

FURTHER OBSERVATIONS ON THE FINE STRUCTURE OF THE PARIETAL EYE OF LIZARDS

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ABSTRACT

An electron microscopical study of the third eye of the Western Fence Lizard, *Sceloporus occidentalis*, fixed with 1 per cent osmium tetroxide, pH 7.4–7.6, for 16 to 20 hours at 0°C., revealed the following new facts. The fibrillar system of the retinal photoreceptor consists of nine double fibrils enclosed in a sheath. Pigment cells and lens cells possess similar systems. Two short cylindrical centrioles are associated with the fibrillar apparatus: one, from which striated rootlets extend inward, lies at the base of the fibrils, with the other at an oblique angle to the axis of the system. A Golgi complex, whorls of endoplasmic reticulum, lipid (?) droplets, and other organelles and inclusions in the photoreceptors are described. An axon leads from the base of the photoreceptor into the nervous layer of the retina which consists of many nerve fibers and large ganglion cells. Although the pattern of neural connections has not yet been determined, some synapses were found. The parietal nerve consists of about 250 non-medullated fibers. The capsule of the eye usually has a layer of iridocytes, which contain rows of guanine (?) rods. A few parietal eyes of the Granite Night Lizard, *Xantusia henshawi*, were also examined. Large lipid (?) droplets occur in the bases of their receptor processes.

INTRODUCTION

The third eye of lizards has been a subject of study for a very long time. The early literature on the anatomy, embryology, and possible function of the parietal eye is discussed by Tilney and Warren (31) and Gladstone and Wakeley (16). Further review with the development of a theory that the eye functions as a dosimeter of solar radiation, was given by Stebbins and Eakin (27). Recent studies on the structure of the eye are cited in Steyn's (30) latest publication in which he presents his own findings with the light microscope, some of which do not agree with the results of electron microscopy done simultaneously and independently by Eakin and Westfall (13) and by Steyn (29). These latter investigators reported in preliminary notes that the receptor processes, which extend from the surface of the retina into

the lumen of the eye, are similar structurally to the rods and cones of the lateral eye. We present here additional observations on the ultrastructure of the retina, lens, capsule, and nerve of the reptilian parietal eye.

MATERIAL AND METHODS

Third eyes of the Western Fence Lizard, *Sceloporus occidentalis*, and of the Granite Night Lizard, *Xantusia henshawi*, were studied by electron microscopy. The former species was collected in large numbers in the Berkeley hills and in the Carmel Valley, California, using a noose of copper wire (10). Immature animals of snout-vent length of about 55 mm. were preferred because of ease in removing the eye. The few adult specimens of the latter type were collected by Dr. Robert Glaser near Banning, Riverside County,

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California. Unless otherwise stated all descriptions and figures pertain to *Sceloporus occidentalis*.

The third eye was obtained as follows. An animal was decapitated, the interparietal scale was removed, and an incision was made in the skin with a sharp scalpel following the contour lines of the scale. The skin, which is differentiated into a clear cornea over the eye (see Fig. 1 of either 27 or 13) is slowly peeled away from cranium, meninges, and lens of the eye. Unless care is taken in removing the skin, the lens ruptures and the retina everts. In *Sceloporus* the parietal foramen of the cranium is large, especially in juveniles and immatures. Through this foramen one may easily cut the meninges surrounding the eye with microscalpel (2) and microscissors (9). The square of meninges, bearing the eye in its center, is transferred with forceps to a drop of cold-blooded Ringer's solution from which it is pipetted into the fixative, 1 per cent osmium tetroxide buffered to pH 7.4-7.6 with veronal acetate, for 16 to 20 hours at 0°C. The specimens, embedded in 1:4 methyl-butyl methacrylate, were sectioned at less than 90 μ m with a Porter-Blum microtome and diamond knife (4, 37) and examined in an RCA-EMU-3 electron microscope.

Procedures were modified slightly for *Xantusia*. Owing to a small parietal foramen in this form the eye could not be extirpated as described above. A square of skin, bearing the eye in the center, plus meninges and perhaps fragments of the cranial roof was removed with microscalpel and scissors and fixed at room temperature for 2 hours in an osmium tetroxide (1 per cent)—sucrose (0.34 M) solution buffered to pH 7.5. Postfixation followed at room temperature for 1 hour in a formalin (10 per cent)—sucrose (0.34 M) solution.

RESULTS

Photoreceptors: The retina of the parietal eye contains three primary elements: photoreceptors (see discussion), pigment cells, and ganglion cells. The photoreceptors are long narrow units, interspersed among the pigment cells, bearing at their distal (luminal) ends processes which extend into the cavity of the eye. Each process, one per cell, consists of an outer and an inner segment joined together by a narrow connecting piece.¹ The

¹ In general, we have adopted terms applied to structures in the lateral eye without asserting any homologies. The designation connecting cilium (6), however, for the narrow junction between outer and inner segments of a photoreceptor is, in our opinion, undesirable because this short element is not a cilium, albeit derived from the base of one.

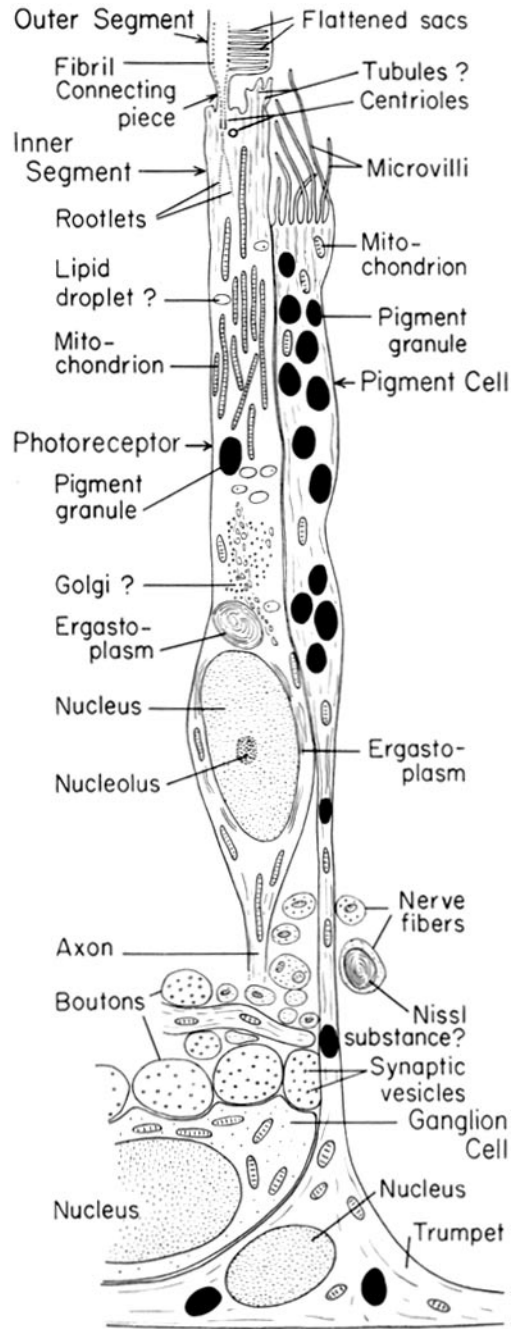


FIGURE I

Three principal elements in the retina of the parietal eye of *Sceloporus occidentalis*: photoreceptor (base only of outer segment shown), pigment cell (minus its fibrillar apparatus), and ganglion cell.

outer segment is essentially a stack of many discs or flattened sacs which were described in our preliminary note (13). At the base of the segment and to one side of the sacs lies the fibrillar apparatus (see Fig. 1). As seen in cross-section (Fig. 2) it appears to consist of a ring of nine double fibrils enclosed in a sheath. The resolution in our electron micrographs does not enable us to determine if the sheath also possesses nine filaments. Central fibers are probably lacking. Most cross-sections of the fibrillar system, as the one in Fig. 2, show a core of very low electron density. Tokuyasu and Yamada (32) did not find central fibers in rods of the kitten; De Robertis (6) reports, however, a central pair of faint filaments in the connecting piece of some rabbit rods. Each fibril appears to be striated (Figs. 3, 5), a feature owing possibly to an artifact of fixation.

