all evidences of approaches to crystallinity and the solid state.

As one element in the still unknown mechanism whereby the absorption of light by a molecule of visual pigment induces a nervous response, the possibility must be considered that the absorbed energy migrates from the site of absorption to some other point in the outer segment at which it has its effect. Two main types of process must be considered: exciton migration—the transfer of electronic excitation from molecule to molecule without further absorption or emission of light (radiationless transfer, inductive resonance); and photoconduction. Both types of process can occur in sufficiently concentrated systems of randomly oriented molecules, but occur much more readily as the solid state is approached.

Exciton Migration

Hagins and Jennings (22) have examined the possibility of exciton migration in frog rods, in which rhodopsin is unusually concentrated and highly oriented, and conditions are correspondingly favorable for this process. They concluded that, even here, exciton migration appears theoretically improbable; and could find no experimental evidence that it operates over distances comparable with the dimensions of the outer segment.

In *Necturus*, the much greater dilution of visual pigment in the rods, together with the possibility that it is concentrated in micelles, make exciton migration over long distances still less likely. Ordinarily, inductive resonance is assumed to be limited to intermolecular distances of at most 100 A. Even within a single micelle, this process might have only limited scope. If, as we have calculated, a lamellar micelle contains perhaps 50 molecules of porphyropsin, they should be on the average about 40 A apart. This may already represent close packing; for as pointed out above, if porphyropsin has a molecular weight of about 40,000, its diameter (if it is spherical) should be about 40 A.[7]

Such conjugated proteins as the visual pigments must always present difficulties for exciton migration. This process involves interactions among the chromophores, which in conjugated proteins even under conditions of close packing are held widely apart by the intervening protein. Clustering a group of such molecules about a center might bring their chromophores closer together, but only a few molecules could enter such an arrangement. We may conclude that though some exciton migration might occur within a lamellar particle, it is very unlikely to carry much further within the outer segment.

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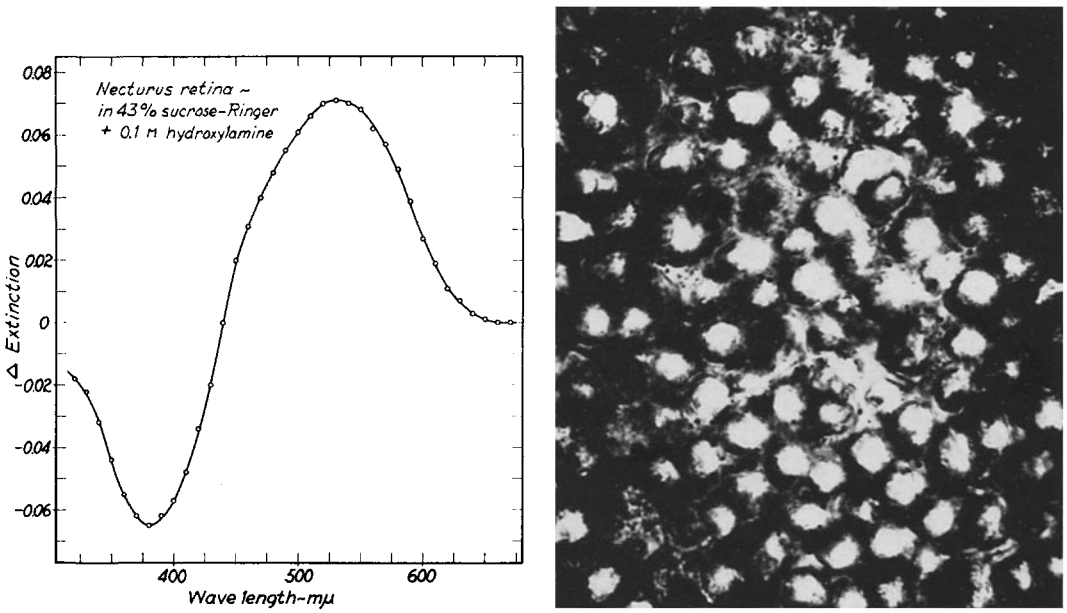
[7] Hagins and Jennings (22), in a computation necessarily filled with approximations, have estimated that the separation between adjacent rhodopsin molecules at which there would be a 50 per cent chance of exciton transfer between them is about 19 A. This estimate is based on an assumed lifetime for excited rhodopsin of about 10^{-9} second. If a long-lived (metastable) excited state of rhodopsin were found, that might increase its lifetime as much as a million times, *i.e.*, to perhaps 10^{-3} sec. Since, however, the critical transfer radius is proportional to the sixth root of the lifetime, even this much increase in the lifetime would increase the transfer radius only 10 times, *i.e.*, to about 190 A. That is quite surely, however, an over-optimistic estimate on all counts.



