STUDIES ON THE EYES OF ANCHOVIES *ANCHOA MITCHILLI* AND *A. HEPSETUS* (ENGRAULIDAE) WITH PARTICULAR REFERENCE TO THE PIGMENT EPITHELIUM

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A study has been made of the eyes of the anchovies *Anchoa mitchilli* and *A. hepsetus* (Engraulidae) with particular reference to the pigment epithelium. Eyes of dark (d.a.) and light (l.a.) adapted fish were investigated by optical and electron microscopy. In light adapted eyes the pigment epithelium is radially extended with the vitread region of the cells inserted amongst the cones and lying in vertical rows above them. They are separated from adjacent rows of pigment epithelial cells by masses of rods arranged in similar vertical sheets. The vitread end of the cell, where it fits between the cones, is cuneate in shape. In l.a. eyes lateral lobes develop from the cuneate portion of the cell, extend into the spaces between the cone rows, and serve to shield the lateral margins of the cones. On dark adaptation of the eye the lateral lobes are withdrawn as the cell shortens radially.

The cytoplasm of the pigment epithelium contains numerous myeloid bodies composed of stacks of smooth endoplasmic reticulum distributed throughout the cell. In l.a. eyes masses of less well ordered e.r. cisternae and tubules are also common, especially in the vitread region among the tapetal material and melanosomes. Mitochondria, some large vacuoles, lysosomes containing ingested portions of rod outer segments, and also occasionally melanosomes and tapetal material occur in the basal region of the cell in the vicinity of the nucleus. Structurally differentiated ground substance and ribosomes are poorly represented throughout all regions.

Spherical and cylindrical melanosomes and tapetal material composed of guanine are plentiful within the cell. In the l.a. state melanosomes occur in the vitread region, at the level of the cones, but in the d.a. state they become concentrated in the basal region forming a dark scleral zone throughout the tissue. The tapetal material consists of approximately isodiametric crystallites, truncate needles and thin almost square platelets. All forms are strongly birefringent. The crystallites are distributed throughout the l.a. cell but in the d.a. condition they become concentrated mainly in the middle region forming a prominent whitish zone throughout the tissue vitread to the melanosome layer. In l.a. eyes the needles are arranged axially and are distributed mainly around the periphery of the cell in the vitread region. On dark adaptation of the eye some of the needles are dispersed amongst the crystallites in the middle region. The platelets are grouped into two stacks which remain essentially unaltered in organization and location during photomechanical movements of the eye. The stacks are grouped into a V in the vitread cuneate region with the platelets abutting the cone units and lying in the same vertical plane as the cone lamellae. Each stack is composed of several regularly spaced rows with each row made up of two files of platelets. The average thickness of platelets measured from vertical sections through stacks is about 82 nm and the distance between platelets of adjacent rows is about 112 nm. A cisterna of endoplasmic reticulum runs between each row and is connected to the membrane sacs of adjacent platelets by tubular connections. Non-membrane cross-bridging material also links the cisterna and platelet sacs. The consequence of the organization of the platelet stacks as a system for the reflexion of light into the cones by constructive interference is discussed.

1. Introduction

The pigment epithelium of the vertebrate eye consists of a basal continuum of cells from which processes penetrate vitread among the photoreceptors. Studies with the electron microscope (see, for example, Yamada 1958, 1961; Porter & Yamada 1960; Dowling & Gibbons 1962; Engström 1963; Moyer 1969; Ishikawa & Yamada 1970; Dickenson & Hollenberg 1971; Fineran & Nicol 1974; Berman, Schwell & Feeny 1974; Nguyen-Legros 1975*a*, *b*) have shown the form and contents of pigment epithelium cells and their relationship to the photoreceptors more clearly than was previously possible using traditional optical microscopy and paraffin methods of specimen preparation. The importance of modern methods in revealing the diverse functions of the pigment epithelium has recently been emphasized by Spencer (1972). It is now apparent

that there are great variations in the organization of pigment epithelia among vertebrates, even within related groups. Variations of structure are most pronounced in those eyes exhibiting a retinal tapetum lucidum (reflecting layer) such as is found in many teleosts (Arnott, Nicol & Querfeld 1972; Arnott, Best, Ito & Nicol 1974; Locket 1971, 1974; Nicol & Arnott 1973; Nicol, Arnott & Best 1973), some nocturnal birds (Nicol & Arnott 1974) and other vertebrates (Walls 1942).

In a recent survey of the tapeta lucida of bony fishes Nicol *et al.* (1973) briefly examined the eyes of the bay and striped anchovy (*Anchoa mitchilli* and *A. hepsetus*, respectively) and found a white diffusing tapetum. Zyznar & Nicol (1973) have since confirmed that this tapetum is composed of guanine. The electron micrograph published by Nicol *et al.* (1973) of a larval eye of *A. mitchilli* does not, however, show a diffuse tapetum but a system of regularly stacked platelets arranged around the perimeter of the cell process and extending between the photoreceptors. They suggested that the platelets might function as some kind of a reflector. Most retinal tapeta lucida of teleosts contain scattered tapetal material in the form of minute crystals (see Walls 1942) or tapetal spheres (Arnott *et al.* 1972; Nicol & Arnott 1973), that forms a layer which functions as a diffuse reflector for the back scattering of light to rods during dark or dim light vision.

The ordered stacking of guanine platelets in pigment epithelial cells of *A. mitchilli* and *A. hepsetus*, their regular association with the photoreceptors, and the vertical orientation of photoreceptor disk lamellae are unusual features and have prompted us to make a detailed study of these eyes. In a preliminary report (Fineran & Nicol 1976), we have shown that the platelets of the pigment epithelium are associated with the cones and not with rod outer segments as was at first believed (Nicol *et al.* 1973). Furthermore, the cones are arranged in vertical rows consisting of alternately placed long cones and short cones with a bilobed outer segment (bifid cones). The outer segment of the long cone, and portion of its ellipsoid, are united with two outer segment lobes of the two adjacent bifid cones to form a wedge-shape structure designated the 'cone unit' (Fineran & Nicol 1976). In vertical section these units give the cone rows a saw-toothed appearance, with the cone units interdigitating with the processes of the pigment epithelium (figure 24, plate 6). We have also demonstrated that the disk lamellae of the cones run longitudinally with respect to the axis of the cell and that lamellae of the long cone lie at right angles to those of the bifid cone. In the present paper our general observations on the retina and detailed studies on the pigment epithelium cells of *A. mitchilli* and *A. hepsetus* are described.

2. Materials and methods

Eyes were obtained from *Anchoa mitchilli* Cuv. *et.* Val. and *A. hepsetus* L. caught in the environs of Port Aransas, Texas. *Anchoa hepsetus* was caught by surface trawling from inshore waters of the Gulf of Mexico; *A. mitchilli* was collected from intracoastal waters by trawling or by seining from the shore. Specimens of *A. mitchilli* ranged in length from 3.5 to 5.5 cm; those of *A. hepsetus* were mostly 12–16 cm long. Dark adapted eyes were secured from fish caught on a dark night or by holding live fish for 1 h in the dark in the laboratory. The dark adapted eyes were opened under dim red light and fixed for 1–2 h. They were then excised and placed in fresh fixative for a further 4 h.

(*a*) *Light microscopy*

Eyes for conventional light microscopy were fixed in Bouin's and Zenker's fluids, embedded in wax and celloidin and sectioned at 5–10 μm. Sections were stained with Heidenhain's iron haematoxylin and eosin, Chlorozal Black E, Ehrlich's haematoxylin and eosin/orange G and Biebrich scarlet, and Mallory's triple stain. Some sections were treated with 2 % oxalic acid and then with Chlorox bleach (1/1000) prior to staining.

Sections for light microscopy were also prepared from Araldite-embedded material processed for electron microscopy (see below). They were cut between 1–3 μm using glass knives on Sorvall Porter-Blum Mt-1 and LKB Ultrotome II microtomes and stained either in methylene blue/azure B (see Juniper, Cox, Gilchrist & Williams 1970) or in Paragon multiple stain for frozen sections (Paragon Co. Inc. N.Y.). The sections were mounted in Permount (Fisher Scientific Co., New Jersey). Some material was also embedded in TAAB resin (TAAB Laboratories, Reading, England) and methacrylate JB-4 plastic (Polysciences Inc. Pennsylvania, U.S.A.). In general, material embedded in Araldite proved the most satisfactory and was superior to that prepared for conventional light microscopy for studying cellular detail. The serially sectioned wax embedded specimens were nevertheless useful for survey work on the histology of the eye, especially when used in conjunction with the epoxy resin embedded material.

Teased preparations of the pigment epithelium were examined for the form and contents of individual pigment epithelial cells. In addition, pieces of retinae were digested with pronase to obtain preparations of the tapetal material.

Sections, individual cells and isolated tapetal components were examined and photographed by transmitted light with Reichert Zetopan and Leitz Ortholux microscopes by using bright field, polarizing, phase contrast and Nomarsky interference optics. The retinal surface of fresh eyes was also studied by superior illumination, with a stereo and compound microscope.

Spectrum reflexion of tapeta was measured with a Leitz MPV photometer and digital multimeter; light was supplied by a Jarrell-Ash Monochromator. The dissected eye (retina *in situ*) was mounted either on a dissecting microscope ($\times 6$ objective, $\times 20$ ocular), and illuminated from above by a prism at an angle of about 30°; or under a Leitz Ultropak ($\times 0.38$ objective 0.12 n.a., $\times 25$ ocular). Reflexion of aluminium foil, a front surface mirror and a freshly deposited surface of MgO were measured under similar conditions.

(*b*) *Electron microscopy*

Eyes to be fixed for transmission electron microscopy were dissected either in the fixative or in teleost Ringer. Some eyes were fixed whole with only portions of the iris punctured to allow easier penetration of the fixative into the eye cup, but with others the lens was removed. The retina of light adapted (l.a.) eyes was usually further dissected into segments taken from selected regions, but with dark adapted (d.a.) eyes it was generally found best to leave as much of the retina as intact as possible to prevent the pigment epithelium splitting through the tapetal layer.

Specimens were fixed mostly in 2.5 % glutaraldehyde (e.m. grade), made up in either 0.1 M phosphate or sodium cacodylate buffer (pH 7.2), both containing 5 % sucrose, for 4–6 h followed by three rinses in the same buffer containing sucrose (the last change overnight). The material was then post fixed in buffered 2 % osmium tetroxide for 6 h. The schedule was

carried out either at room temperature (20 °C) or in the cold (4 °C). Specimens were dehydrated in ethanol increasing at 10 % concentrations, with 10 min in each, until the absolute ethanol stage when they were left for 1 h with three changes during that time. The specimens were then soaked in propylene oxide for 10 min and progressively infiltrated with Araldite following established procedures (Fineran & Bullock 1972). Occasionally, material was prepared in Karnovsky's (1965) fixative, containing NaCl, and processed rapidly according to the schedule outlined by Hayat (1972). Thin sections were cut with glass and diamond knives on an LKB Ultrotome II, mounted on Colloidin/carbon and Formvar coated 100 mesh copper grids and post stained in uranyl acetate followed by lead citrate. Also some material was block stained with uranyl acetate during the dehydration stage. Sections were examined using Hitachi HS-7S, HS-8, and Siemens Elmiskok I electron microscopes.

Measurements of isolated tapetal crystals and melanosomes were made from electron micrographs taken at fixed magnifications and calibrated, without any change in the microscope, against a grating replica having 1102 lines per mm (E. Fullam, Inc. Schenectady, N.Y.). The measurements of the thickness of tapetal platelets and of their spacing within the stack were made from electron micrographs, at a fixed magnification and calibrated against the grating replica, of unstained sections mounted on support film.

Preparations for scanning electron microscopy included isolated tapetal material, melanosomes and portions of ruptured pigment epithelium cells; the suspensions containing these fragments were spread on coverslips. On drying, the coverslips were broken and pieces of them mounted on aluminium stubs with silver conductive paint, coated with gold:palladium (60:40) on an omnirotory stage in a Denton DV 502 evaporator, and examined in an AMR 1000 scanning electron microscope. The micrographs were recorded with Polaroid film.

3. Observations and results

(*a*) *Appearance of intact eyes and fresh tissues*

The intact eye is roughly subspherical with the spherical lens situated slightly anteriorly (figure 1, plate 1). A prominent stratum argenteum covers the iris and most of the eye cup which, when viewed with reflected light under the stereoscopic microscope, exhibits fine vertical striations. The stratum argenteum in the dorsal anterior rim position of the eye cup is covered by a layer of dark pigmented tissue; more diffuse pigmentation extends from this area around the remainder of the rim and down the back of the eye. Adjacent to the optic nerve, and anterior to it, the argenteum is absent and the underlying dark tissues of the retina and covering layers are visible. The optic nerve emerges from the back of the eye in a posterior dorsal position. The choroid fissue lies in a posterior ventral position with a slight anterior orientation on the inside of the eye cup.

The tissues of the retina seen macroscopically from within the eye appear black in l.a. eyes (figure 8, plate 2), often with a faint green tinge in the anterior region. In d.a. fish the eye shows eyeshine and the fundus is creamy white (figure 1, plate 1). When l.a. eyes are cut into pieces the pigment epithelium adheres strongly to the retina and contains whitish streaks running radially. In d.a. eyes the pigment epithelium contains a prominent whitish tangential band which often splits when the retina is cut into segments.

A regular organization of the retina is revealed when the inside of the eye is viewed microscopically by superior illumination (figures 6 and 7, plate 2.). Fine striae are observed, consisting

of alternating light and dark bands, which run dorso-ventrally across the retina. The bright bands are yellow green and contain light and dark spots closely spaced (figure 7). The bright spots are a mixture of green, yellow and orange, they measure approximately 8 μm in diameter, they sometimes alternate with those of adjacent rows (figure 7) and they correspond to stacks of flat crystals in the pigment epithelium cell processes. In the l.a. eye of *Anchoa mitchilli* the green reflecting area as seen in the central fundus is surrounded by a zone appearing dull orange and sloping upwards towards the periphery. The appearance of the latter (i.e. the orange zone) is in some way related to the direction of illumination and viewing.

(*b*) *Histology of the retina*

The retina varies in thickness in different parts of the eye cup; the anterior ventral region is almost half the thickness of the anterior dorsal and posterior regions. A small depression, probably representing a fovea, was noted in sections through the posterior ventral region. The retina consists of the usual layers of tissue with a particularly well developed horizontal cell layer (figure 2, plate 1). In the anterior ventral and dorsal half of the retina the outer nuclear layer is almost twice the thickness of the inner layer whereas in the posterior ventral region both layers are of a similar thickness. The photoreceptor layer and pigment epithelium constitute between half to one third the thickness of the retina, but their appearance varies greatly depending on the plane of sectioning and whether the eye is light or dark adapted.

The photoreceptors consist of rods and two types of cone cells, namely long cones and short bifid cones (Fineran & Nicol 1976). The identification of these two types of cones has been resolved satisfactorily only from electron microscopy. A detailed description of the photoreceptors will be given in a later paper.