The fibrils converge at the base of the outer segment, pass through the connecting piece, and continue into the inner segment for a short distance where they terminate in a short electron-dense cylinder, the axial centriole (c_1 , Fig. 3). A second body, the oblique centriole, lies nearby and at an angle to the axis of the fibrillar system. Serial sections suggest that it also is a short cylinder which appears in cross or oblique sections as a dark circle or oval. Striated rootlets extend from the axial centriole into the basal part of the inner segment (Figs. 3, 5, 6). This account of striated roots agrees with that of Sjöstrand (25) for the guinea pig rod, except that he represents the root as a single cross-banded filament, whereas in the parietal eye photoreceptor there seem to be at least two rootlets, like those figured by Fawcett and Porter (15) in intestinal epithelial cells of the fresh water mussel, *Elliptio*.

Distal to the nuclei of the photoreceptors (*i.e.* toward the lumen) there are masses of endoplasmic reticulum or ergastoplasm consisting of granular double membranes arranged in whorls or piles (Fig. 4). Similar whorls occur in large nerve fibers within the nervous layer of the retina (see below). In *Xantusia* such structures are found also adjacent to nuclei in the outer zone of the retina just inside the capsule of the eye (Fig. 18). Profiles of relatively straight reticular membranes may be seen encircling the nuclei of photoreceptors. Whether the fine parallel membranes in the inner segment of the receptor process, earlier described (13) as tubules, are endoplasmic reticulum is not clear.

Adjacent to the ergastoplasmic whorls lie chains of agranular membranes and vesicles which are tentatively designated Golgi apparatus. In this same region of the cell one frequently observes ellipsoid bodies, of larger size than mitochondria, which exhibit a moderately electron-dense unstructured interior and a well defined limiting membrane. In one instance of an eye fixed in potassium permanganate several of these oval bodies are in contact with a pile of reticular membranes (Fig. 11). These elliptical structures occur from the level of the Golgi and whorled organelles into the inner segment of the receptor process. In *Xantusia* the range of size is from 0.23 to 2.10 μ in length. (see Fig. 13). Usually one large oval body lies in the base of the inner segment of the sensory process (Fig. 12). Within these ovals one frequently observes smaller bodies of similar nature and pigment granules also. Another feature of *Xantusia*, not seen in *Sceloporus*, is the presence of very large irregular masses of electron-dense material distal to the nuclei of photoreceptors (Fig. 14).

Pigment Cells: These retinal elements are exceedingly elongate cells which parallel the photoreceptors (see Figs. 1 and 4). At their distal (luminal) ends they bear many microvilli; basally the cells flare out into trumpet pieces (18, 30) which abut on the connective tissue capsule of the eye, like the trumpet pieces of the Müller's fibers in the lateral eye. Usually the nucleus is situated in the trumpet piece. The microvilli, system of tubules, mitochondria, and pigment granules were described earlier (13). Incidentally there are fewer pigment granules in the retina of *Xantusia henshawi*, a crevice-dwelling form, than in that of *Sceloporus*. The pigment cells possess fibrillar systems of ciliary origin, each consisting of nine peripheral fibers enclosed in a sheath and two centrioles. These features are shown in Figs. 7 and 8. Central filaments have not been observed. At the luminal surface the cell membrane is invaginated about the base of the cilium, forming a circumciliary space (not figured), similar to that in protozoans (21, 14). In this respect the fibrillar apparatus of the pigment cell differs from that of the photoreceptor. At most the membrane of the connecting piece of the latter is only slightly recessed into the inner segment of the cell process (see Fig. 3). The nature of the distal part of the cilium of the pigment cell and its relation to the microvilli have not yet been deter-

mined. The cilium is not shown, therefore, in Fig. 1.

The width of the pigment cell is varied. In general the distal part is relatively wide, sometimes wider than the photoreceptor, and packed with pigment granules. At this point it may be stated that, contrary to the observations of Nowikoff (18) on *Lacerta* and *Anguis* and Dendy (5) on *Sphenodon*, pigment granules are sometimes seen in photoreceptors. At the level of the nuclei of the photoreceptors the pigment cell is usually very narrow, presumably because of the pressure of the adjacent nuclei. Pigment granules, if present in

this narrow region, are oriented lengthwise and lie in single file. Here also appear double-layered membranes (Fig. 4), which are probably folds in the cell membrane.

Lens: The cells of the clear lens are elongate elements, extending in parallel array from the lumen of the eye to the basement membrane of the cornea. The position of the large ovoid nuclei varies, but they usually lie in the basal halves of the cells (see Fig. 1 of either 27 or 13). The basal (luminal) ends of the cells exhibit a labyrinth of very irregular microvilli (Fig. 10) which project into the cavity of the eye. In the midst of the

Explanation of Figures

All figures pertain to *Sceloporus occidentalis* unless otherwise stated.

KEY TO ABBREVIATIONS

<i>a</i> , axon	<i>m</i> , mitochondria
<i>b</i> , bouton	<i>mv</i> , microvilli
<i>c</i> ₁ , axial centriole	<i>n</i> , nucleus
<i>c</i> ₂ , oblique centriole	<i>nf</i> , nerve fibers
<i>cm</i> , cell membrane	<i>nm</i> , nuclear membrane
<i>cp</i> , connecting piece	<i>nu</i> , nucleolus
<i>cs</i> , circumciliary space	<i>os</i> , outer segment of receptor process
<i>ct</i> , connective tissue of capsule	<i>p</i> , perineurium
<i>d</i> , dendrite	<i>pc</i> , pigment cell
<i>e</i> , endoplasmic reticulum (ergastoplasm)	<i>pg</i> , pigment granule
<i>f</i> , fibrils	<i>r</i> , rootlets
<i>fs</i> , flattened sacs	<i>rc</i> , photoreceptor
<i>g</i> , guanine (?) rods	<i>s</i> , sheath
<i>ga</i> , Golgi apparatus	<i>sn</i> , sheath cell nuclei
<i>gc</i> , ganglion cell	<i>sv</i> , synaptic vesicles
<i>i</i> , iridocyte	<i>t</i> , tubules
<i>is</i> , inner segment of receptor process	<i>v</i> , vesicles
<i>l</i> , lumen of the eye	<i>x</i> , body of unknown nature
<i>ld</i> , lipid droplets	