a *b*

FIGURE 12 *a*. The spectrum of porphyropsin *in situ*. Difference spectrum of porphyropsin (λ_{max} 525 mμ), measured in a circular area of *Necturus* retina about 0.17 mm in diameter. The retina was mounted in 43 per cent sucrose in Ringer solution containing 0.1 M hydroxylamine. The spectrum shows the difference in absorption between the unbleached and bleached retina, the minimum at about 383 mμ marking the appearance of retinene$_2$ oxime as a product of bleaching. *b*. Photomicrograph of a portion of *Necturus* retina mounted in Ringer solution for such a measurement, showing the ends of the rods and cones brilliantly lighted, whereas little light comes through the spaces among them. The outer segments, and to a degree the visual cells as a whole, act as light pipes which convey most of the radiation transmitted through the retina, and so are primarily responsible for the measured absorption.

Migration of Electrons: Photoconduction

This involves the possibility that the absorption of light by porphyropsin results in a liberation of electrons, which, with the residue of positive ion radicals or "holes," might be available for charge separation or the conduction of an electric current. It is hard to say anything definite about this possibility that might not be questioned by one worker or another in the field. Nevertheless, it should be pointed out that this typically solid state phenomenon, if it exists in rods at all, would be expected to be limited to the double-membrane discs, the only elements of rod structure that appear to approach solid state organization. Even here, the great dilution of photopigment molecules and gross heterogeneity of composition argue against photoconduction. It is a far cry from the outer segment or any of its formed elements to such melts of crystalline β-carotene as Rosenberg (29) has proposed as photoconductive models of rod structure. Recently, Rosenberg *et al.* (30) have reported that dried layers of washed fragments of sheep rods exhibit semi-conduction and photoconduction; yet the photoconduction reported was very small, and the time constants very slow (10 to 15 seconds), far too slow to account for visual excitation, which is completed within a fraction of a second even in cold-blooded animals.[8] It should be recalled also that a dark-adapted

[8] Commoner *et al.* (5) have reported similarly long time constants for the rise and decay of electron spin resonance signals in chloroplasts; in *Chlorella*, for example, about 10 sec for the rise and 11 and 20 sec for two decay processes. Such slow rates are no hindrance in photosynthesis, but would utterly frustrate vision and are wholly incompatible with the time constants of the visual response and its decay.

human rod is stimulated by the absorption of a single photon, presumably by a single molecule of rhodopsin, an event that could be expected to liberate only one photoelectron or positive hole. Altogether, it seems as yet unlikely that this type of process contributes in an important way to visual excitation.

The outcome of this discussion is that exciton migration and photoconduction over distances comparable with the dimensions of outer segments lack adequate demonstration and seem theoretically unlikely. One cannot dismiss the possibility that such processes may occur, but one is in no position simply to assume them, for example on the basis of presumed analogies between outer segments and chloroplasts (*cf.* discussion in 39). Indeed, as will appear below, it would be easier to understand rod excitation if some migration process conveyed molecular excitation from the interior of a double membrane disc to its edge, where it might interact with the plasma membrane; but as yet even such short migrations of molecular excitation remain questionable.

The Site of Excitation

For these reasons, we prefer to assume that the processes responsible for excitation occur at or very near the site of absorption of light, and are then communicated by some type of axial structure to the whole receptor cell. Three such axial structures need to be considered: the plasma membrane, the ciliary process, and the dendrites.