(i) *Dark adapted eyes*

Transverse sections through the photoreceptor region of d.a. eye show four distinct layers (figure 2, plate 1). The two outermost layers are made up of the pigment epithelium and the innermost ones of the photoreceptors and scleral processes of the pigment epithelium. The outermost layer of the pigment epithelium is heavily pigmented due to the presence of numerous melanosomes (figure 4, plate 1; figure 17). Abutting the melanosome layer there is a broad zone of tapetal material, the outermost region of which consists of a diffuse mass of

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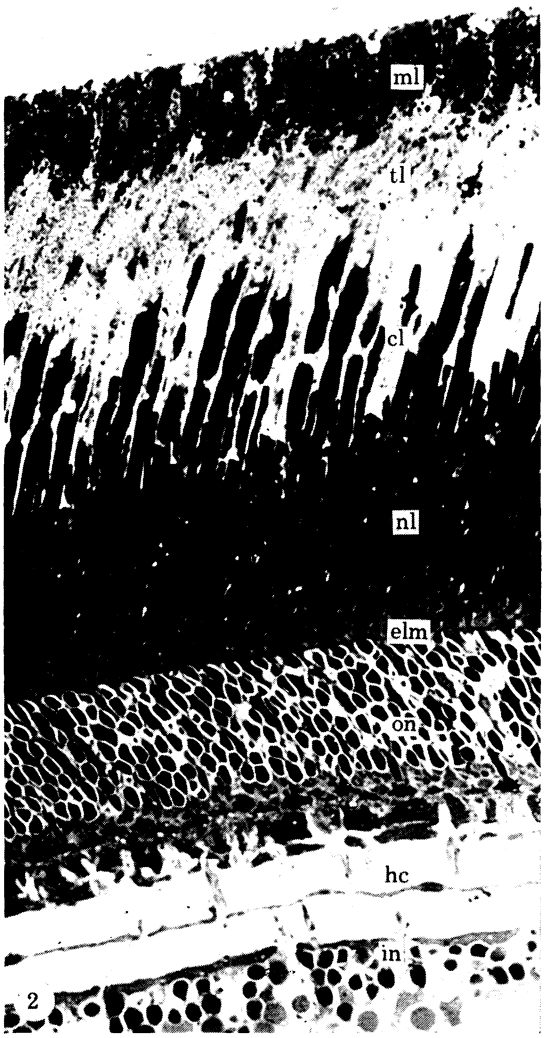
Description of plate 1

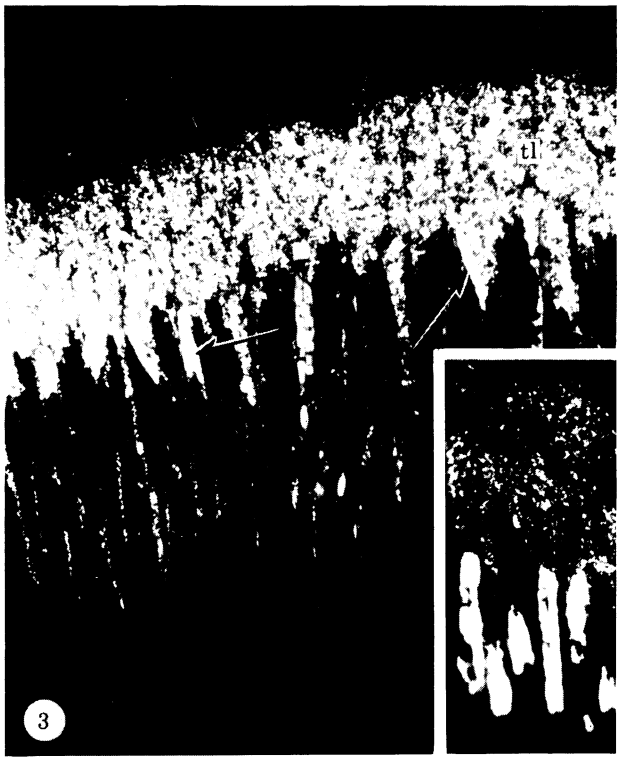
Figure 1. Head of *Anchoa mitchilli* showing a dark adapted (d.a.) eye with prominent eyeshine. (Magn. × 10.)

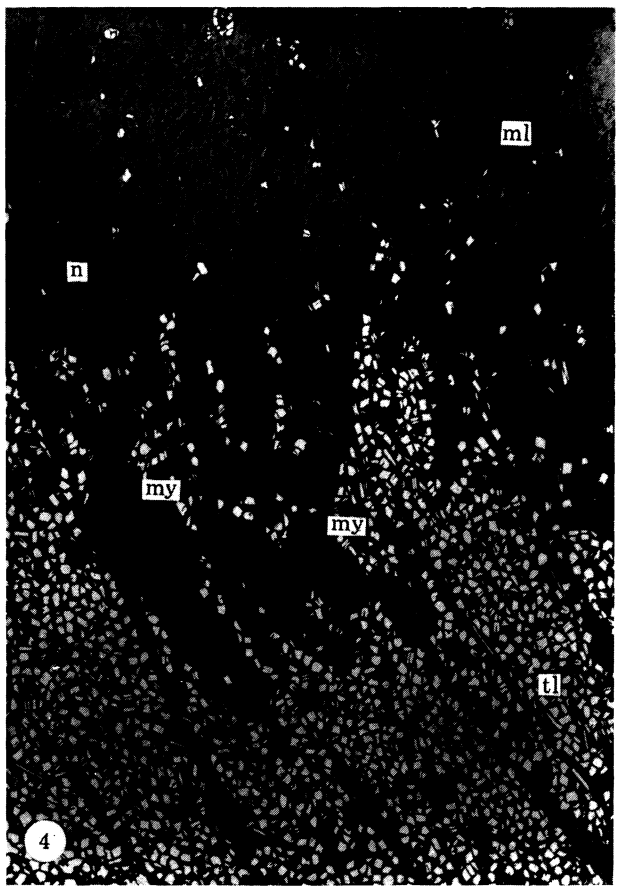
Figure 2. Transverse horizontal section of a d.a. eye showing the dark scleral band of melanosomes separated from the photoreceptors by a broad tapetal layer. Processes of the pigment epithelium interdigitate with the cones whereas the rods form a compact layer abutting the external limiting membrane. Epoxy resin embedded material cut at 2 μm and stained in methylene blue/azure blue. (Magn. × 540.)

Figure 3. Transverse vertical section of a d.a. eye viewed with polarized light microscopy. The tapetal crystallites form a broad tangential layer of diffuse birefringent material scleral to the stacks of platelets (arrows) facing the cone units. (Magn. × 540.) *Insert:* transverse horizontal section showing the birefringence of the platelet stacks and their rectangular form more clearly in this plane of section. Epoxy resin embedded material cut at 2 μm. (Magn. × 540.)

Figure 4. Thin section through portion of the melanosome and tapetal layers of a d.a. eye showing the radial orientation of cylindrical melanosomes interspersed with spherical ones, and the close packing of the tapetal crystallites. Note the radial elongation of the nucleus and the occurrence of large myeloid-like bodies between the melanosomes and tapetal layers. (Magn. × 3300.)







FIGURES 1–4. For description see opposite.

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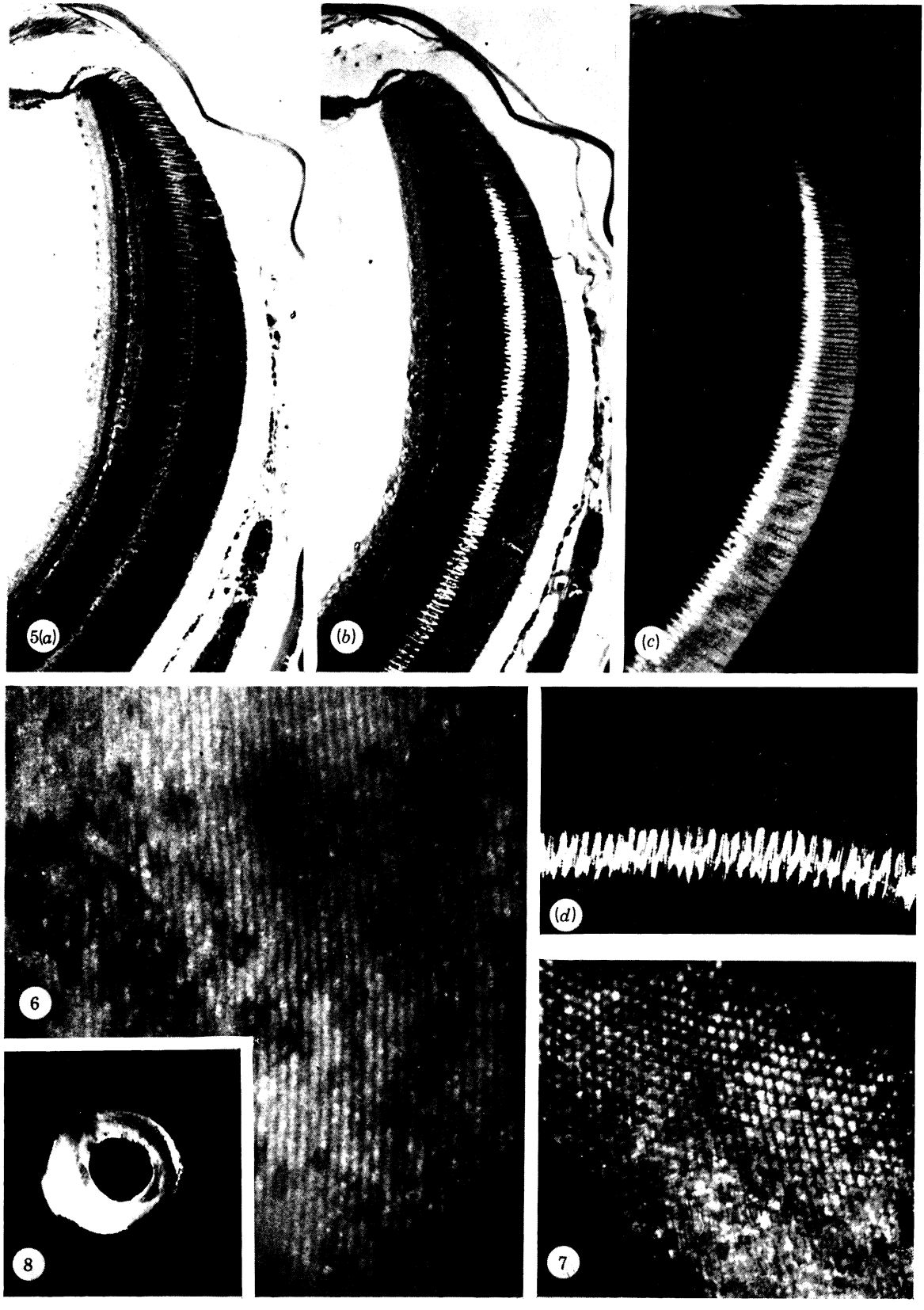

FIGURES 5–8. For description see opposite.

crystals. The inner region of the tapetum is dominated by stacks of platelets lying alongside the cone units (figure 3, plate 1). The tapetal material is strongly birefringent when viewed with polarized light microscopy (figure 3, plate 1; figure 13, plate 4). In both tangential and transverse vertical sections the greatest amount of birefringence of the platelet stacks is found towards the tips of the cone units (figure 13, plate 4). In tangential section each cone unit has associated with it two distinct birefringent stacks (figure 13, insert). Just scleral to the tips of the cones, the adjacent stacks meet and the non-birefringent areas between and within each stack appear as a dark cross within the combined birefringent area. Vitread to the tips of the cone units the platelet stacks progressively separate and also become smaller (figure 13). In d.a. eyes the two innermost layers of the photoreceptor region consist of a scleral layer dominated by cone unit outer segments and their ellipsoids, and an inner layer made up of rods (figure 2, plate 1).

(ii) *Light adapted eyes*

The photoreceptor region of l.a. eyes shows two distinct layers in transverse section (figure 9, plate 3). The outermost layer consists of the pigment epithelium and the outer segments of rods; the inner layer is composed of cones and rod inner segments penetrated by vitread processes of the pigment epithelium. The cones and pigment epithelial cells lie on the same axis, interdigitating to form vertical sheets of tissue which run dorso-ventrally across the eye, separated by similar vertical sheets of rods. This vertical arrangement of the pigment epithelium and photoreceptors is shown strikingly in tangential sections of the retina (figure 12, plate 4). The rows of pigment epithelium cells are aligned in the same direction as the striations of the stratum argenteum. Transverse sections viewed with polarized light microscopy show masses of diffuse birefringent crystals throughout the length of the rows with slabs of more strongly birefringent material, representing the stacks of platelets, facing the cones (figure 5, plate 2; figure 10, plate 3). On rotating the analyser from zero (extinction) to the position of maximum intensity, birefringence is shown first by the platelets, followed later by the crystallites (see figures 5*a–c*). Figure 5*b* (partially crossed nicols) shows strongly birefringent platelet stacks with only a small amount of birefringence in the crystallites. Figure 5*c* (fully crossed nicols) shows strong birefringence in both platelets and crystallites. In fully l.a. eyes the pigment

Description of plate 2

Figure 5. Transverse vertical section through the retina of a light adapted (l.a.) eye viewed by brightfield and polarized light microscopy. (*a*) Brightfield microscopy showing the various layers of the retina; (*b*) partly crossed nicols showing the appearance of strongly birefringent stacks of platelets abutting the cone units; (*c*) crossed nicols showing the appearance of other birefringent material (crystallites) scleral to the platelet stacks; (*d*) higher power detail of the platelet stacks overarching the cone units (partly crossed nicols). In (*a*)–(*c*), note the paucity of birefringent tapetal material towards the margin of the retina. Paraffin wax embedded material of *A. mitchilli* cut at 5 μm, bleached and stained with Ehrlich's haematoxylin and eosin. (Magn. *a–c* × 125, *d*, × 330.)

Figure 6. Surface of the retina of a l.a. eye of *A. mitchilli* viewed by reflected light showing vertically orientated green and white strips. The green (dark) strips correspond to the rows of rods revealed by transmission optical and electron microscopy; the white strips correspond to the rows of cones and their superimposed rows of pigment epithelium cell processes. (Magn. × 176.)

Figure 7. Surface of the retina of a d.a. eye viewed by reflected light. The green strips are less pronounced compared with the l.a. condition (figure 6) but the white areas are more distinct consisting of squarish bright masses. Each bright square corresponds to the pair of platelet stacks surmounting a cone unit (cf figure 24 plate 6). (Magn. × 176.)

Figure 8. A light adapted whole eye of *A. mitchilli* showing the black appearance of the retina. (Magn. × 10.)

epithelium shows a marked vitread zonation of melanosomes at the level of the cone units (figure 9, plate 3; figure 16; figure 37, plate 11).

(*c*) *Histology of the pigment epithelium*

The pigment epithelium forms a continuous tissue sclerally but vitreally the cells become variously separated according to whether the eye is light or dark adapted. In d.a. eyes only the most vitread regions inserted between the cone units remain apart, whereas in the l.a. state the cells are largely separated to their basal region through the intrusion of rods (figure 9, plate 3).

In tangential view the basal region of the pigment epithelium forms a discernible hexagonal array with the cells tending to lie in dorso-ventral rows corresponding to those of the cone complexes directly beneath them. In their vitread region the rows are more clearly defined, and at the level of the cone units the cells of one row regularly alternate with those of adjacent rows (figure 12, plate 4) in the same manner as the cone units. Throughout the greater part of the retina there is a 1:1 ratio between the numbers of pigment epithelium cells and cone units.

The basal region of the pigment epithelium is held together by a system of well developed desmosomes (figures 16 and 17). These form a narrow tangential band at the level of the nucleus, less well developed at the corners of the cell. The organization of the desmosome varies along its length but resembles that found in the eyes of other fish (Fineran & Nicol 1974). The vitread region of adjacent pigment epithelium cells in the same row, and the cone unit with which they interdigitate, are also held firmly together but no special junctional complexes have been recognized at this level.