FIGURE 2

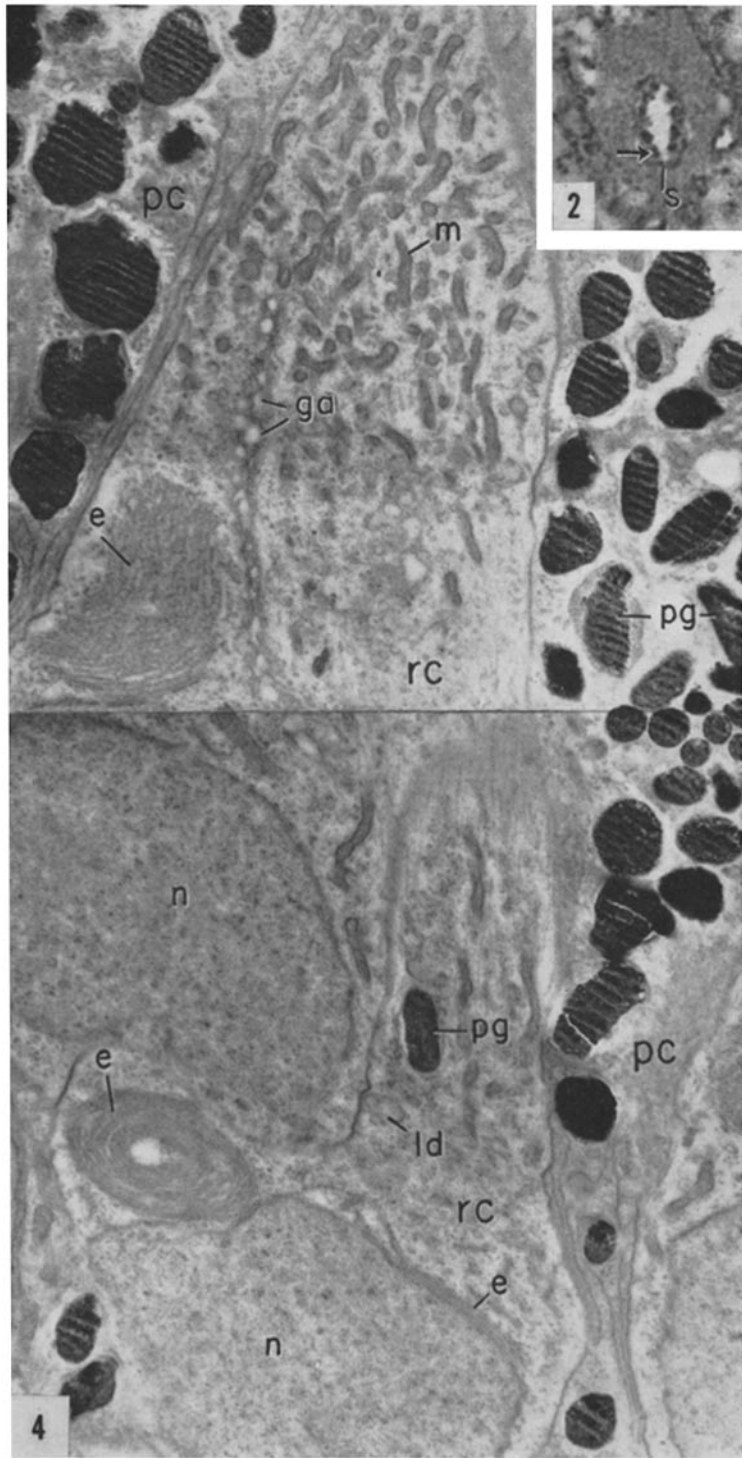
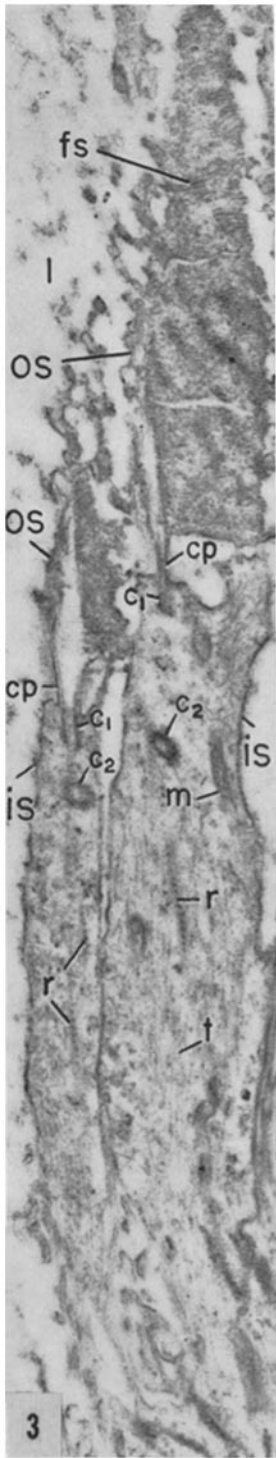
Cross-section of the fibrillar apparatus of the receptor process of *Xantusia*. The number of fibrils is not clearly shown, but there appear to be nine enclosed in a sheath (*s*). There is a suggestion in some (see arrow) of a double nature. $\times 29,000$.

FIGURE 3

Two receptor processes showing their outer (*os*) and inner (*is*) segments joined by a connecting piece (*cp*). Discs in outer segment are not clear; for this feature see Fig. 2 of Eakin and Westfall (13). $\times 9,000$.

FIGURE 4

Region of the retina between the nervous layer and the receptor processes (see Fig. 1) showing two receptor cells (*rc*) between two pigment cells (*pc*). $\times 9,000$.



labyrinth associated with each cell is a cilium (see arrows in Fig. 10), consisting of a ring of nine double filaments enclosed in a sheath. There is sometimes a suggestion of central fibers in transverse sections of the cilium beyond the surface of the cell (Fig. 9). One observes circumciliary spaces (Fig. 10) about the bases of the cilia, similar to but larger and deeper than those of the pigment cells. Fig. 10 shows also the roughly polygonal shape of the lens cells, their heavy cell membranes, and the many small vesicles (possibly indentations of the cell membrane) in the microvilli and in the bases of the cells. Here and there are lateral irregularities similar to the interdigitating processes observed in the developing lens of the mouse (3). Between the vesicular border zone and the nucleus each lens cell contains many small mitochondria. The distal ends of the cells (not figured) are characterized by thin cell membranes, a central cytoplasmic mass that is fibrous in appearance, and numerous small mitochondria lying in a band beneath the cell membrane.

Neural Structures: The photoreceptor terminates basally in a fiber (Fig. 19) which we are tentatively calling an axon, although the cell from

which it comes is not a neuron and its connections and ultrastructure have not been fully analyzed. It has been seen with the light microscope (5, 18, 30, 33). These axons, in which mitochondria are oriented lengthwise, cannot be traced far in single sections presumably because of their tortuous course into and through the nervous layer of the retina. The relationships of the many nerve fibers in this layer have not been determined. In general, our micrographs strikingly resemble Sjöstrand's (24) electron micrographs of the outer molecular layer of the guinea pig retina in which bipolar neurons synapse with the foot-pieces of the rods and cones. Presumably a clarification of the neural connections in the parietal eye will require model reconstructions of a large number of serial sections. The nerve fibers vary considerably in diameter, from 0.25 to 1.25 μ . In many of the larger fibers laminated whorls (Nissl substance?), like those described above, may be seen (Fig. 15). Except for their granular nature these structures resemble the onion-like bodies in the perikaryon of the rat superior cervical ganglion (20).

Ganglion cells are large neurons in or externally adjacent to the nervous layer (Fig. 15). Their

FIGURES 5 and 6

Longitudinal sections of connecting pieces showing the striated nature of the fibrils (*f*) and the rootlets (*r*). $\times 23,000$ (Fig. 5) and $\times 24,000$ (Fig. 6).

FIGURE 7

Longitudinal section of the fibrillar apparatus of a pigment cell showing the axial (*c*₁) and oblique (*c*₂) centrioles and pigment granules (*pg*). $\times 15,000$.