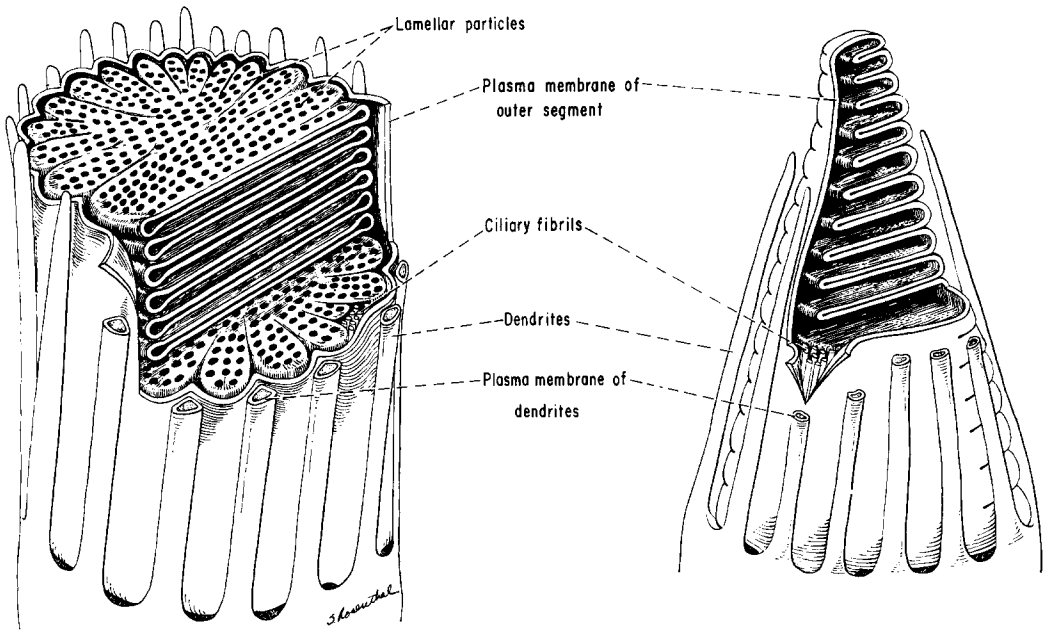


FIGURE 13 Diagram to show structural relations between rod and cone outer segments in *Necturus*. In the rod all edges of the double membrane discs involve a differentiated rim structure. The discs are cut into lobules by deep longitudinal fissures; and the disc membranes contain a system of deeply staining micelles in regular array. The stack of double layers is completely enclosed within a plasma membrane, continuous with that of the inner segment, and reflected also over the dendrites. In the cones this common plasma membrane is infolded repeatedly on the side opposite from the cilium to form the double layers. The cone double layers lack the special rim structure, fissures, and lamellar micelles.

What later was identified as the *ciliary process* (9) has been suggested for this role by Sjöstrand (32). This view is favored by a number of considerations: (a) The cilium is a universal element of rod and cone structure. (b) There is some evidence that in olfactory cells the cilia possibly constitute the sensing element (Ottoson, 27; *cf.* also 6, 18). (c) The ciliary processes, at least in *Necturus*, penetrate considerable distances into the outer segments. On the other hand, even in *Necturus* the

ciliary process seems to extend only about halfway up the rod outer segments, and hence could conduct excitation only from its proximal portions, leaving the distal half functionless.

The same point may be made for the *dendrites*, which also appear to extend only about halfway along the rod outer segments. A more basic consideration, however, weighs against them as the both rods and cones the *plasma membrane* of the outer segment is the probable site of neural excitation. In the cones, in which infoldings of the plasma membrane constitute the transverse layers, the absorption of light by a visual pigment in the membrane may represent a direct attack upon its structure, resulting in its depolarization. In the rods, one should have to assume that some device