(i) *Three dimensional form of the cell*

Each pigment epithelium cell is elongated radially and is divisible into three regions: basal, middle and vitread (figures 14–17; figure 19, plate 5). The shape and relative length of these regions varies depending on whether the eye is light or dark adapted. The basal region alters least of all in shape and usually exhibits a polygonal outline in cross-section with a flat to slightly rounded basal end in transverse section. The middle region is short in d.a. eyes (figures 15 and 17; figure 19, plate 5) and similar in cross-section to the basal portion, but in l.a. eyes it becomes greatly elongated and narrowed (figures 14 and 16), with an oval to round outline in cross-section. The vitread region of the cell in both l.a. and d.a. eyes spans the length of the cone unit, and where it fits between adjacent units of a row its form is wedge-shaped (figures 14–17), resembling that of the cone unit itself. However, the shape of the truncate portion varies slightly along its length being somewhat convex where it lies against the long cone outer segment

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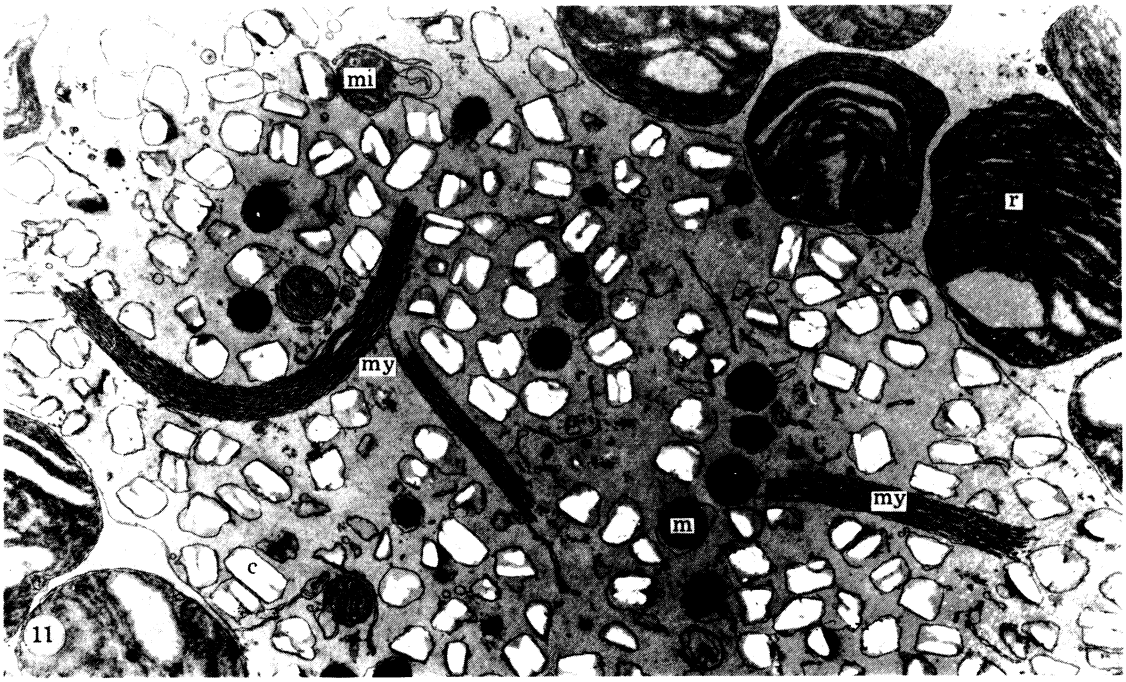
Description of plate 3

Figure 9. Transverse horizontal section through the photoreceptor layer of an l.a. eye showing radial rows of interdigitating pigment epithelium cells and cone units, separated by rows of rods. Phase contrast optics. (Magn. × 870.)

Figure 10. Transverse horizontal section through the pigment epithelium and photoreceptors of an l.a. eye viewed with polarized light microscopy. The radial rows of pigment epithelium cells are strongly birefringent due to the presence of numerous tapetal crystallites. Note the lower concentration of crystallites in the basal region and the strong birefringent areas, representing the stacks of platelets, abutting the cones. (Magn. × 870.)

Figure 11. Transverse section through a pigment epithelium cell just scleral to the stacks of platelets showing myeloid bodies, melanosomes, crystallites and a few mitochondria. (Magn. × 7000.)



FIGURES 9–11. For description see opposite.

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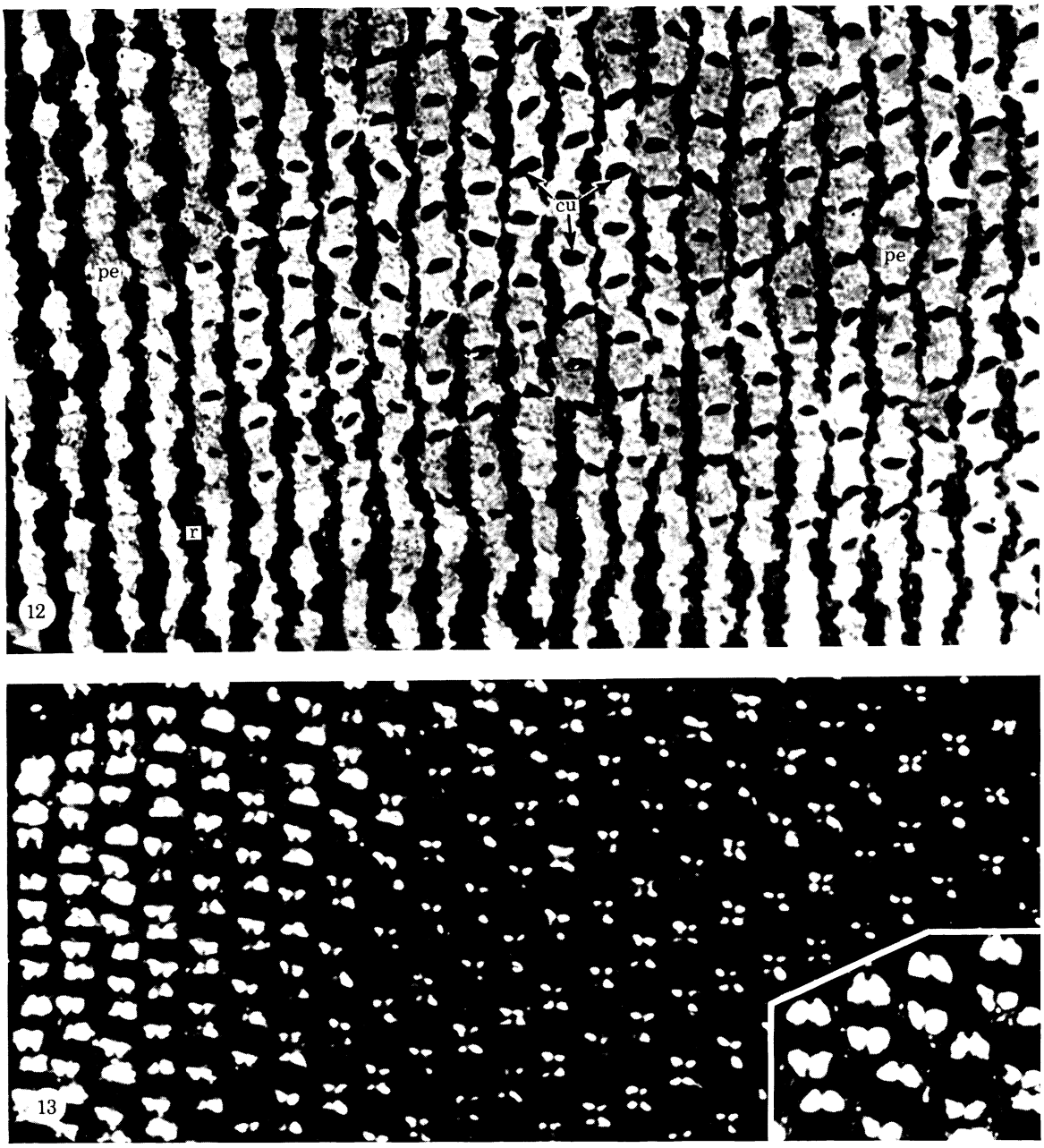

FIGURES 12 AND 13. For description see opposite.

and more attenuated where it is inserted between the lobes of the bifid cone (figure 24, plate 6). In l.a. eyes the vitread region is further developed into two pairs of lateral lobes (figure 14) which extend into the space between the cone rows. These lobes partly surround the adjacent cone units and at the level of the bifid cone outer segments they are typically separated by a large space from the cones (figure 36, plate 10). Vitread to the level of the cone units, each pair of lobes continues to converge and then forms a laterally flattened tapering process (figure 14) extending between the cone rows and reaching to about midway along the ellipsoid of the cones (see figure 6 in Fineran & Nicol 1976). On dark adaptation of the eye these vitread processes and the lateral lobes are withdrawn leaving only the truncate portion of the cell (figure 15). This truncate region maintains a constant association with adjacent cone units in both the light and dark adapted conditions and during the photo-mechanical movements of the retina.

(*d*) *Cytology of pigment epithelium cells*

Structurally differentiated ground substance, or cytoplasmic matrix, and ribosomes are poorly differentiated in pigment epithelium cells. Throughout the vitread, middle and much of the basal regions the ground substance appears electron transparent and is interspersed with only sparse finely granular material. Near the nucleus, the ground substance is a little better developed but associated ribosomes are still relatively uncommon, either free or attached to membranes.

The nucleus is ovoid and is situated in the basal region of the cell at the level of the intercellular junctions (figure 4, plate 1), and resembles that of other pigment epithelial cells (Moyer 1969). In l.a. eyes the nucleus shows a tangential orientation whereas in d.a. eyes its alignment is radial with respect to the retina (figure 4, plate 1; figures 16 and 17).

Stacks of closely packed smooth cisternae, resembling the myeloid bodies of other workers (Moyer 1969), are a very common feature of the pigment epithelium cells (figure 11, plate 3). Occasionally they occur near the nucleus but more often they are widely distributed throughout the cell, occurring within the vitread lobes (figure 36, plate 10), near the stacks of guanine platelets (figure 11, plate 3; figures 26 and 27, plates 7 and 8) and in the middle and basal

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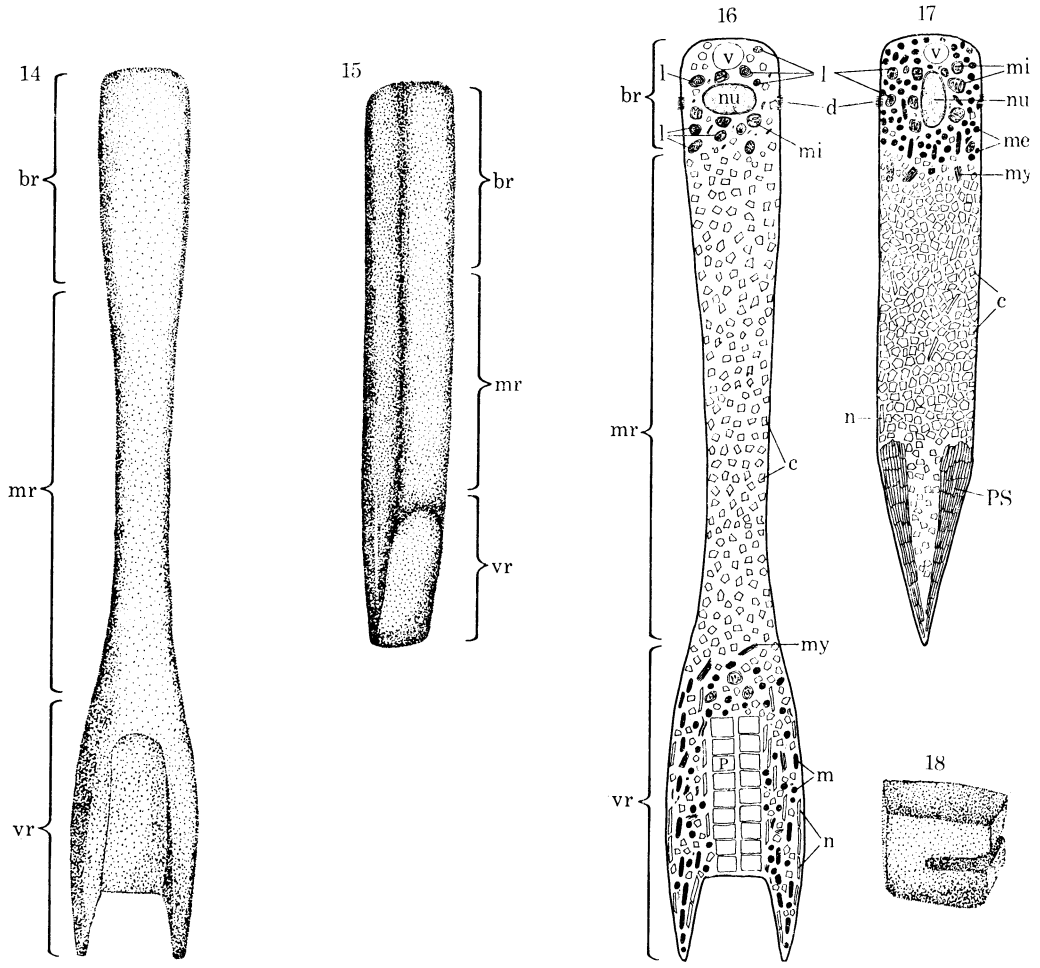
Description of plate 4

Figure 12. Tangential section through the photoreceptor layer of an l.a. eye showing the rows of rods and pigment epithelium cells that run vertically throughout the eye. The section passes at slightly different levels due to the curvature of the eye cup. On the left and bottom of the micrograph the pigment epithelium has been cut scleral to the cones but in the centre the tips of the cone units have been sectioned transversely and lie in the same rows as those of the pigment epithelium cells. (Magn. × 1270.)

Figure 13. Tangential section through the photoreceptor layer of a d.a. eye, cut at various levels through the cone units, viewed with polarized light microscopy. The section has the same orientation as figure 12, with the rows of pigment epithelium cells running vertically as in the eye cup. The rows are seen more clearly on the left where the stacks of platelets are best developed at the level of the tips of the cone units. Vitreally, the amount of birefringent material diminishes and the two stacks within the same cell come closer together (as shown by the progressively deeper levels from left to right in the micrograph). Between rows the pigment epithelium cells are arranged alternately and because of this and the greater separation of the stacks associated with each cone the overall arrangement of the stacks assumes more of an hexagonal pattern at deeper levels. (Magn. × 1130.) *Insert:* a group of stacks cut towards the tips of the cones showing the two distinct birefringent areas within each stack. The paucity of birefringent material, other than the stacks of platelets, is due partly to the scleral withdrawal of many of the needles and crystallites from around the cones on dark adaptation of the eye. (Magn. × 2000.)



FIGURES 14 AND 15. Three dimensional representations of pigment epithelium cells from l.a. and d.a. eyes, respectively. Note the elongated narrowed middle region and the vitread lobes in the cell of the l.a. eye, and the shorter simpler form of the cell in the d.a. eye.

FIGURES 16 and 17. Schematic representations of pigment epithelium cells from d.a. and l.a. eyes, respectively, showing the distribution of cell components. Figure 16, transverse horizontal view. Figure 17, transverse vertical view.

FIGURE 18. Three dimensional reconstruction of a typical tapetal crystallite.