FIGURE 8

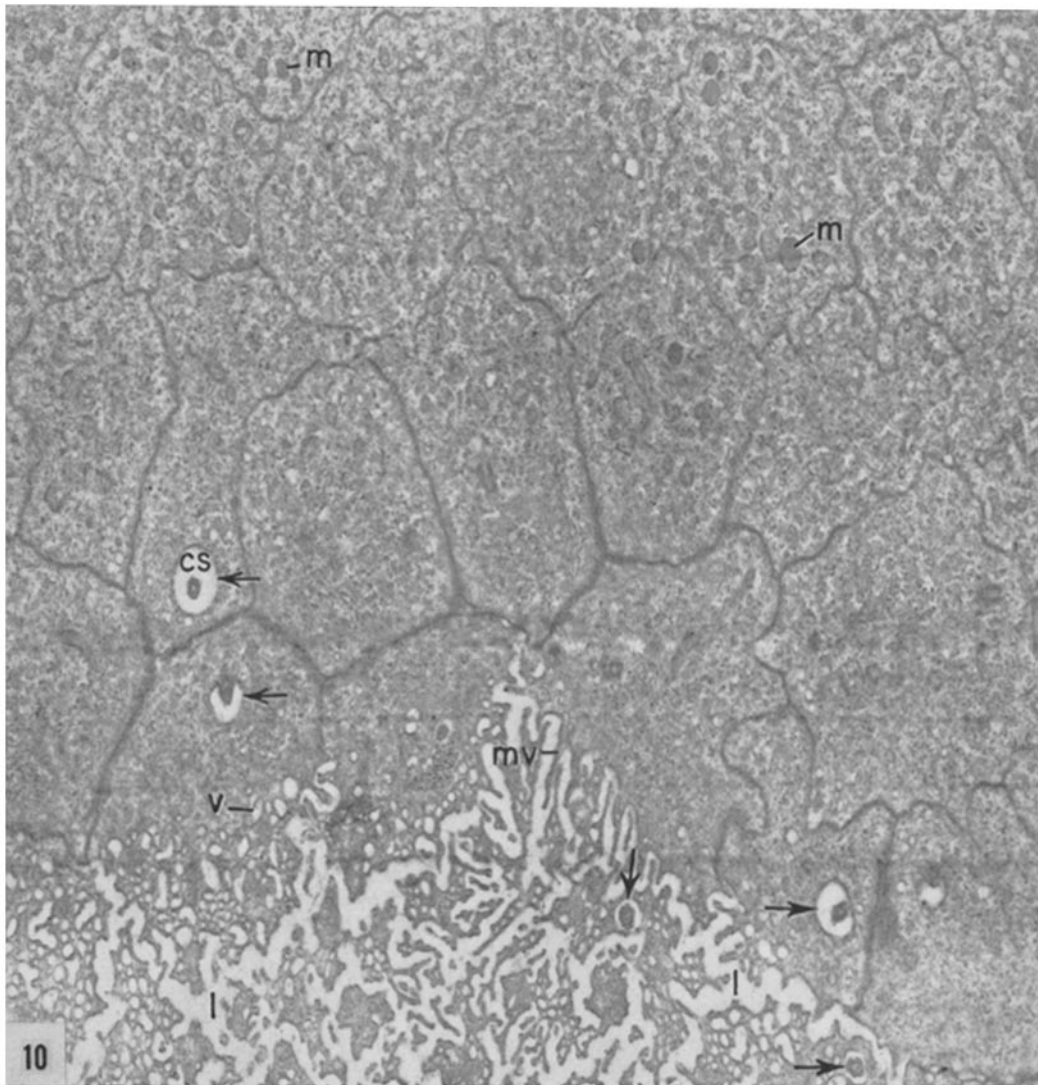
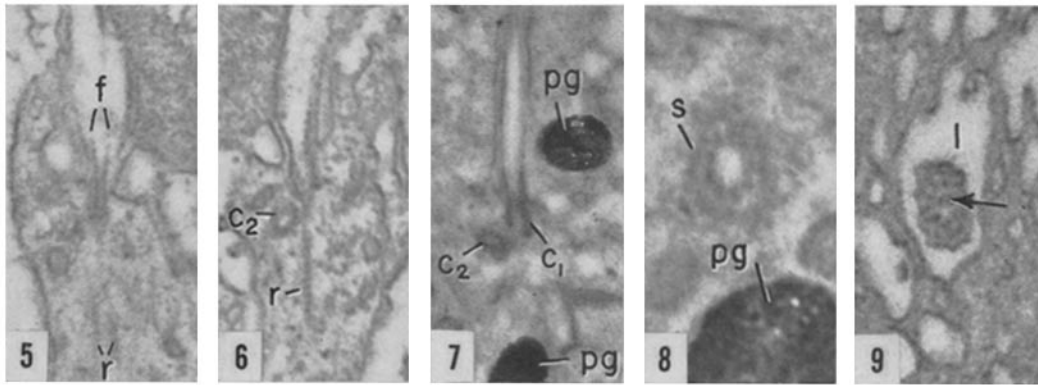
Cross-section of the fibrillar apparatus of a pigment cell showing nine double peripheral fibers in a sheath (*s*). $\times 33,000$.

FIGURE 9

Cross-section of a cilium of a lens cell situated in the lumen (*l*) of the eye. The arrow indicates the presence of central fibers within the nine double peripheral fibers. $\times 37,000$.

FIGURE 10

Oblique section of the lens showing roughly polygonal shape and heavy membranes of the cells. At the luminal surface of the lens many vesicles (*v*) or indentations of cell membranes are found, and numerous microvilli (*mv*) project into the cavity (*l*) of the eye. Arrows indicate cilia. A circumciliary space (*cs*) is found around the cilia below the cell surface. $\times 12,000$.



cytoplasm is considerably less dense than that of neighboring pigment cells and they possess numerous mitochondria. Nerve fibers are intimately associated with them. Those which are large and contain many synaptic vesicles are regarded as foot-pieces or boutons, the enlarged termini of axons. In Figs. 15 and 16 one observes that the axons or their boutons are unusually thick in certain places. Because of the similarity of these thick areas to the active points in synaptic membranes of other neural connections (see 7), they might be axosomatic and axodendritic synapses.

The gross anatomy of the parietal nerve has recently been described (12) in *Sceloporus* and several other lizards. The fine structure of the nerve is presented in Fig. 20, a cross-sectional view of the nerve lying immediately outside the capsule of the eye. One sees the nuclei of three sheath cells and about 250 to 300 non-medullated nerve fibers. Pigment granules are also found in the nerve, presumably in the cytoplasm of sheath cells. It may be recalled here that Nowikoff (18) mentioned pigment cells (*Bindegewebschromatophoren*) as occurring in the parietal nerve of *Lacerta* and *Anguis*. The nerve is enclosed in a thick perineurium which exhibits faint striations (probably collagen fibers). Fig. 21 shows the connection of the nerve to the retina of the eye. Bundles of nerve fibers, two of which may be observed in the upper left hand corner of the figure, converge and assemble into a cord, the nerve, which in this view appears as a ventral mid-line bulge of the retina into the capsule of the eye. As the nerve fibers leave the retina they bend and course posteriorly. Thus, some fibers (nf_1) have

been cut longitudinally, others (nf_2) in cross-section. Note that many of the latter are seen suspended within a sheath cell by infoldings (mesaxons) of its cell membrane. The nerve is covered with perineurium, outside of which is the iridocyte layer of the capsule. In other sections one observes retinal pigment cells among the converging nerve fibers.

Capsule: The capsule of the parietal eye usually contains flat iridocytes (Fig. 15) covering the ventral and lateral surfaces of the eye. At the junction of capsule and meninges the iridocytes are much less flattened, some being ovoid or almost spherical (Fig. 17). They are also present on the under surface of the meninges adjacent to the eye. In the cytoplasm of the iridocytes are rows of rectangular blocks, assumed to be guanine because of their seeming brittleness and because of the silvery appearance of the capsule of the eye in the living animal. The arrangement of the blocks, in rows parallel to the surface of the retina, is similar to that of long rods in the tapetum lucidum of the kitten, as figured by Bernstein and Pease (1). These workers concluded, however, that the tapetal inclusions in the cat are melanin products. In the capsule of some eyes of *Sceloporus occidentalis* the guanine appears to be reduced or absent. Stebbins (unpublished) found in examining over 400 animals that this condition was present in about one out of every four animals.