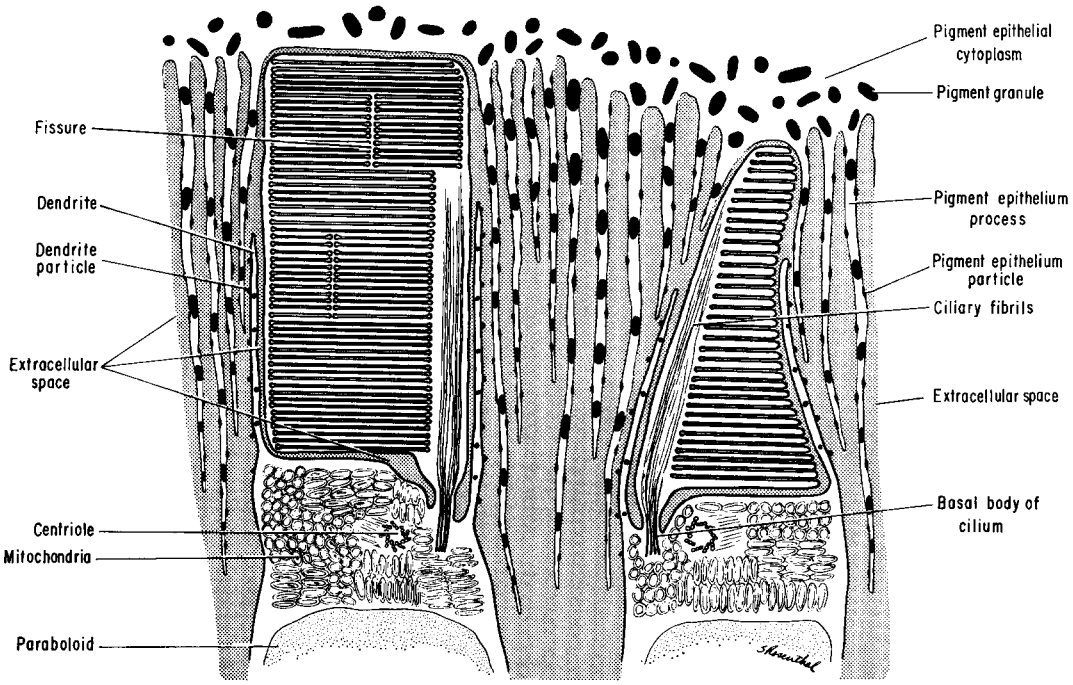


FIGURE 14 Diagram to show relationships of rod and cone outer segments to the inner segments and the pigment epithelium.

site of rod excitation. Their relation to the outer segment superficially resembles that of the dendritic process of the eccentric cell to the rhabdomeres in a *Limulus* ommatidium (25). The difficulty in the present instance is that, unlike the dendritic process in *Limulus*, the dendrites of the rods and cones are parts of the same cell as the outer segments. Their plasma membranes are extensions of the plasma membrane that surrounds the outer and inner segments of a rod or cone. For an excitatory process to reach the plasma membrane of a dendrite, it should first have to cross another portion of the same plasma membrane covering the outer segment. If the point of rod excitation is to affect the plasma membrane, why not do this on the first encounter?

These considerations lead us to suppose that in conveys the effects of light from the site of absorption in a double-membrane disc to its edge, where they could affect the plasma membrane. This possibility focuses attention upon the differentiated rim structure which surrounds each double-membrane disc, the special business of which may be to collect the effects of light absorption from the disc as a whole, and mediate their interaction with the plasma membrane.

Roles of the Cilium, Dendrites, and Pigment Epithelial Processes

If this is the role of the plasma membrane, what of the other organelles discussed above?

The *ciliary process* may be mainly concerned with the development of the outer segments in the

embryo, and their regeneration in the adult (*cf.* 10, 11, 14). The plasma membrane of the outer segment begins as the plasma membrane of the cilium; and without a basal body in the inner segment from which a ciliary process can spring, no outer segment can apparently be formed. So for example, when, at an advanced stage of vitamin A deficiency in the rat, the outer segments of the rods are lost, they can be regenerated on administration of vitamin A as long as inner segments remain. When at a still later stage of vitamin A deficiency the inner segments also are lost, outer segments can no longer be regenerated (16, 14).

The *dendrites* may be mainly concerned with exchanges of material between the inner and outer segments. The structure of an outer segment, rod, or cone, would otherwise make metabolic communication with the inner segment difficult. The dendrites originate in the region of the ellipsoid, with its tightly packed mitochondria, and so are well placed to convey mitochondrial products, perhaps among them ATP, to the outer segments. It is possible that the dendrite particles play a part in such exchanges. In the rods, communication between the dendrites and the interior of the outer segment must be greatly facilitated by the deep fissures, in the mouths of which the dendrites lie.

Yet if a wide cytoplasmic channel is needed running from the inner segment axially up the outer segment, why not provide this *within* the outer segment, rather than through an external system of dendrites? The answer may be that a large increase in the bulk of cytoplasm within the outer segment would necessarily separate the edges of the double-membrane discs further from the plasma membrane, and so should make more difficult such interactions between these structures as we suggest may be involved in visual excitation.

This consideration is closely connected with another: the presence of fissures in almost all types of rod outer segment, and their absence in cones, where, to our knowledge, they have not yet been observed. It has been suggested that fissures, by increasing the surface of the outer segment, facilitate interchanges of material with adjoining structures (38); but if such a spinning-out of surface is needed in rods, why not also in cones, particularly in such large cones as those of *Necturus*? Perhaps that question can be answered as follows: All material must be conveyed to and from the shaft of the outer segment through the extracellular fluid. In rods, this lies outside the plasma membrane, and can penetrate to the interior of the rod only through whatever fissures are provided. By the same token, fissures would have no place in cones, in which the space between each pair of transverse membranes is extracellular.

The *pigment epithelial processes* seem clearly to be concerned with exchanges of material between the outer segments and the pigment epithelium. These tissues have been shown directly to exchange vitamin A (13); and presumably they exchange other metabolites. The particles we have described lying in the membranes of the processes may have a part in such transactions.

It may be noted finally that these two overlapping systems of microvilli—the dendrites and pigment epithelium processes—may communicate not only with the outer segments but with one another, so connecting the inner segments of the receptor cells with the pigment epithelium. Metabolically and electrically, such a connection may play a significant role in retinal function.

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