DESCRIPTION OF PLATE 5

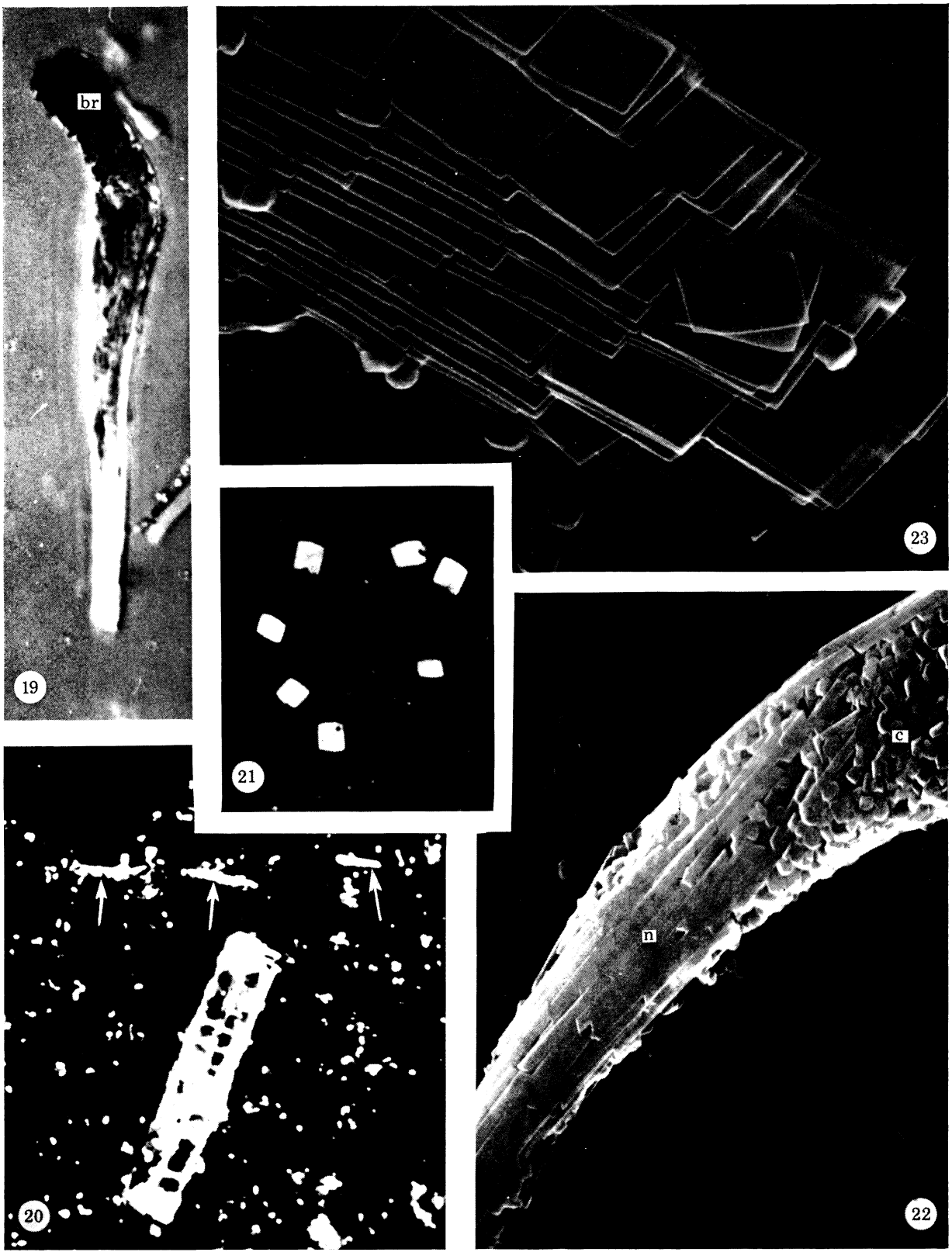
FIGURE 19. A whole pigment epithelium cell isolated from a d.a. eye. Note the concentration of black pigmented material in the basal region and the truncate vitread end of the cell with its stacks of birefringent platelets. Nomarski interference optics. (Magn. × 1400.)

FIGURE 20. Teased preparation of tapetal material under polarized light microscopy. Scattered crystallites and groups of needles (arrows) surround an intact stack of platelets. The two files of platelets making up each row within the stack are discernible. (Magn. × 2400.)

FIGURE 21. An isolated group of tapetal platelets in face view showing strong birefringence. Polarized light microscopy. (Magn. × 2500.)

FIGURE 22. Scanning electron micrograph of the vitread portion of an isolated pigment epithelium cell showing the peripheral palisade of needles. Where the needles have been removed the underlying crystallites and melanosomes are exposed. (Magn. × 6000.)

FIGURE 23. Portion of an isolated and partly disrupted stack of platelets seen by scanning electron microscopy. The thin film type of construction of the stack is clearly evident. A few crystallites are scattered nearby. (Magn. × 12000.)



FIGURES 19–23. For description see opposite.

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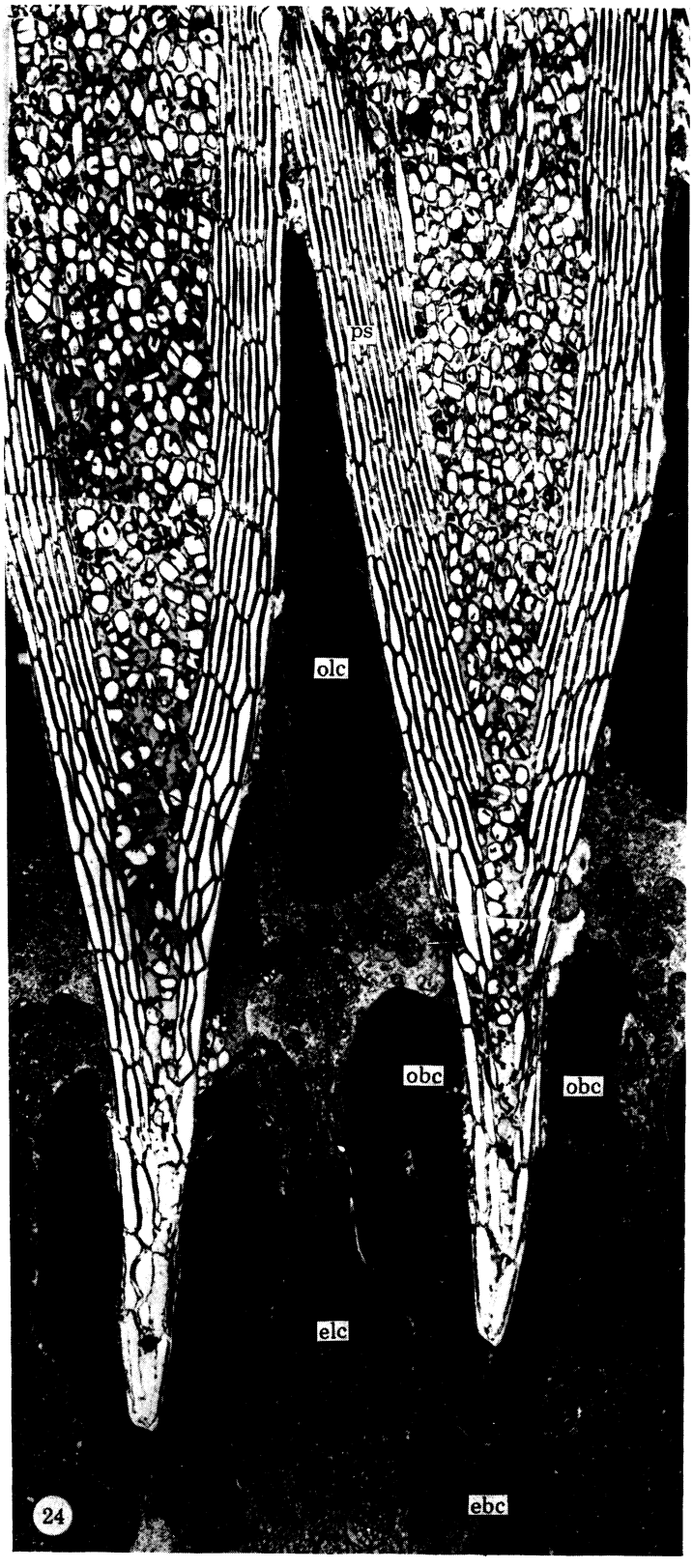

FIGURE 24. For description see opposite.

regions. Their frequency seems to be lowest in l.a. eyes in the long middle region of the cell. In d.a. eyes the vitread region is devoid of myeloid bodies and instead they accumulate in the now shortened middle region. Optical preparations of d.a. eyes often show an intense staining (with methylene blue/azure blue) of short thread-like structures (figure 17) scattered among the crystallites of the middle region, and on examination with the electron microscope these are found to be myeloid bodies. Particularly large rounded myeloid-like bodies are commonly found adjoining the melanosome layer of d.a. eyes (figure 4, plate 1). The number of cisternae making up each myeloid body is variable, ranging from 2 to over 20, with about 12 being a common condition in those of l.a. eyes. The stacks vary greatly in length and are either straight, crescentic or occasionally circular in section. Three dimensionally these curved stacks probably represents a tube or cup-shaped structure cut transversely. The membranes of myeloid bodies are mostly confined to the stack, but sometimes one or two may extend into the adjacent cytoplasm, occasionally forming associations with other organelles. Relationships have been noted with mitochondria but whether these are fortuitous or functional is not known.

Profiles of mainly short smooth endoplasmic reticulum (e.r.) cisternae, vesicles and/or tubules occur throughout the pigment epithelium cell (figure 11). At the level of the tapetal stacks in l.a. eyes the profiles are more numerous than elsewhere and often occur as diffuse lamellae distributed among the needles and crystallites (figure 26, plate 7). In some cells there is a gradation from the loose lamellar stacks to the compact myeloid bodies (figure 27, plate 8) suggestive of an inter-relationship between the two structures. Golgi bodies also occur throughout the pigment epithelium cell but they are often difficult to distinguish from myeloid bodies. Ishikawa & Yamada (1970) have also experienced difficulty in distinguishing Golgi bodies from e.r. unless they were treated by a special staining technique in the pigment epithelium of the rat.

Mitochondria are best developed within the basal region of the cell in the vicinity of the nucleus and lysosomes. Further from this area they are sparse and often less differentiated. In l.a. eyes a small group of mitochondria also occurs just scleral to the stacks of platelets in a position between the middle and vitread regions (figure 11, plate 3; figure 16). Mitochondria are largely absent within the vitread lobes and between the stacks of platelets of l.a. eyes (figures 24, 26 and 27, plates 6–8; figure 36, plate 11). On dark adaptation of the eye, mitochondria move from the vitread and middle zones into the basal region.

The internal appearance of mitochondria varies according to how well they have been fixed and on their state of activity. However, no correlation has so far been noted between their state of activity, judged by the appearance of the matrix and cristae, and the photo-adapted state of the eye. In general, mitochondria of the basal region exhibit an active state irrespective of whether the eye is light or dark adapted.

Description of plate 6

Figure 24. Low power electron micrograph montage from a transverse vertical section of the retina showing the vitread region of two pigment epithelium cells and their associated cone units. The tapetal stacks surmounting the cones are composed of regularly spaced platelets arranged in vertical rows. The greatest depth of platelets occurs towards the tips of the cone units; vitreally, the number of rows of platelets diminishes and is least where they abut the outer segment lobes of the bifid cones. *Note:* the slight variation in shape of the truncate vitread region throughout its length, the somewhat tiered arrangement of platelets in parts of the stacks, and the abundance of crystallites between stacks. In this particular eye the retina is only recently light adapted and the melanosomes have not yet migrated into the vitread region of the pigment epithelium cells. (Magn. × 4000.)

Large spherical vacuoles are frequently present in the basal region of the pigment epithelium adjacent to Bruch's membrane. They are often larger in l.a. than in d.a. eyes (figures 16 and 17). A few small vacuoles occur among the larger ones and appear to fuse with them. It is possible that the large vacuoles occasionally fuse with the cell membrane and release their contents to the extra-cellular space. The contents of most vacuoles are electron transparent after glutaraldehyde fixation, but scattered profiles of membranes may also be present, suggestive of lysosomal activity at some stage during the ontogeny of the organelle. A few of the large vacuoles occasionally show invaginations containing cytoplasmic material reminiscent of autophagic activity by their limiting membrane.

In addition to the large vacuolar bodies, structures more readily recognizable as lysosomes or autophagic vacuoles are common in the basal region of the pigment epithelium of both l.a. and d.a. eyes (figure 34, plate 9). Very occasionally small lysosomes are present in the vitread region of l.a. eyes but normally they are absent here and in the middle region of the cell. The contents of lysosomes are diverse, reflecting the nature of the cytoplasmic components engulfed and their state of breakdown. A lysosome commonly found in l.a. eyes contains spirals or compacted stacks of membranes suggestive of ingested rod outer segments (figure 34, plate 9). In fully d.a. eyes this type of lysosome is less frequent and is mostly represented by a form showing a more advanced breakdown of the membranes. The commonest type of lysosome in d.a. eyes contains both spherical and cylindrical melanosomes at various stages of disintegration. Another type of lysosome found more particularly in d.a. eyes towards Bruch's membrane contains guanine crystallites and needles. Occasionally melanosomes and guanine crystals occur within the same lysosomal compartment.

(i) *Tapetal material*

Pigment epithelium cells contain abundant tapetal material composed of guanine (Nicol *et al.* 1973; Zyznar & Nicol 1973). The deposits occur in three distinct forms: platelets (figures 21, 23, 24–29, plates 5–8), needles (figure 22, plate 5; figures 30 and 33, plate 9), and crystallites (figure 24, plate 6; figures 30 and 32, plate 9). All forms are strongly birefringent under polarized light (figures 3, 10 and 13, plates 1, 3 and 4; figures 19–21, plate 5). In teased preparations crystallites are the most abundant tapetal deposit (figure 20, plate 5; figures 30 and 32, plate 9), platelets are fewer and usually remain in groups (figures 20 and 23, plate 5), whereas needles are the least prominent form (figures 20, 30 and 33, plates 5 and 9).

Platelets are square to rectangular (1.5–3 μm × 1.5–3 μm) in shape (figure 26, plate 7). Their basic outline is clearly visible with polarized light microscopy (figure 21, plate 5); their thickness can be determined from electron microscope preparations of thin sections (see p. 335). Needles

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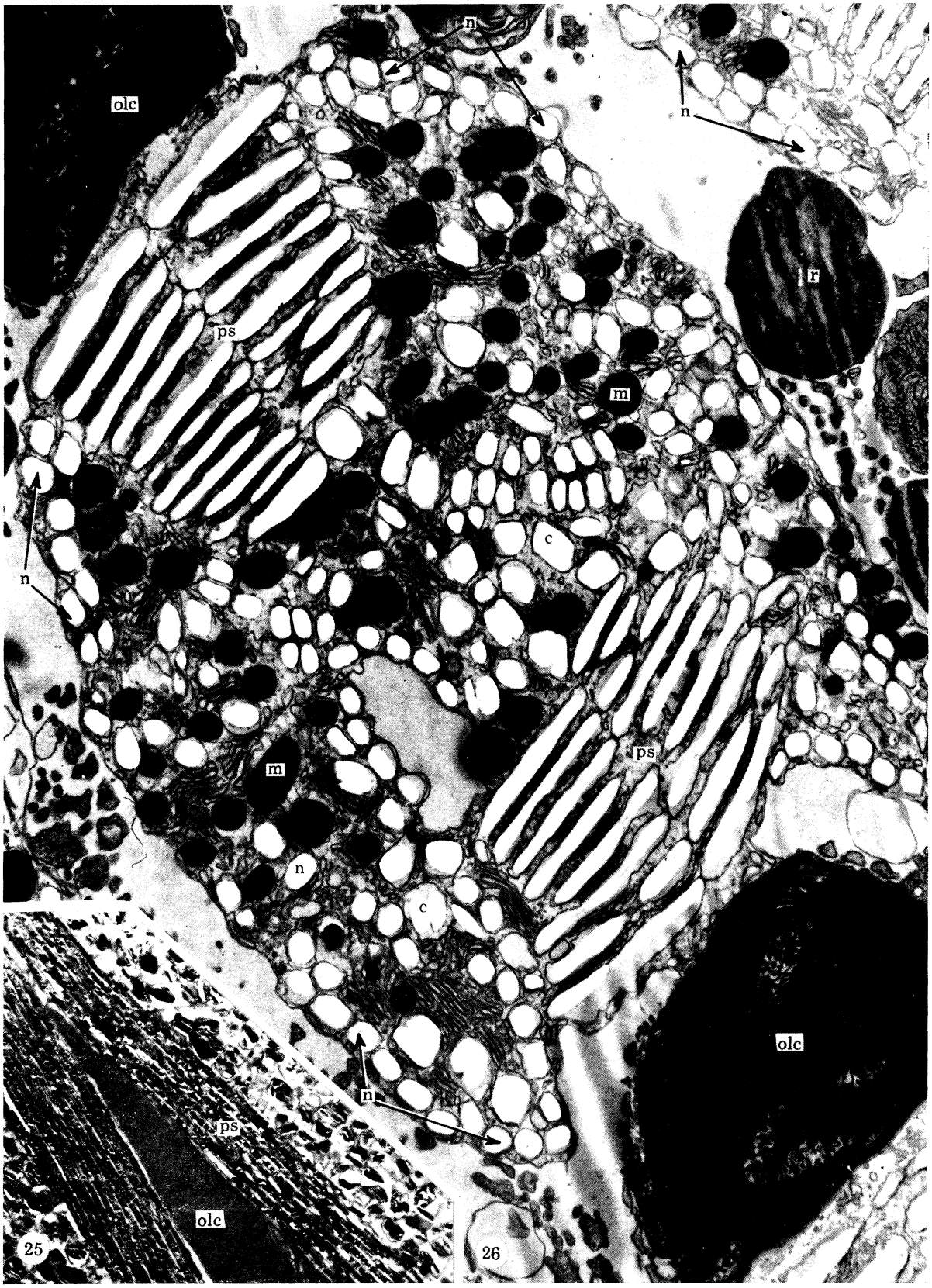
Description of plate 7

Figure 25. Transverse vertical section through the tip of a cone unit and its surmounting stacks of platelets in an unstained preparation. Compare the greater electron density, and somewhat shattered appearance of the platelets and neighbouring crystallites, with that shown in figure 24 of a post stained section from the same specimen. (Magn. × 8000.)