DISCUSSION

Whether or not the reptilian third eye is functional is a controversial subject. Recently Roth

FIGURE 11

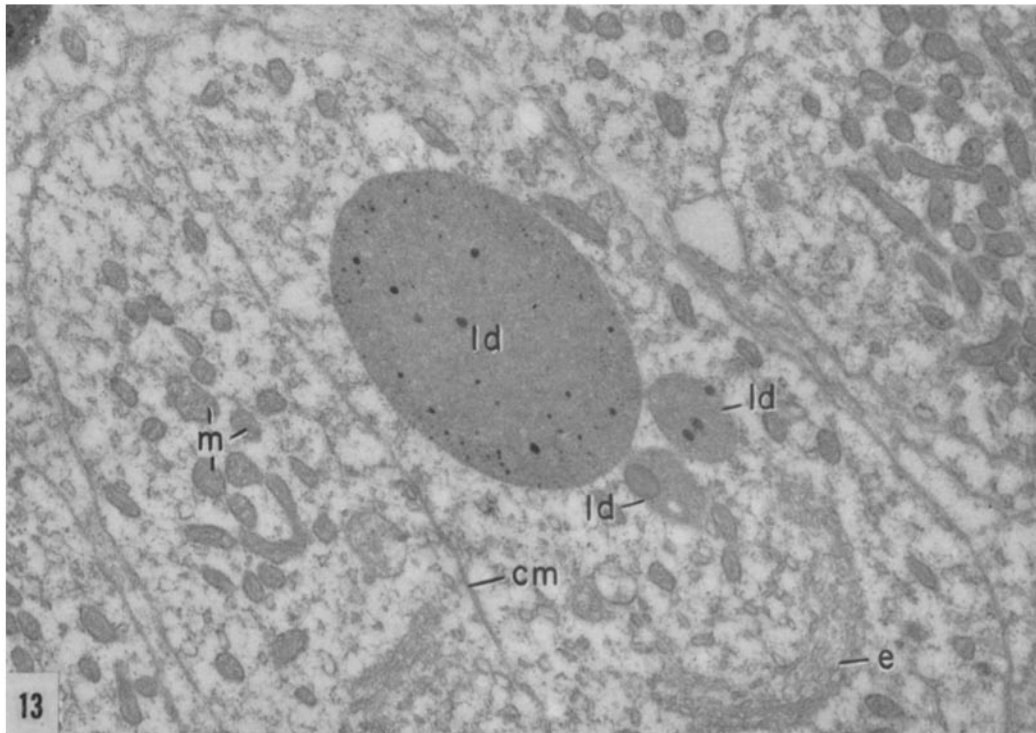
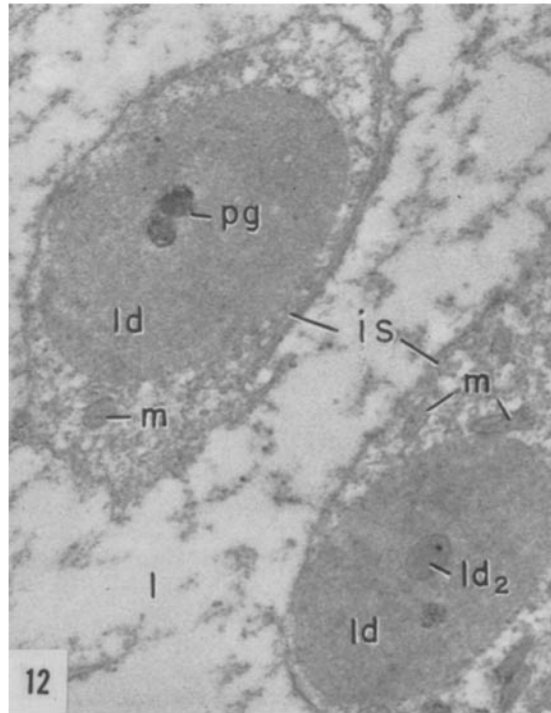
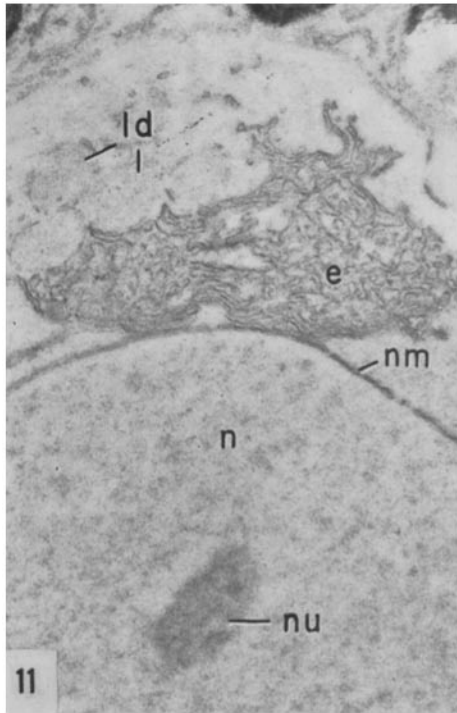
Part of a photoreceptor showing a pile of endoplasmic reticulum or ergastoplasm (e) distal to the nucleus (n). Immediately adjacent to the ergastoplasm are ovoid droplets (ld), presumed to be lipid in nature. Tissue fixed in 0.6 per cent buffered $KMnO_4$. $\times 19,000$.

FIGURE 12

Inner segments (is) of two receptor processes of *Xantusia* showing a large lipid (?) droplet in the base of each process. Pigment granules (pg) or smaller droplets (ld_2) frequently occur as inclusions in the large droplets. $\times 29,000$.

FIGURE 13

Oblique section through several photoreceptors of *Xantusia* distal to their nuclei. Lipid (?) droplets (ld) of several sizes are shown in the vicinity of a mass of endoplasmic reticulum (e). $\times 26,000$.



and Braun (22) concluded from experiments on *Anguis fragilis* that the parietal eye is neither a photoreceptor nor a thermoreceptor. On the other hand, there is increasing evidence that the third eye of the lizard is functional and not merely a *vestige* of an ancient and large median eye (see 26). In the first place, the anatomical features of the parietal eye in the following list indicate that it is light sensitive: the transparency of the cornea and lens, the fine structure of the receptor processes, the arrangement of organelles in the sensory cells, the relationship of pigment cells to photoreceptors, and the presence of nerve fibers from the receptors, ganglion cells, and a nerve connecting the eye to the habenular region of the brain (12). Secondly, the behavioral studies of Stebbins and Eakin (27) and of Glaser (17) suggest that the eye functions as a dosimeter of solar radiation. Thirdly, Eakin and Quay (11) have shown that PAS-positive material (substances stainable with the periodic acid-Schiff technique) accumulates in the parietal eye of a dark-adapted lizard and that it becomes markedly reduced in the eye of a light-adapted animal. For these reasons we have called the sensory units in the retina of the parietal eye photoreceptors.

It is hoped that critical proof of this sensitivity may be obtained soon in the form of a biochemical demonstration of a photopigment in the retina of the third eye and of physiological evidence of nerve impulses in the parietal nerve upon stimulation of the eye with light. Eakin and Crescitelli (un-

published) were unable to demonstrate a photosensitive substance in digitonin extracts of as many as 110 parietal eyes of *Sceloporus occidentalis*. Although the sample was large in number of animals, representing incidentally a considerable collecting effort, the actual amount of tissue for analysis was small. Moreover, digitonin may not be a suitable solvent for the particular pigment which we believe to be present in the outer segments of the receptor processes. Finally, if the sensory cells are "conelike" in photopigment concentration and sensitivity level (see below), then it is not surprising that the first effort to demonstrate a photosensitive pigment was unsuccessful. Lateral-eye cone-photopigments are present in such low concentration as to be invisible to the human eye, in great contrast to the rhodopsin and porphyropsin of lateral-eye rods. As for a demonstration of neural conduction, the physiologist should be able to insert his electrodes into the parietal nerve, especially in *Sceloporus* juveniles or immatures, in which there is a large parietal foramen in the cranium and in which the nerve runs under the skin for some distance caudal to the eye.

We are impressed not only with the anatomical complexity of the parietal eye but also with the remarkable similarity of its photoreceptors to the rods and cones of the lateral eye. The agreement in detail extends to the arrangement of discs or flattened sacs in the outer segments of the receptor processes, the fibrillar structure of the

FIGURE 14

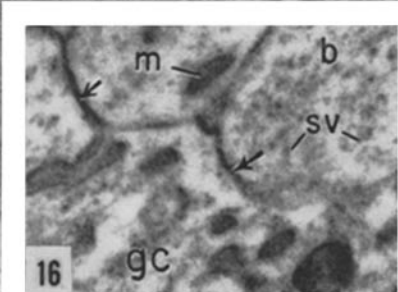
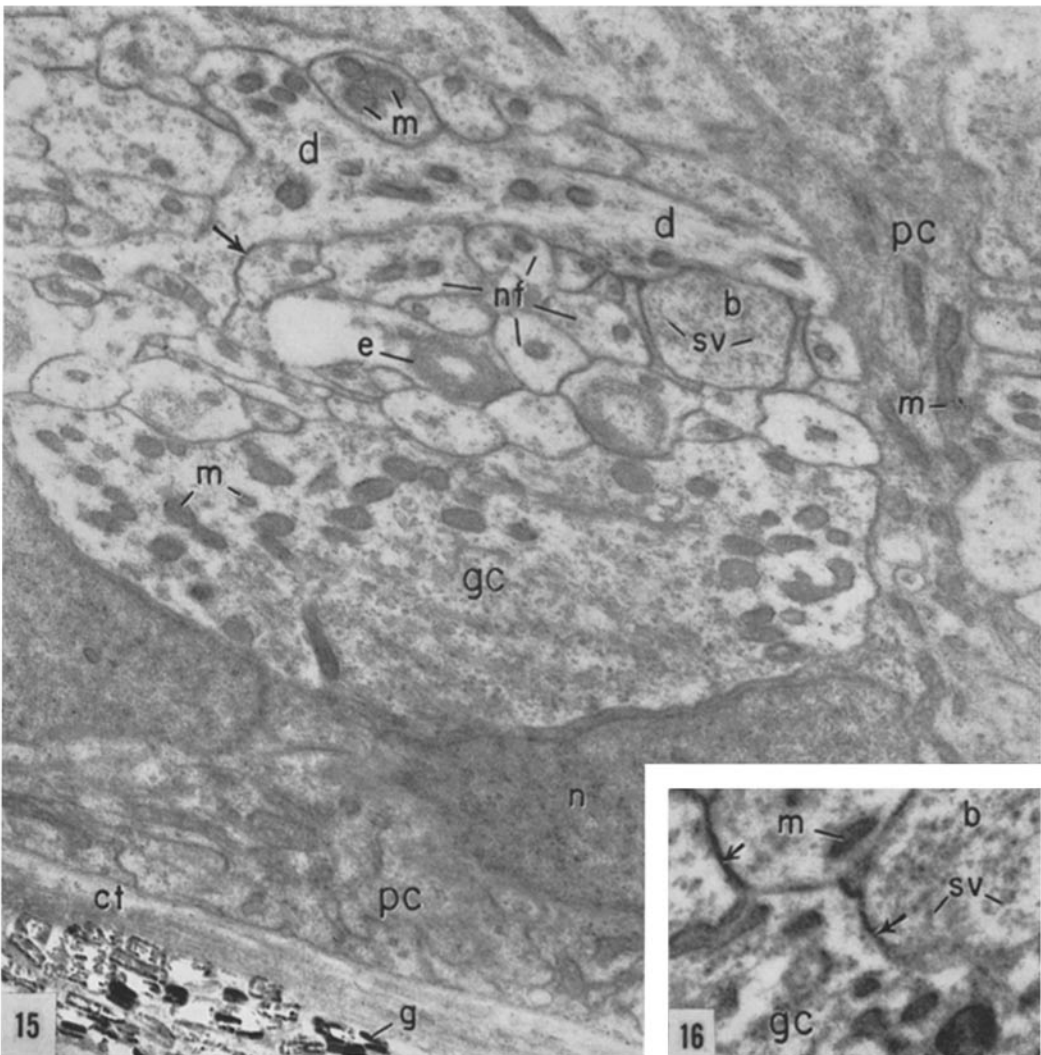
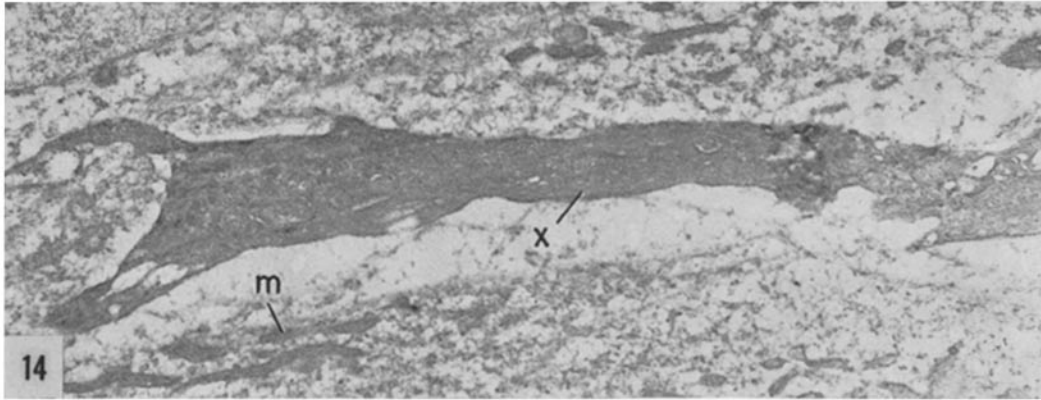
Large mass of unknown material (*x*) lying distal to the nucleus of a *Xantusia* photoreceptor. $\times 17,000$.

FIGURE 15

Proximal (outer) zone of the retina showing the trumpet, with nucleus (*n*), of a pigment cell (*pc*); a ganglion cell (*gc*); a dendrite (*d*) and many other nerve fibers (*nf*), axons perhaps, which variously contain synaptic vesicles (*sv*), mitochondria (*m*), and whorls of ergastoplasm (*e*, Nissl substance ?); a bouton (*b*), the enlarged terminus of an axon. Arrow indicates a thick membrane between a dendrite and an axon, which may represent an active synaptic point (axodendritic synapse). Covering the retina is a capsule consisting of an inner layer of connective tissue (*ct*) and an outer layer of iridocytes containing rows of guanine (?) rods (*g*). $\times 13,000$.

FIGURE 16

Boutons (*b*) of two axons in synaptic relation (axosomatic) with a ganglion cell (*gc*). Arrows point to thickenings in the synaptic membranes, which are presumed to be active points. $\times 22,000$.



connecting piece, the presence of striated roots extending basally from the axial centriole, the orientation and distribution of mitochondria, the position of the nucleus, and the nature of the nerve fiber. True, one might expect the median and lateral-eye receptors to have common features of a general sort, but the high degree of similarity is striking and affords further support of the theory of the endodermal origin of photoreceptors (34, 35).

The third eye develops as a vesicle from the roof of the neural tube in close association with the epiphysial evagination (18, 28, 33). The embryonic outpocketing is lined with endodermal cells. After the parietal vesicle becomes separated from the epiphysis and "migrates" to its definitive position under the skin the central part of the dorsal wall differentiates into the crystalline lens. The retina arises from the ventral and lateral walls of the vesicle, and because there is no invagination of the vesicle to form a cup, as in the development of the lateral eye, the cilia of the prospective retinal cells project into the lumen of the eye, as do also those of the lens cells. The luminal end of each presumptive photoreceptor extends beyond the limiting membrane into the cavity of the vesicle, bearing the cilium (flagellum) at its tip. The cilium subsequently becomes the outer segment and connecting piece of the receptor process and the part of the sensory cell projecting into the lumen becomes the inner seg-

ment of the process. Topographically the retina of the parietal eye corresponds not with the retina of the lateral eye but with its pigment epithelium, and the lens of the parietal eye, unlike that of the lateral eye, is derived from neural ectoderm (18, 28, 33).