Figure 26. Transverse section of a pigment epithelium cell from a tangential section of the retina at a level through the long cone outer segments. A stack of platelets lies against each cone unit and is constructed of two files of platelets. Palisades of transversely cut needles are present at the periphery of the cell and scattered needles interspersed with crystallites and melanosomes also occur elsewhere. (Magn. × 14000.)



FIGURES 25 AND 26. For description see opposite.

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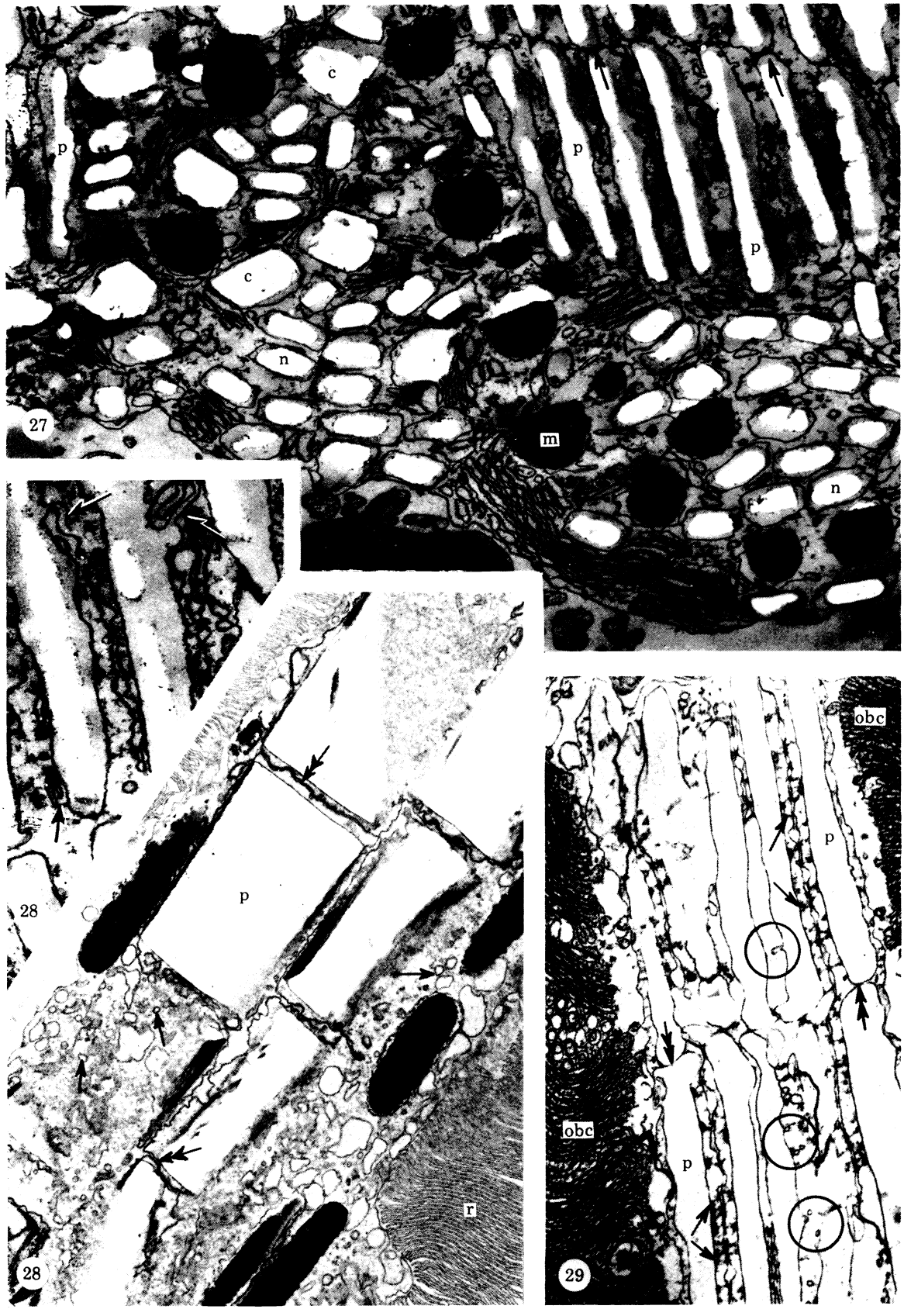

FIGURES 27–29. For description see opposite.

have parallel truncated ends and mostly measure 3–4 μm long, by 0.12 μm wide, with a thickness of 0.061 μm. Their morphology is best visualized by scanning and transmission electron microscopy (figures 30 and 33, plate 9).

Crystallites are the smallest tapetal components and they have the most variable morphology. Their apparent shape depends on the direction from which they are viewed as whole crystals (figures 30 and 32, plate 9), or on the plane in which they are cut as thin sections (figure 11, plate 3). Few details of their morphology are visible by optical microscopy alone (figure 20, plate 5) and even with the scanning electron microscope their form is not always clear (figure 30, plate 9). Transmission electron microscopy of isolated whole crystals provides sharp outline views (figure 32, plate 9) whereas thin sections show more of their internal structure (figure 11, plate 3). Most crystallites are approximately isodiametric and have a mean diameter of 0.5 μm. Crystallites usually have two broad parallel faces bounded by four to six sides seen in face view. In lateral view some may also be slightly chevron shaped (figure 32, plate 9). Thin sectioning shows that a lateral groove or notch extends part way around the crystal, in approximately an equatorial plane, penetrating to about one third of its thickness (figure 11, plate 3). The three-dimensional form of a typical crystallite is shown in figure 18.

The contents of platelets, needles and crystallites are electron dense in unstained sections examined by transmission electron microscopy (figure 25, plate 7). However, under the full intensity of the electron beam many of the guanine crystals are unstable and partial evaporation often changes them slightly in size and shape. When thin sections are stained by lead citrate followed by uranyl acetate the guanine is removed leaving electron transparent areas where the deposits lay (figure 11, plate 3; figures 24 and 26, plates 6 and 7). For convenience these areas will be referred to as either platelets, needles or crystallites respectively, from here on. Rohrlich (1974) has noted a similar dissolution of iridophore crystals of *Anolis* after alkaline lead staining; the problem is also discussed by Setoguti (1967). The removal of the quanine, especially from the stacks of platelets, greatly weakens the thin section which often splits under the beam in the absence of a support film.

Each crystalline deposit lies in a sac surrounded by a single unit membrane which is often somewhat undulatory or loose-fitting. The sac completely envelopes crystallites and needles (figures 11 and 27, plates 3 and 8) and there is little sign of any continuity of membranes from one structure to another. Platelets are also completely surrounded by a membrane but differ

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Description of plate 8

Figure 27. Transverse section through portion of a pigment epithelium cell at the scleral end of a stack of platelets. The tapetal material and melanosomes are contained within single membrane bounded sacs and a cisterna of e.r. runs between each row of platelets. Note the close contact between the sacs of the two files (arrows) and the smaller more rectangular form of the needles in contrast to that of the crystallites. (Magn. × 20000.)

Figure 28. Transverse horizontal section through portion of a stack showing the close association among the membrane sacs (double barbed arrows) of a file and the rectangular form of individual platelets, especially where they have been cut tangentially throughout the structure. Where the section has passed between the platelet and the e.r. cisterna the tubular membrane connections between the platelet sac and the e.r. are revealed (single barbed arrows). (Magn. × 16000.) *Insert:* Portion of a stack of platelets showing the membrane connections (arrows) between the platelet sac and that of the e.r. between the rows. (Magn. × 30000.)

Figure 29. Transverse section through a stack of platelets bordering a bifid cone outer segment showing e.r. running between the rows and the cross-bridge connections (single barbed arrows) with the platelet sacs. Also note the close contact between the sacs of the two files (double barbed arrows) and the tubular membrane connections between the sacs and the e.r. (circled areas). (Magn. × 25000.)

from needles and crystallites in that the sacs of adjacent platelets between rows are interconnected (described below). In l.a. eyes the limiting membrane mainly closely surrounds the platelet (figure 27, plate 8) but in d.a. eyes there is apparently a small amount of lateral extension of the sacs which causes the platelets of adjoining files to be pulled slightly apart.

The tapetal material appears to be a relatively stable component of the cell. Once the stacks of platelets are fully formed there is no apparent indication of any addition or replacement of individual platelets, and the same condition apparently obtains for the main population of needles and crystallites which, presumably, are formed at the margin of the retina during cytogenesis. However, there is some indication that lysosomal degradation of crystallites, and very occasionally also of needles, does occur. In the basal region crystallites are occasionally noted in sacs containing a dense matrix (figure 34, plate 9), but whether these are newly formed crystallites or those at the beginning of their lysosomal break-down has not been investigated.

(ii) *Arrangement and distribution of tapetal material*

The following observations summarize the general distribution of tapetal material within pigment epithelium cells based on an examination of tissues from all parts of the eye. Some variation in the amount of tapetal material has been noted within different regions of the same eye and among different fish but no attempt has been made to study this systematically. The chief variation in adult eyes is a decrease in the amount of tapetal material at the very margins of the retina (figure 5, plate 2). Other cells with poorly developed stacks of platelets have been noted, in optical preparations studied by polarized light microscopy, in a small area near the anterior dorsal region of the choroid fissure. The same preparations also indicate a paucity of crystallites in a region between the fovea and the choroid fissure.

(A) *Platelets.* Platelets are confined to the vitread region of the cell in both light and dark adapted eyes. The platelets form two clearly defined stacks (figure 26, plate 7) arranged in the form of a V (figure 24, plate 6) inserted into the truncate vitread end of the cell where it fits between two cone units of a row. Each arm of the V lies parallel to the tangential face of the adjacent cone unit so that the two arms join at the vitread end of the cell between two cone units. The two stacks of platelets seen flanking a single cone unit in transverse vertical (figure 24, plate 6) or tangential sections (see figure 4 in Fineran & Nicol 1976) are therefore derived from two adjacent pigment epithelium cells. This condition is in contrast to the situation found in

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Description of plate 9

Figure 30. Scanning electron micrograph of a group of isolated crystallites and needles. (Magn. × 12000.)

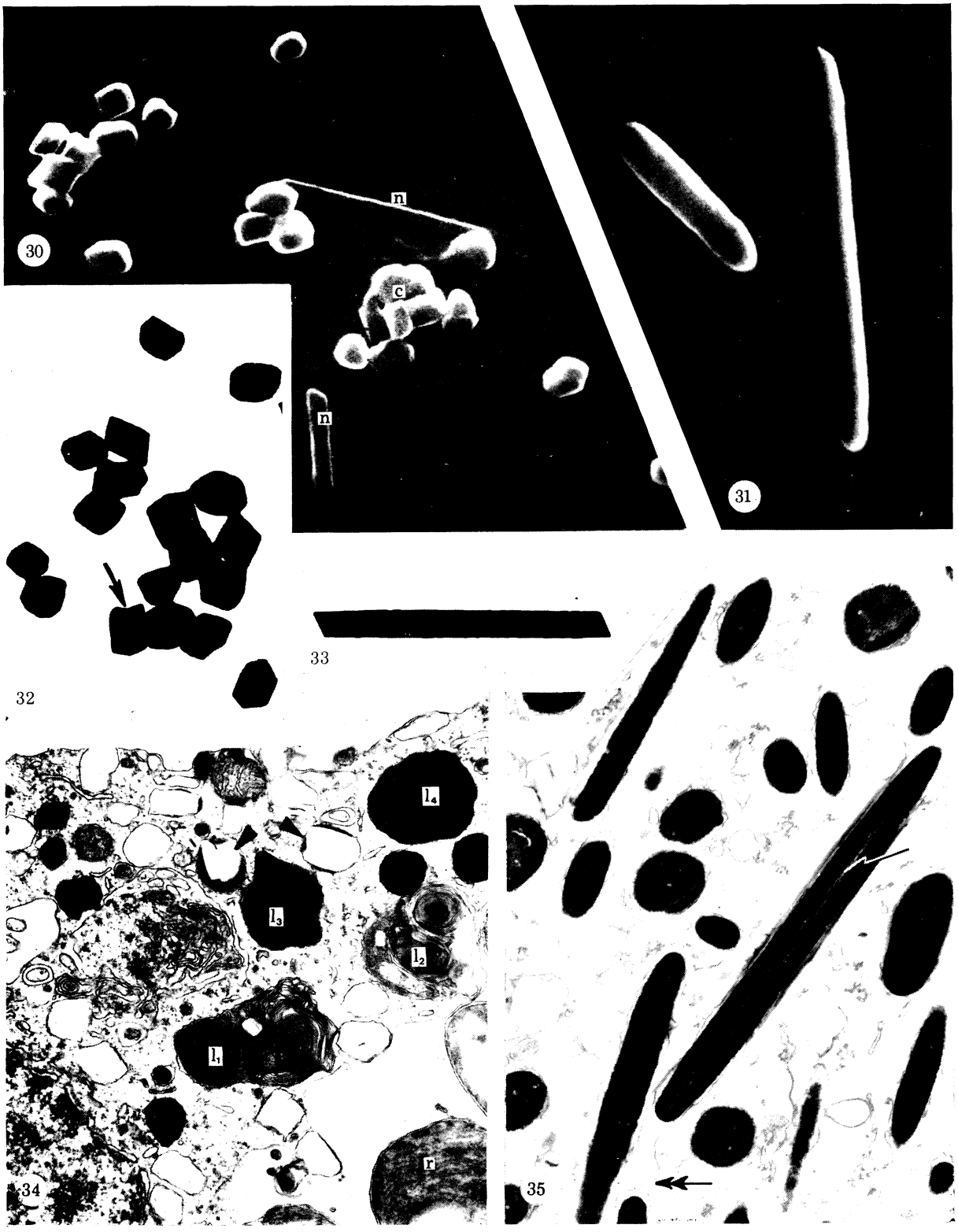
Figure 31. Two isolated cylindrical melanosomes shown by scanning electron microscopy. (Magn. × 24000.)

Figure 32. A group of isolated crystallites visualized by transmission electron microscopy. Note the somewhat chevron shape shown by some of the crystallites in lateral view (arrow). (Magn. × 14000.)

Figure 33. Transmission electron micrograph of an isolated needle. (Magn. × 14000.)

Figure 34. Portion of cytoplasm from the basal region of a pigment epithelium cell of an l.a. eye showing lysosomes. L_1 and L_2 contain spirals of membranes; L_3 and L_4 have a dense non-membraneous matrix with some darker globular areas suggestive of disintegrating melanosomes. Some crystallites in sacs containing a dense matrix occur near the lysosomes (arrows). (Magn. × 16000.)

Figure 35. Melanosomes in thin section from the basal region of a cell from a d.a. eye The cylindrical melanosomes exhibit a central core (single barbed arrow) and the larger spherical forms show small translucent areas scattered amongst the dense matrix. Note the contact between the membrane sacs of two of the cylindrical melanosomes (double barbed arrow). (Magn. × 41000.)



FIGURES 30–35. For description see opposite.

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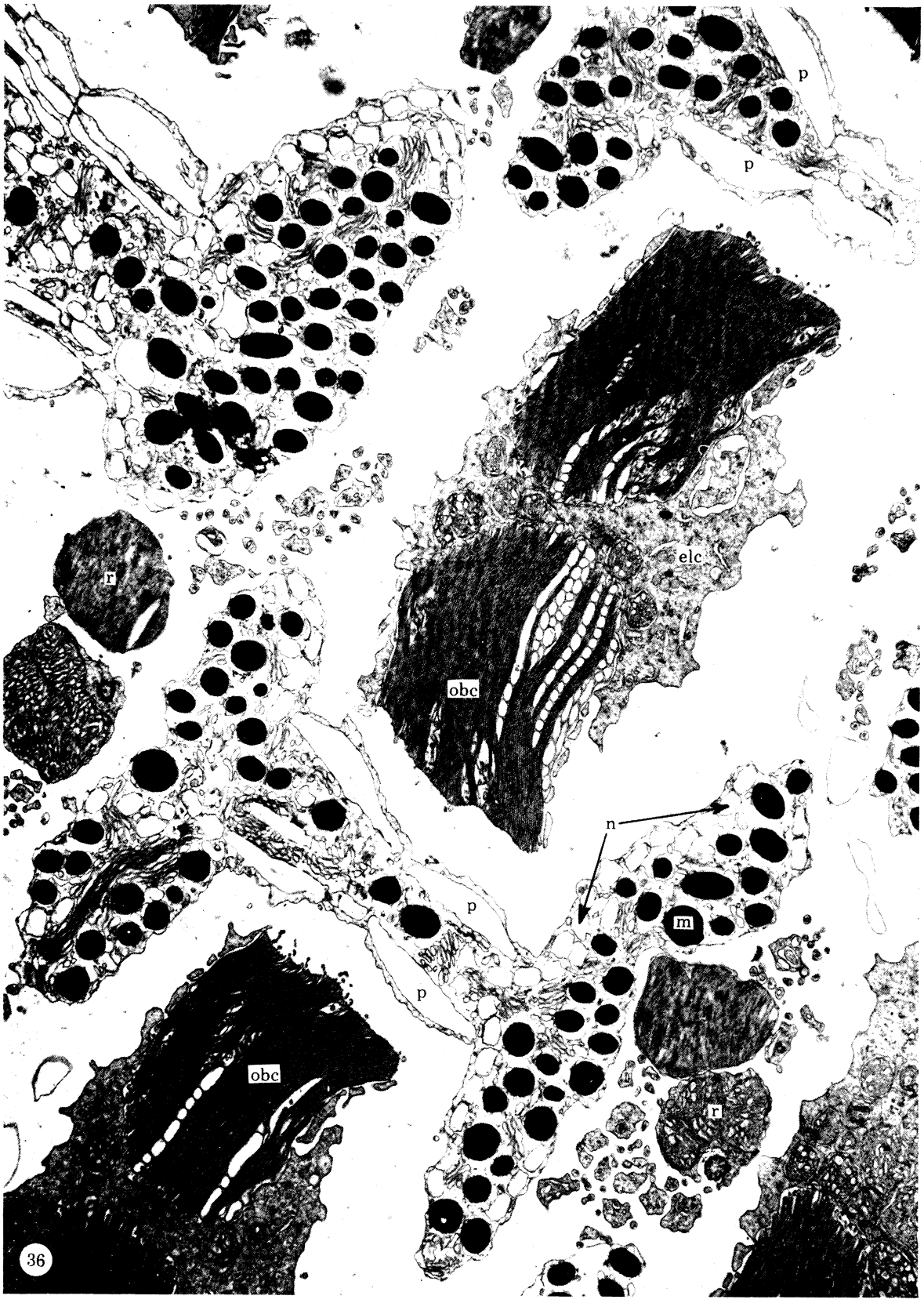

FIGURE 36. For description see opposite.

many fish where the cone outer segment is completely enclosed by a single pigment epithelium cell, as for example in the long cones of certain wrasses, Labridae (Fineran & Nicol 1974). Each stack of platelets is approximately rectangular when seen in face view in isolated (figure 20, plate 5) and sectioned material (figure 37, plate 11).

The stacks are composed of orderly rows of regularly spaced platelets and their enveloping sacs. However, the interpretation of the structure of the stacks is difficult unless the plane of sectioning is carefully controlled so as to pass either transversely to the stack (figure 26, plate 7) or in a vertical plane corresponding to that of the cone row (figure 24, plate 6). The organization of the stacks is most difficult to decipher in horizontal section due to the fact that the stacks are invariably cut in an oblique tangential direction through the rows of platelets. This results in various deception patterns in the arrangement of platelets (figure 37, plate 11).

The average thickness of platelets measured from transverse vertical sections through stacks (figure 25, plate 7) was about 82–83 nm and the distance between platelets of adjacent rows was about 112 nm. However, even in unstained sections there is often some alteration in the thickness of platelets and intervening cytoplasm caused through swelling and shattering during cutting. At the scleral end of the cone unit, each bordering stack is composed of usually between 10–15 rows of platelets (figure 24, plate 6). Vitread the number of rows diminished as the adjacent stacks converge until in the crotch between the adjacent cone units there are normally only two rows shared between the stacks. The parallel arrangement of platelets within the stacks of *Anchoa* is reminiscent of that shown by Rohrlich (1974) in the iridophore scale of *Holocentrus*.

The rows within a stack are composed of two files of platelets (figure 26, plate 7). The platelets of one file lie mainly opposite or slightly alternate to those of the adjacent file of the same row (figure 16; figure 28, plate 8). Between rows the platelets show a similar relationship. This is seen best in transverse sections (figure 24, plate 6), where the platelets of stack often have a tiered or slightly staggered formation. However, this arrangement is seldom precisely regular throughout a stack and is usually most conspicuous towards its scleral end.

During the radial shortening of the pigment epithelium, on dark adaptation of the eye, the stacks of platelets stay in the same position at the vitread end of the cell and the basic arrangement of the rows and files remains unchanged. However, the platelets of adjacent rows may separate slightly, although their membrane sacs remain in contact. This small amount of tangential displacement of the files explains the enhanced non-birefringent space seen in d.a. eyes from cross-sections of a stack in optical preparations (figure 13, plate 4).

The spatial relationship of platelets within a stack in both l.a. and d.a. eyes is maintained by an intricate system of membrane contacts, membrane continuities and membrane to membrane cross-bridges. Each row of platelets across and throughout the files is firmly held together by

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Description of plate 10

Figure 36. Tangential section through the retina of an l.a. eye at the level of the vitread lobes of the pigment epithelium cells and the outer segments of the bifid cones. Palisades of needles are well developed along the margins of the lobes, especially adjacent to the space separating the lobes from the cone unit. Melanosomes and profiles of membranous material are also abundant within the lobes. The cuneate tips of the cells, inserted between adjacent cone units of a row and connecting the lobes, contain only a few rows of platelets at this level together with some melanosomes, needles and short stacks of membranes. Note the vertical orientation of bifid cone lamellae and their alignment parallel to the cone row. (Magn. × 5000.)

a system of contacts developed between the three margins of each platelet sac and those of the abutting platelets (figures 27 and 28, plate 8). The structure of these membrane to membrane contacts has not been determined. Throughout a row of platelets there is an absence of membrane continuities, so that each platelet sac represents an individual compartment within the row. The various rows of a stack are separated by cytoplasm containing a single sheet of smooth e.r. placed equidistantly and mostly running throughout the extent of the stack (figures 26, 27 and 29, plates 7 and 8). In section, each sheet consists of a cisterna and tubules which probably represent fenestrated portions of the membrane system. At various points throughout the length of this cisterna membrane connections extend on either side to the platelet sacs of adjacent rows, but in any one cross section only a few connections may be evident (insert figure 28, plate 8). Oblique tangential sections through a stack indicate that these connections consist of short tubular struts (figures 28 and 29, plate 8). In addition to direct membrane continuities, cross-bridges occur between the sacs and the e.r. system running between the rows of platelets. The cross-bridges are equally developed on both sides of the cisterna and appear as dark finely granular material embedded in an otherwise structureless ground substance (figure 29, plate 8). They are easily broken and disrupted by poor fixation and probably represent protein material.

(B) *Needles.* In l.a. eyes needles occur mostly in the vitread region of the cell and show a radial orientation (figure 16). Tangential sections of the retina therefore show the needles cut transversely (figures 26 and 27, plates 7 and 8). In cross-section needles can be distinguished from crystallites by their smaller more rectangular form and by an absence of a notch. In transverse sections of the retina needles are cut in various longitudinal directions (figures 24 and 37, plates 6 and 11) and are easily mistaken for platelets cut longitudinally, except that platelets are confined to stacks where needles are absent.

Needles show a less well ordered arrangement than platelets and are more generally distributed throughout the vitread region. They commonly occupy a peripheral position forming a palisade-like layer, usually one row deep, of closely packed crystals (figures 22 and 26, plates 5 and 7). Within a layer the needles are placed with their narrower longitudinal edges abutting. In vertical distribution the arrangement of needles varies from opposite to alternate (figure 22, plate 5). The truncate end of one needle is usually matched by that of the abutting one so that the palisade is closely fitting throughout. In l.a. eyes palisades of needles frequently line the vitread lobes facing the lateral margins of the enclosed cone unit, particularly at the level of the bifid cone outer segments (figure 36, plate 10). Groups of needles may also occasionally flank the lateral margins of platelet stacks (figure 27, plate 8).

On dark adaptation of the eye a large proportion of the needles are withdrawn from the vitread region, on retraction of the lobes, and come to lie scleral to the platelet stacks. Here in

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Description of plate 11

Figure 37. Transverse horizontal section through the vitread region of a group of pigment epithelium cells and associated cone units of adjacent rows in an l.a. eye. The pigment epithelium cells and cones lie in the same vertical rows that run throughout the retina separated by rows of rods. Melanosomes, needles and stacks of platelets are intimately associated with the cone units. Each stack of platelets shows its construction of two files, but because the plane of sectioning passes obliquely through the successive rows of platelets various superficial patterns of their arrangement are produced. (Magn. × 4000.)

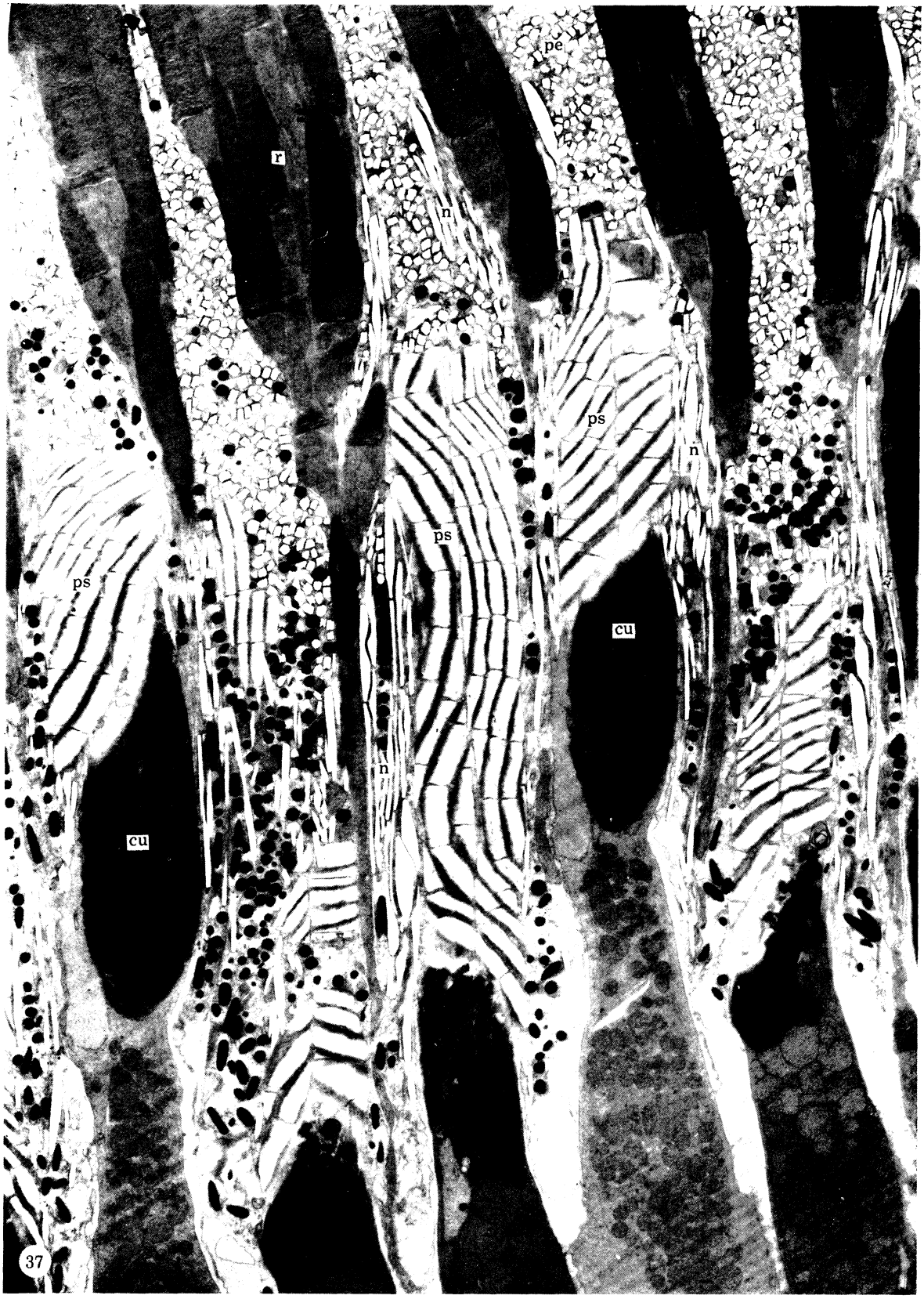

FIGURE 37. For description see opposite.

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the shortened middle region of the cell the needles are distributed, apparently randomly and mostly singly, among the numerous crystallites (figure 4, plate 1; figure 17).

(C) *Crystallites.* Crystallites of l.a. eyes are distributed throughout the length of the pigment epithelium cell. They sometimes fill the remaining spaces among the needles and melanosomes in the vitread lobes and between the two stacks of platelets (figures 24 and 26, plates 6 and 7); they are most abundant in the middle region of the cell being the only tapetal material present (figures 11 and 37, plates 3 and 11). Crystallites are also common in the basal region but they become less frequent in the vicinity of Bruch's membrane (figure 10, plate 3). The orientation of crystallites appears to be random throughout the cell and the masses show similar amounts of birefringence irrespective of the plane of section in which they are viewed.

In d.a. eyes a few crystallites occur between and alongside the stacks of platelets but during dark adaptation most are withdrawn from the vitread end (figure 13, plate 4) and come to lie in the middle region of the cell (figure 3, plate 1; figure 17). A large portion of crystallites is also withdrawn at the same time from the basal region into the middle region. As a result of these migrations and the general shortening of the middle region the density of crystallites is greatly increased (figure 4, plate 1; compare figures 16 and 17). The development of this dense band of crystallites in the middle region is largely responsible for the eyeshine of d.a. eyes. The crystallites in this broad tapetal reflecting layer show no apparent preferred orientation and the masses exhibit a similar degree of birefringence whether viewed in transverse or tangential sections.

(iii) *Comparison of crystals in tapetum with those of iris and stratum argenteum*

Needles and platelets occur in the iris and stratum argenteum but crystallites are absent. Needles of the stratum argenteum are longer and more variable in length than those of the tapetum while the needles of the iris are even longer than those of the argenteum. The platelets of the iris and argenteum are similar in form, consisting of flat elongated hexagonal structures of variable size, and therefore differ from those of the pigment epithelium.