Nowikoff (18), working before the development of the electron microscope, made some remarkable observations on the processes of the sensory cells (*Sehzellen*) of the parietal eye in *Lacerta agilis* and *Anguis fragilis*. He described long tapering extensions from the luminal ends of both retinal and lens cells. Those of the receptors contained fine fibrils, of varying length, connected to basal bodies. Nowikoff postulated a ciliary nature and origin of these structures. He believed, however, that each process was derived from not one but several cilia fused together. Lacking the high degree of optical resolution required he did not see the discs or flattened sacs which we described (13), and he concluded that there was no specialized receptor structure (*Sehelement*) in the sensory cells. Nevertheless, he assumed the presence of a photosensitive substance distributed throughout the cell. Dendy (5) noted short processes from the luminal ends of the "sense-cells" in *Sphenodon punctatus*, but he stated that they exhibit "no sort of special differentiation." Steyn (30) described sensory cells in *Cordylus polyzonus* which project into the cavity of the parietal eye in the form of processes with inner and outer segments. The outer

FIGURE 17

A semispherical iridocyte (*i*), at the junction of the capsule of the parietal eye and the meninges, with its nucleus (*n*) surrounded by rows of guanine (?) rods (*g*). $\times 8,000$.

FIGURE 18

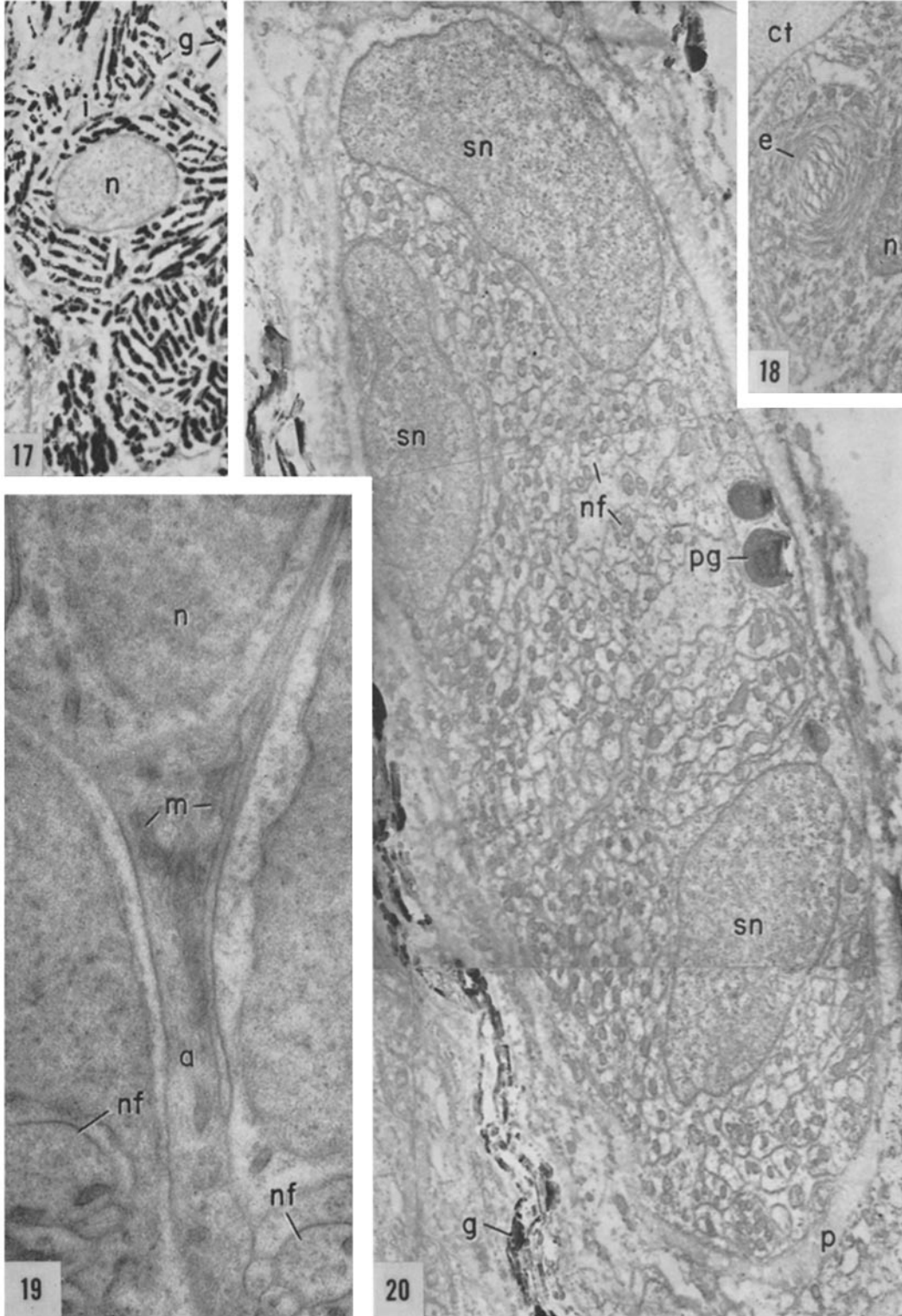
A part of the outer zone of the retina of *Xantusia* showing a whorl of endoplasmic reticulum (*e*) near the nucleus (*n*) of a cell, which may be a ganglion cell. $\times 8,000$.

FIGURE 19

Basal end of a photoreceptor terminating in an axon (*a*), with lengthwise oriented mitochondria (*m*), which is soon lost among a large number of nerve fibers (*nf*). $\times 15,000$.

FIGURE 20

A cross-section of the parietal nerve containing three sheath cell nuclei (*sn*), pigment granules (*pg*), and 250 to 300 non-medullated nerve fibers (*nf*) within a perineurium (*p*), the faint cross striations of which may be collagen fibers. The nerve lies just outside the guanine-*g*) containing capsule of the eye. $\times 15,000$.



segment was observed to exhibit "variable appearance according to cyclic phases of an apocrine secretory process." This picture was not referred to in Steyn's (29) preliminary note on the ultrastructure of the sensory cells of the pineal eye of the same animal. We suggest that some of the features observed by Steyn (30) and Trost (33) using the light microscope may be artifacts.

Now let us see in what respects the photoreceptors of the parietal eye resemble either the rods or cones of the lateral eye. It was noted above that the unsuccessful attempt (Eakin and Crescitielli, unpublished) to extract a photopigment from parietal eyes of the fence lizard *may* suggest that the pigment is present in very low concentration. If true, this would be significant because the most important difference between rods and cones lies in the relative concentration of their photopigments: high in the former, low in the latter.

Secondly, a comparison of glycogen distribution is interesting. Eakin and Quay (11) recently showed that the region of the parietal eye photoreceptor distal to its nucleus contains strongly PAS-positive granules. Because prior treatment with amylase rendered the photoreceptor completely negative to the PAS reaction, these workers concluded that the stainable material is a glycogen-like polysaccharide. Cones of the lateral eye, on the other hand, have long been known to contain glycogen, particularly in the paraboloid, usually a solid or semi-solid body distal to the nucleus. Applying the PAS-technique to the rabbit retina Sidman and Wislocki (23) found that the cone paraboloid was strongly positive. In fact the paraboloid was negative to all other stains used by these investigators. Rods may have paraboloids, according to Walls (36), but only when they have had a peculiar history.