(iv) *Melanosomes*

Melanosomes are prominent structures of the pigment epithelium, and they participate in retinomotor movements. They occur throughout the retina and are most abundant, proportionately, in cells at the very margins. In teased preparations for optical microscopy melanosomes are distinguishable from guanine crystals by their dark colour under transmitted white light and by an absence of birefringence under polarized light. Under both the scanning and transmission electron microscopes, melanosomes are more rounded than guanine crystallites and vary in shape from spheres to short and long cylinders with rounded ends (figures 11 and 31, plates 3 and 9). The spherical forms measure 0.4 μm in diameter and appear to constitute a separate class of melanosome. Cylindrical melanosomes are from 1 to 3.5 μm long (average 2–3 μm) and have a diameter of 0.3 μm. Thin sectioning also indicates differences between the spherical and cylindrical melanosomes (figure 35, plate 9). Spherical melanosomes have contents slightly less electron dense than the cylinders and frequently contain a few small pockets of translucent material. Cylindrical melanosomes often have a dense outer zone enclosing a less dense central core. In contrast to guanine crystals, both types of melanosomes are unaffected by block staining of the tissue with heavy metals during dehydration and post staining of the thin sections.

Melanosomes are surrounded by a single limiting membrane (figures 11 and 35, plates 3 and 9). This membrane is a somewhat loose envelope resembling that of most other fish (Arnott *et al.* 1972) but differing from the membrane sac of melanosomes and red pigment cylinders in the eyes of New Zealand parrot fish (Fineran & Nicol 1974). There is no sign of any continuity between the membrane of one melanosome and that of another but the membrane sacs of cylindrical melanosomes are sometimes closely apposed, in this respect resembling the plate sacs (figure 35, plate 9).

In the l.a. state melanosomes are concentrated towards the vitread end of the cell (figures 9 and 37, plates 3 and 11; figure 16) and are responsible for the black appearance of the retina (figure 8, plate 2). They are variously distributed between the stacks of platelets and behind the peripheral rows of needles (figures 26 and 27, plates 7 and 8). Melanosomes are most abundant in the cell processes extending between the cone rows. The cylindrical forms are radially aligned in the processes and in the region of the stacks they lie alongside the rows of platelets (figure 28, plate 8). The spherical melanosomes are distributed, apparently randomly, among the cylindrical ones. In l.a. eyes few melanosomes occur in the basal and middle regions of the cell, and most terminate a short distance scleral to the stacks of platelets. During dark adaptation of the eye both the spherical and cylindrical melanosomes are withdrawn from the vitread end of the cell and become concentrated in the basal region (figure 4, plate 1) where they are responsible for the dark band seen in histological sections of d.a. eyes (figure 2, plate 1). Within the band the melanosomes are randomly distributed except that the cylindrical forms show a preferred radial orientation.

The migration of melanosomes during photomechanical movements of the pigment epithelium is generally slower than the changes accompanying the shortening or lengthening of the cell. Thus, in recently d.a. eyes a few melanosomes may still occur at the vitread end of the cell and in the middle region amongst the tapetal material. When d.a. eyes become light adapted the migration of melanosomes from the basal region is comparatively slow and occurs after the elongation of the middle region. This accounts for the condition shown in figure 24, plate 6, where the melanosomes have not yet migrated to the vitread region at the level of the cone units (cf. figures 36 and 37, plates 10 and 11).

Melanosomes appear to be relatively stable components of the cell and most of them may arise at the margin of the retina during the ontogeny of the cell. However, as lysosomal activity indicates some destruction of melanosomes there is undoubtedly also a system for their renewal. Sections through the basal region of the cell near the nucleus sometimes show small vesicles with electron dense contents, but we have not studied these sufficiently to determine whether they represent premelanosomes or young lysosomes. According to Turner, Taylor & Tchen (1975), melanosome formation in goldfish melanophores is from multivesicular bodies derived from fused e.r. and Golgi vesicles.

(*e*) *Reflexion of the retina*

Reflexion from a small area of l.a. retina gives a broad spectrum curve, with maxima in the yellow green or yellow region (λ_{max} 560–580 nm), varying with the specimen (figure 38). Reflexion as measured is low, it does not represent the reflectivity of the tapetal surface because of multiple reflexion, scattering and absorption which takes place within the tissue. The white retina of a d.a. eye reflects well above 500 nm; there is considerable absorption of short wavelength (figure 39).

4. Discussion

(*a*) *Inter-relationship between pigment epithelium cells and cones*

Pigment epithelium cells of *Anchoa mitchilli* and *A. hepsetus* have essentially the same organization, and undoubtedly the same function, and may therefore be discussed together. In most vertebrate eyes the processes of the pigment epithelium penetrate vitread amongst the photoreceptors but the pattern which they form has seldom been described. In some eyes, especially those where the photoreceptors show no regular pattern, there may in fact be no special pattern of the processes, but in other eyes patterns which exist may well have been missed because only transverse sections were examined. In our earlier work on the eyes of *Pseudolabrus* (Fineran & Nicol 1974) we demonstrated the importance of studying serial tangential sections to determine the pattern of cones and pigment epithelium cells in the retina. In the present study serial tangential sections have also been used extensively for both optical and electron microscopy and we have shown a similar relationship between pigment epithelium cells and cones. Whereas in *Pseudolabrus* there is a 1:1 ratio between pigment epithelium cells and long single cones, in *Anchoa* this ratio exists between the pigment epithelium cells and the cone units. This suggests that relationships such as these might be common in eyes where the cones form mosaics. However, it is interesting to note that in both these examples the 1:1 ratio is not of the total number of pigment epithelium cells to the total number of cones but of the total number of pigment epithelium cells to certain groups of cones. Thus in *Pseudolabrus* there are four pigment epithelium cells associated with thirteen cone cells in an area of retina bounded by four long single cones. In contrast, in *Anchoa* there is one pigment epithelium cell for every two cone cells, and the relationship is further complicated as each cone unit is formed from one single cone and one lobe from each of the two adjacent bifid cones. The relationship between pigment epithelium cells and cones therefore appears to be dictated by differences in the actual pattern and grouping of the cones rather than by the total number of cones present.

In *Anchoa* the relationship of pigment epithelium cells to the cone unit is unusual in that the vitread end of the cell does not completely enclose the cones in the way that it does in *Pseudolabrus*, and apparently also in many other fish. Recently, Steinberg & Wood (1974) have described how cone outer segments of the cat are ensheathed by processes of the pigment epithelium cell. In *Anchoa* the cone unit adjoins two pigment epithelium cells which together form a structural and functional entity overarching it. Furthermore, the pigment epithelium cells overlying a row of cones are all linked in the same direction throughout the row and move together during photomechanical movements of the retina but between rows they are separated, especially in the l.a. state, through the intrusion of rods.

The relationship between the vitread truncate end of a pigment epithelium cell and its two adjacent cone units is a constant one. This wedge-shaped portion of the cell has the same form in both l.a. and d.a. eyes and remains unaltered during photomechanical movements. Such an intimate relationship is no doubt linked to the functioning of the cone unit in the perception of light. We suggest that this regular association ensures the least amount of disturbance to the stacks of guanine platelets and their orientation relative to the photosensitive membranes of the cones. The guanine platelets bordering the cone units within adjacent pigment epithelium cells are believed to reflect light back into the cone system and this is discussed in greater detail later. For a fish living in an environment subject to frequent changes of light

intensities, it would be advantageous, as in *Anchoa*, to have the cone associated tapetal material move with the cones during photomechanical movements within the eye. The association maintains the alignment of the reflectors relative to the cones and synchronizes their movement.

Although the cuneate vitread portion of the pigment epithelium cell remains relatively static in shape the margins bordering the space between the rows of cones change form during photo-adaption of the eye. The development of lateral lobes at the level of the cone units in l.a. eyes and their vitread extension, reaching to the lower ellipsoid region of the cones, are interpreted as modifications that provide lateral shielding of the cones. The lobes of adjacent pigment epithelium cells in the same row usually meet to shield completely the outer segment of the long cone whereas at the level of the bifid cones the lobes part somewhat but, nevertheless, still provide a complete lateral shielding of the bifid cone outer segments. At this level only the ellipsoid of the long cone is directly exposed to the space between the cone rows, but some shielding is still furnished by the pigment epithelium cell of the adjacent row because of the alternate arrangement of cells between rows. The extensions of the lobes vitread to the cone units offer lateral shielding for the bifid cone outer segment at its proximal end where it links adjacent cone units. As far as we are aware, no other instance has been described in which the vitread region of a pigment epithelial cell develops additional processes for shielding cones in the l.a. state. In *Stizostedion*, which has a retinal tapetum, processes of several epithelial cells surround the cone outer segments and are situated so as to reflect light into them (Zyznar & Ali 1975).

On dark adaptation of the eye the lateral lobes and their vitread extensions are withdrawn leaving only the simple cuneate process of the pigment epithelial cell inserted between the cone units. Retraction of the lobes accompanies the scleral movement of the cones when, presumably, the functional rôle of the latter as photoreceptors is interrupted. The withdrawal of the lobes probably allows a closer and more precise packing of cones and pigment epithelial cells, and a redistribution of their contents in relation to the functioning of the pigment epithelium as a tapetum lucidum during dim light vision.

(*b*) *Cytoplasmic organization of pigment epithelium cells*

Pigment epithelium cells of *Anchoa* show a marked gradation of cytoplasmic organization throughout the length of the cell. The location of the nucleus in the basal region and the concentration here of mitochondria, lysosomes, vacuoles and the relatively few ribosomes indicates that this is metabolically the most active zone of the cell. Judged by the lack of differentiation of the ground substance, the absence of ribosomes and paucity of mitochondria, the vitread region in contrast must be relatively inactive in terms of cell metabolism. The few mitochondria which do occur just scleral to the tips of the cone units in l.a. eyes may represent a small isolated centre for respiration associated with the formation and retraction of the lobes during photo-adaptation of the eye.

The general appearance of the vitread and middle regions of the pigment epithelium cell with their numerous melanosomes, abundant tapetal material and masses of smooth membranes suggest that these regions are more concerned with structural functions of the cell rather than as sites for active metabolism. The vitread and middle regions are important areas for the concentration of tapetal material and melanosomes in relation to the rôle which these components play in the perception of light by the photoreceptors.

The occurrence of lysosomes in vertebrate pigment epithelium cells is becoming increasingly well documented (Dowling & Gibbons 1962; Moyer 1969; Ishikawa & Yamada 1970). As in other cells and tissues (see Dingle & Fell 1969), the function of lysosomes in the pigment epithelium is to provide a site for the breakdown of redundant or malfunctional cell components so that their constituent molecules may be recycled. Lysosomal activity in pigment epithelial cells is diverse in the sense that the lysosomes must cope with the catabolism of their own cell components as well as outer segment portions of photoreceptors. The ability of pigment epithelial cells of the frog to phagocytize extra-cellular material, other than rod outer segments, has been demonstrated recently by Hollyfield & Ward (1974*a*) for the uptake of polystyrene spheres. However, these workers failed to get ingestion of pasteurized bacteria (Hollyfield & Ward 1974*b*).

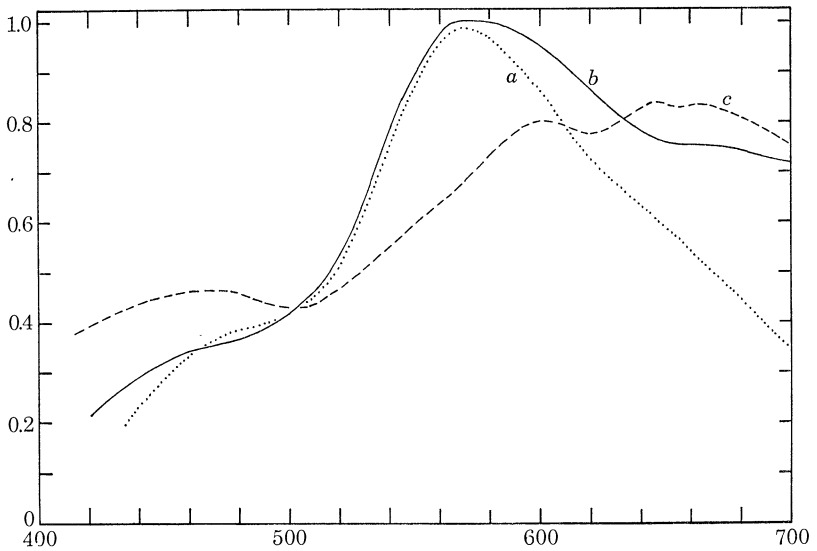

FIGURE 38. Spectrum reflexion of light-adapted retinae of *Anchoa*. Curves: *a*, yellow-green region of the retina of *A. hepsetus*; *b*, *c*, yellow-green and yellow-orange regions, respectively, of retinae of *A. mitchilli*. The ordinates are arbitrary values.

In *Anchoa* the lysosomes contain a diversity of cell debris and various stages of degradation are recognizable. Our observations further suggest that lysosomal activity may vary between the light and dark adapted states of the retina and the length of time the eye has been adapted to its particular condition. In fully l.a. eyes lysosomes mainly contain small compact stacks or tightly wound spirals of membranes derived from rod outer segments. With increasing time in the l.a. state the contents of many lysosomes are progressively digested and ultimately appear homogeneous without any trace of the original membranes. However, we have been unable to determine whether portions of rod outer segments are engulfed by pigment epithelial cells immediately after photomechanical movements have ceased or continuously throughout the period of light adaptation of the eye. In the d.a. state there is probably little opportunity for detached portions of rods to be engulfed by the pigment epithelium because of the spatial separation of the cells.

During the d.a. period of the eye the lysosomal activity of the basal region of the pigment epithelial cell appears to be involved mainly in the degradation of its own components. This is shown by an increasing number of lysosomes containing melanosomes and a few with guanine

crystals. The breakdown of these components (and other cellular debris) at this stage is understandable as it coincides with their increased concentration in the basal region. Shortly after an eye has become dark adapted lysosomes containing fragments of rod outer segments may still be evident but these progressively disappear during the d.a. state.

Myeloid bodies are compact stacks of smooth membranes derived from endoplasmic reticulum. The occurrence of myeloid bodies in a variety of vertebrates has been the subject of a recent discussion by Nguyen-Legros (1975*a*, *b*). They were once confused with phagosomes or lysosomes (see Cohen 1969) but, in fact, they can readily be distinguished because they have no surrounding membrane. In addition, the work of Young & Bok (1969) has shown that while. labelled protein is transported from rods to phagosomes there is no transfer to myeloid bodies. Whereas phagosomes have been found in most pigment epithelia, myeloid bodies have so far been reported only in certain amphibia (Porter & Yamada 1960), birds and reptiles (Okuda 1962) and more recently in some fishes. In the eye of the sea trout Arnott *et al.* (1972) found structures resembling myeloid bodies but they had difficulty in clearly distinguishing them from phagosomes. They therefore used the term myeloid body and phagosome interchangeably, fully realizing that two classes of structures might be involved. 'Myeloids' are also mentioned by Nicol & Arnott (1973) in the eyes of gars. Cohen (1963) early pointed out an absence of myeloid bodies in eyes of catfish and this has since been confirmed by Arnott *et al.* (1974). In the eyes of *Pseudolabrus* we occasionally found myeloid bodies, typically in association with the nucleus. With *Anchoa* on the other hand, myeloid bodies are numerous and often exceptionally well developed – more so than reported so far from any other fish eye.

There have been few suggestions concerning the functions of myeloid bodies in those vertebrate eyes where they occur (Porter & Yamada 1960). In *Anchoa* the presence of intergrading forms of myeloid bodies and the less ordered e.r., especially in the vitread region of the cell of l.a. eyes, suggests a dynamic relationship between the two membrane systems. The non-stacked system of e.r. membranes is intimately associated with the encapsulation of the tapetal material, and probably also of melanosomes. In the case of the guanine reflectors abutting the cone unit, the e.r. is apparently further responsible for the spatial distribution of platelets within a stack and for assisting in maintaining their position during photomechanical movements of the eye. In such cells where there is abundant membrane material possibly with a low rate of renewal (judged by the poverty of ribosomes for protein synthesis) the myeloid bodies may function as sites for storage of membrane components. It is of interest to note that Larson (1965) has found stacks of endoplasmic reticulum in mature pollen and suggests that it may function in a storage capacity. The presence of preformed stacks of membranes in pigment epithelium cells would ensure an immediate supply of membrane material in a cell with regular and often rapid redistribution of its cell components. The fact that the largest myeloid bodies occur in d.a. eyes of *Anchoa* is suggestive of an accumulation of membranes on the withdrawal and redistribution of needles and crystallites from the vitread region. The surface area of the pigment epithelial cell may possibly be reduced on dark adaptation of the eye as a result of the withdrawal of the vitread lobes and appendages and the considerable radial shortening of the cell. Myeloid bodies may conceivably also be involved here in storing membrane components withdrawn from the cell membrane as its area is reduced.