Thirdly, the large ovoid body at the base of the receptor process in the parietal eye of the night lizard, *Xantusia*, may be a "conelike" feature. The shape and seemingly structureless appearance of this body together with the fact

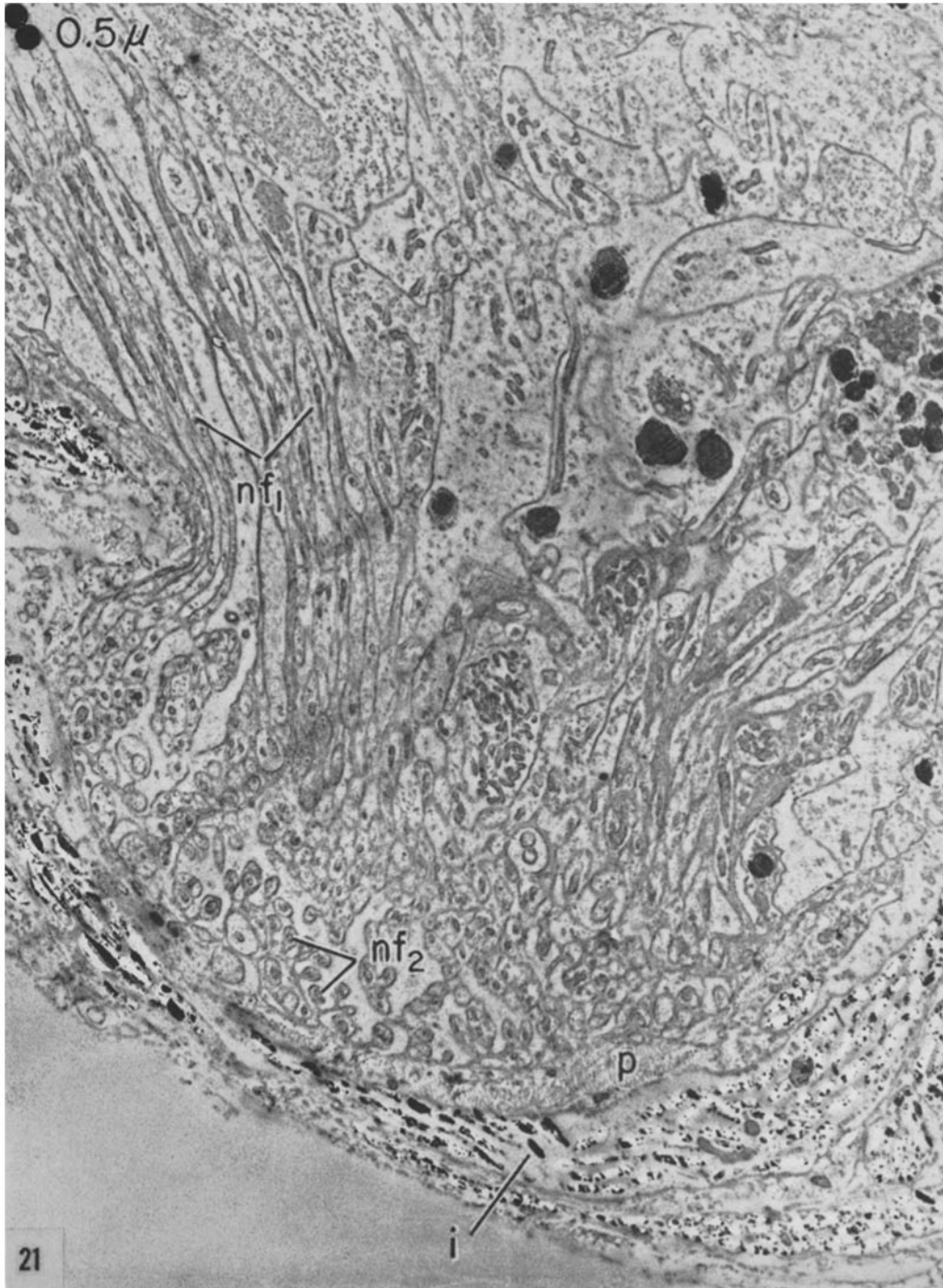
that smaller yet similar ovals and pigment granules are occasionally seen inside it suggest a fluid nature. Although the droplets are not intensively stained by osmium tetroxide they may be lipoidal nevertheless. It is well known that various factors, such as state of aggregation and degree of saturation, affect the affinity between this metal and lipids. Oil-droplets at the bases of the receptor processes are commonly found in lateral-eye cones of higher vertebrates (36); in the cones of *Xantusia* the oil-droplets are present although colorless (36).

Finally, lateral-eye rods and cones may differ in their ultrastructure. De Robertis and Lasansky (8) noted differences in the shapes of the rod and cone sacs and in the lengths of the connecting pieces of the rods and cones of the rabbit. Until these features have been shown to distinguish rods and cones in other forms they can not be generally considered cell characteristics. The discs or sacs and the connecting piece of the parietal eye receptor show some resemblance to the lateral-eye cone with respect to the above points (13).

The presence or absence of a parietal eye nerve in adult lizards is another controversial matter. Steyn (30) described a parietal nerve in embryos of *Cordylus polyzonus* and *Mabuya sulcata*, but he is of the opinion that the nerve atrophies in the adult of these and other forms. Steyn discussed the earlier literature and concluded that workers such as Nowikoff, who reported a nerve in adult *Lacerta* and *Anguis*, had misidentified the structure described and figured. This conclusion seemed justified in view of the recent claim by Roth and Braun (22) that the nerve is absent in adult *Anguis*, one of the forms upon which Nowikoff worked. Eakin and Stebbins (12), however, reported a nerve in the adults of nine different lizards, including *Sceloporus occidentalis*. Also Ortman (19) has recently given evidence that a parietal nerve is present in the adult *Anolis carolinensis*. The electron micrograph here figured (Fig. 20) supports the conclusion that the structure

FIGURE 21

Part of a frontal section of an eye showing the connection of the parietal nerve with the retina. *nf*₁, two bundles of nerve fibers, seen in longitudinal aspect, leaving the retina and entering the parietal nerve; *nf*₂, nerve fibers, seen in cross-section, within the nerve proper. The fibers are supported by infoldings (mesaxons) of the cell membrane of a sheath cell. Diameter of polystyrene balls in upper left-hand corner is 0.5 μ .



described by Eakin and Stebbins (12) in the adult *Sceloporus* is indeed a nerve. Dendy's (5) reservation about the small number (fourteen or fifteen) of fibers reported by Nowikoff (18) to compose the parietal nerve seems to be justified, as we count between 250 and 300 fibers. Moreover, our finding that the fibers of the parietal nerve in *Sceloporus* are non-medullated confirms Dendy's (5) observations on *Sphenodon*; Nowikoff (18) stated, however, that the fibers in *Lacerta* are myelinated.

Nowikoff (18) and Dendy (5) are in agreement that the fibers of the parietal nerve originate from ganglion cells in the retina of the eye. According to Steyn (30), however, the nerve develops largely by outgrowth of fibers from the roof of the embryonic diencephalon "into the retina." The latter picture differs from that of other nerves of special sense, as the neuroblasts giving rise to olfactory, optic, and acoustic fibers lie in the sense organ itself or in cranial ganglia. Incidentally, there is evidence of a ganglion on the parietal nerve of *Callisaurus draconoides* and *Sauromalus obesus* (12)

and of *Anolis carolinensis* (19). The distal connections of the parietal nerve fibers, however, have not yet been determined. Ortman (19) using nerve stains and phase microscopy was unable to trace the fibers in *Anolis* beyond the capsule of the eye. Likewise, Eakin and Stebbins (12), using light microscopy, could not follow the parietal nerve fibers into the retina of *Sceloporus*. Fig. 21 conclusively demonstrates that the fibers in the parietal nerve connect with the nervous layer of the retina. The analysis of their terminal connections, however, will require further electron microscopy.

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