(*c*) *Tapetal material*

Guanine tapetal material is the most striking component of pigment epithelium cells of *Anchoa mitchilli* and *A. hepsetus*. Tapeta lucida composed of guanine are found in other fishes but the morphology and arrangement of the crystals has received scant attention. In the eyes of some scopelarchid deep-sea fishes Locket (1971) has noted that the pigment epithelial cells contain several rows of crystals regularly arranged on the sides of the cell facing the photoreceptors. Nicol *et al.* (1973) found white diffusing tapeta in *A. mitchilli* and *A. hepsetus* but their electron micrograph of a larval eye shows only platelets arranged in stacks alongside the cone units. The present work on adult eyes clearly shows the presence of three different forms of tapetal material each with a characteristic position in the cell. Zyznar & Nicol (1973) found that the anchovy tapetum appeared to contain only guanine. The diversity of tapetal particles in *Anchoa* is undoubtedly associated with the complex organization of the retina, especially in relation to the structure and arrangement of the photoreceptors. The main function of the tapetal material in *Anchoa* is to provide a reflecting system; the crystallites and needles probably behave as diffuse reflectors whereas the platelets behave more as specular reflectors.

(i) *Platelets*

The association of platelets with cone units and their ordered arrangement within a stack is a constant feature of the eyes of *A. mitchilli* and *A. hepsetus*. The positioning of the stacks on each side of the cone unit, corresponding to the longitudinal orientation of the photosensitive cone lamellae, suggests that the platelets play some rôle in absorption of light by the cone system. We believe that the main function of the stacks is to provide a system of reflectors to direct light back into the cones. The stacks consist of superposed quarter wavelength films - the guanine platelets - which by virtue of high refractive index and regular spacing achieve a high reflectivity through constructive interference of light rays reflected from their surfaces (Denton 1970; Denton & Land 1971). The platelets are arranged many layers deep (up to 10–15 layers) bordering much of the cone unit; towards the vitread end of the cone unit the number of layers of platelets bordering the cone unit decreases progressively to one or two (figure 24, plate 6). A single film reflects 8 % of incident light, and reflectivity at maximal wavelength rises rapidly as the number of films increases, reaching 99 % for a perfectly ordered system of 19 films (figure 39). Reflexion from single platelets near the apices of the processes and from single needles, therefore, would be 8 %, while reflexion from stacks of 10 or more crystals at the bases of the V-shaped processes could approach a maximum of over 90 %.

The stacks of platelets surrounding the cones of *Anchoa* resemble the multilayered systems found in many animals that give rise to reflexion and iridescence through constructive interference. Examples are the retinal tapeta of *Scopelarchus* (Locket 1971), the choroidal tapeta of sharks (Best & Nicol 1967) and the coelacanth (Locket 1974), and the iridescent corneas of teleosts (Locket 1972; Lythgoe 1975). The wavelengths reflected most intensely by such a system of platelets are

$$(m-\tfrac{1}{2})\,\lambda = 2(n_1 d_1 + n_2 d_2)\cos\omega,$$

where n is the thickness of a film, d its refractive index, m any integer, and ω the angle of incidence. The colour of the platelets of the anchovy tapetum indicate a thickness of approximately 80 nm, and measured thicknesses in electron micrographs give a median value of about 82 nm; assume a refractive index of 1.82 (Greenstein 1966; Denton & Land 1971). Cytoplasmic

lamellae between crystals in electron micrographs range in thickness from 61 to 166 nm. The mode is about 112 nm; assume a refractive index for cytoplasm of 1.34. Then the optical path between two crystals giving rise to constructive interference is about 596 nm. This value is generally in accord with observations, the colours of small areas in fresh preparations are green yellow to red, and maximal reflectivity occurs in the yellow green, λ_{max} around 570 nm, for greenish areas of the retina, whereas in yellow-orange areas, reflexion progressively rises at long wavelengths (figure 38).

Computed spectra for reflectivity of one to ten regularly arranged quarter wavelength plates, maximum at 550 nm, give broad bands; for multiple films reflexion falls off sharply on either side of the maximum range (figure 40). A computed spectrum for reflectivity of ten somewhat irregularly spaced crystals gives a broad band, falling off gradually at wavelengths on either side of the maximum (figure 39). A curve derived from a mix of spectra like the latter, the spectra

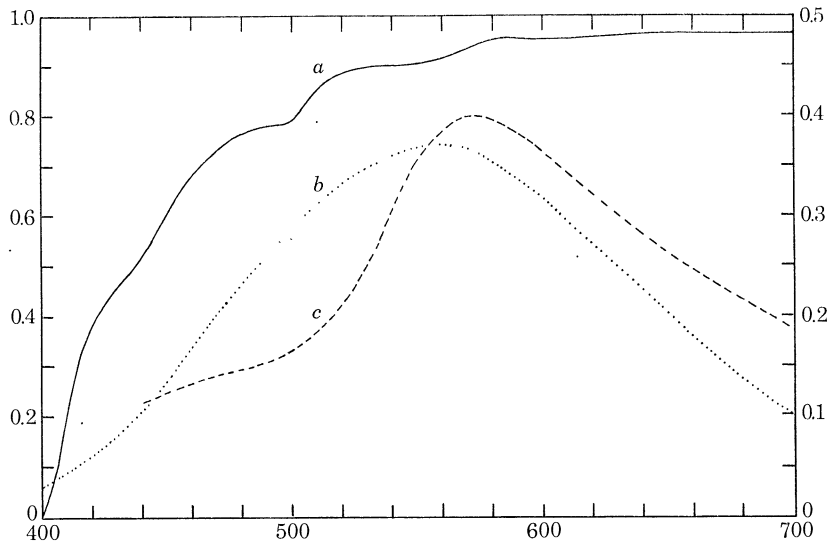

FIGURE 39. Reflexion spectra. (*a*) Reflexion of a d.a. retina of *Anchoa hepsetus* (scale to the right). (*b*) Computed reflectivity of a stack of ten crystals somewhat irregularly spaced to achieve maximal reflexion at 450 (1), 470 (2), 490 (2), 510 (3), 530 (3), 550 (3), 570 (3), 590 (3), 610 (2), 613 (2) and 650 nm (1 crystal). (*c*) Average spectrum of l.a. retinae of *A. hepsetus* and *A. mitchilli* (scales for (*b*) and (*c*) (*c* arbitrary) to the left).

differing in their reflexion maxima, still gives a composite curve resembling that shown in the figure. Now, this reflexion spectrum is not dissimilar from that of the anchovy, except that the latter falls off more rapidly on the short wavelength side of the maximum. Alternatively, a reflexion spectrum generally resembling that of the anchovy is obtainable by summing a series of spectra from regularly arranged platelets, the spectra differing in their reflexion maxima. Of the two explanations the former is preferred because the lamellae between crystals patently vary in thickness. These arguments, however, are based upon a system consisting of ten or more superposed platelets reflecting light at near normal incidence, and are certainly too simplistic. One third of the reflecting surface consists of stacks of four or fewer platelets, reflexion spectra of which are broad curves (as shown in figure 40). Much of the light striking the platelets does so at angles of incidence that are greater than the normal to their surfaces, possibly at angles ω ranging from near normal to about 70°. As ω increases, spectrum reflexion shifts to shorter wavelengths, amounting to 180 nm at 70°. Spectrum reflexion, measured, may

be the result of constructive interference at oblique angles of incidence, and maxima lie at wavelengths shorter than are indicated by measurements of crystal thicknesses and spacing, when normal incidence is assumed. Real spacing of cytoplasmic lamellae, therefore, may be somewhat greater than those measured, possibly due to shrinkage. Finally, some contribution of scattered light from the crystallites may occur. It is difficult to evaluate these factors on the basis of the data available. The reduced reflexion of the anchovy spectrum on the short wavelength side of the maximum could be the result of differential absorption by pigments, possibly bleached cone pigments, since spectra were measured on intact retinae. Because the reflecting platelets lie very obliquely to the longitudinal axes of the cones, multiple reflexion must occur between opposing faces of platelets across the cone outer segments, a situation favouring progressive absorption. The discussion immediately preceding assumes that reflexion is equally effective for light rays vibrating in all planes, and requires modification for polarization, which will be attempted in a later paper when the photoreceptors are described.

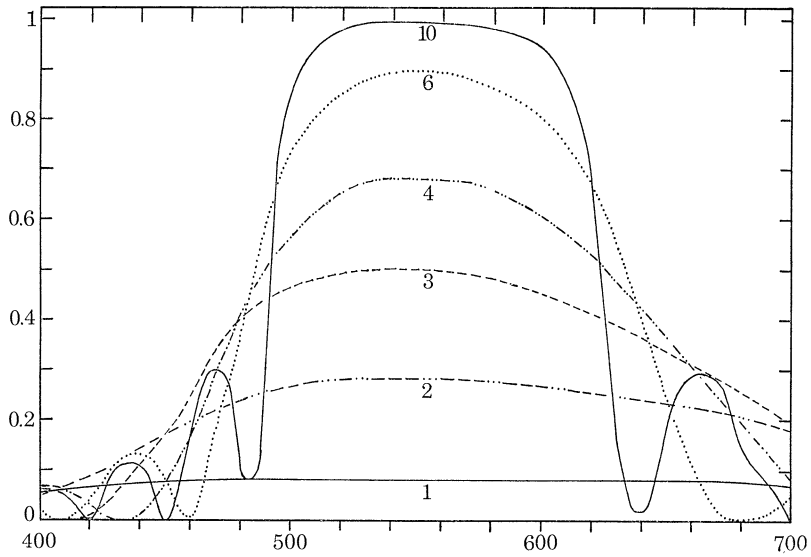

FIGURE 40. Computed reflectivities of stacks of quarter wavelength films having characteristics to give maximal reflexion at 560 nm. Spectra for 1–10 platelets (2–19 films) shown.

The reflexion spectrum of the retina of the d.a. eye shows low reflexion at low wavelengths, rising to a plateau > 500 nm. Absorption of short wavelengths again is indicated. Several factors favour backscattering: the relatively large size of the crystallites, density of packing, high refractive index. The structural arrangement in the d.a. eye suggests that there is some contribution to reflexion from the platelets.

The precise spacing of platelets within and between rows is important if the stack is to function by constructive interference as an efficient reflecting system. To achieve such a regular distribution of platelet reflectors, a system of row spaces and interlocking structures is required. Evidence for the existence of a strong linking of platelets in stacks of *Anchoa* is shown by the way in which the platelets of the stack usually remain together when released from the cells (figures 20 and 23). The complex system of smooth e.r. running between the rows of platelets, the continuities between this e.r. and the envelopes containing the platelets and the cross-bridging material almost certainly serve to maintain the precise spatial relationship of components in the reflecting stacks of *Anchoa*.

The structures which hold the components of vertebrate reflecting systems together have generally proved elusive to demonstrate. The existence of linking structures was inferred as early as 1872 by Schlutze who noted that the bundles of rodlets in the tapetum of the cat remained together when the cells containing them were disrupted. In a study of the reflecting eye of the bush baby *Galago crassicaudatus agisymbanus*, Dartnall *et al.* (1965) found thin flat crystals in the tapetum separated by cytoplasmic lamellae and there was some structural evidence that the crystal stacks were 'keyed' into the cytoplasmic lamellae. Pedler (1963) and Rutschke (1966) have discussed the various hypotheses to explain a structural framework between compound reflectors but the requisite structures had still not been recognized satisfactorily. More recent work on iridophores is, however, beginning to reveal something of this interlocking framework. In an examination of the iridescent cornea of serranid teleosts, Locket (1972) noted within the endothelial cells a series of flat parallel sacs separated by cytoplasmic lamellae which contained a single smooth cisterna (probably e.r.), resembling the cisterna found between the rows of platelets in *Anchoa*. Rohrlich (Rohrlich & Porter 1972; Rohrlich 1974) has specifically studied the linking processes in iridophores from a range of vertebrates and has noted that they contain 6.5 nm filaments joining the parallel stacks of crystals. In addition, she observed 10 nm filaments in several physiologically passive iridophores. Rohrlich suggested that the 6.5 nm filaments might be iridophore actin whereas the 10 nm filaments might function as a cytoskeleton in those structures where they occur.

While there are similarities between the interlocking and spacing systems described by Rohrlich and that of *Anchoa* there appear to be no filaments in our material comparable to those of her iridophores. The cross-bridges in *Anchoa* appear to be fairly stable structures and as the platelets show no marked rearrangement during photo-mechanical movements of the eye they probably function mainly as cytoskeletal locking processes rather than as contractile filaments. However, a little contraction may occur for any fine adjustment of the space between the rows of platelets that might be necessary. We suggest that the supportive structure of a stack is largely provided by the e.r. sac surrounding each platelet and the cisternae which lie between the rows. The tubular membrane connections between the sacs and each cisterna probably serve as the spacers of the system with the various cisternae of the stack linking the whole collection of platelets together. The cross-bridges are interpreted as tying the whole system together and therefore maintaining its stability.

Vertebrate eyes which exhibit a tapeta lucida typically utilize the reflecting material to increase the light sensitivity of the rods during dark or dim light vision. The platelet reflectors of *Anchoa* on the other hand, operate when the cones are functioning at their optimal level during light adaptation of the eye. On dark adaptation of the eye the stacks of platelets move sclerally at the same time as the cone units but unlike the other tapetal material the platelets undergo no basic rearrangement. Although the cones in the d.a. state, with their elongated myoids, are probably non-functional in the perception of light the platelet system may still operate in a general way by reflecting light back through the cones and into the rods which now occupy a vitread position.

(ii) *Needles*

Whereas platelets form a well developed system of reflectors facing the horizontally orientated converging faces of the cone unit, they are conspicuously absent on the vertical more or less parallel sides of the unit facing adjacent cone rows. Probably little or no light enters the cone

unit from this direction because of the shielding provided by the lateral lobes of the pigment epithelial cells extending into the space between the cone rows. However, a small amount of light passing sclerally through the cones may leak laterally from the cone unit and would therefore not be reflected by the platelets. It seems that this stray light may instead be reflected into the cone unit mainly by the tapetal needles. This suggestion is based on the observation that the needles often form a tight palisade layer with the broadest facets of the needles directed towards the cone unit. The needles may also prevent any stray light coming from adjacent rows from entering the cone unit which they surround. Compared with platelets, the needles form a less well ordered reflecting system and so they are probably only of secondary importance in the diffuse reflection of light back into the cones. The removal of most needles from the vitread region of the pigment epithelial cell and their apparent random redistribution within the middle region on dark adapation of the eye further suggests that they form a less precisely ordered system of reflectors. In the d.a. eye needles appear to contribute towards the general diffuse reflecting system developed in the middle region of the cell, largely by the crystallites.

(iii) *Crystallites*

The crystallites of both l.a. and d.a. eyes seem to function mainly as randomly orientated diffuse reflectors. In the l.a. eye the crystallites of the middle and scleral regions probably play little rôle in the visual process and are merely 'stored' in these parts of the cell so as not to interfere with the functioning of the cones and their associated reflectors. Little or no light may reach these parts of the cell because of the efficient system of reflectors surmounting the cones and the abundance of melanosomes in the vitread region. The crystallites which do occur in the vitread region of l.a. eyes appear to function as diffuse reflectors packed amongst the melanosomes and needles.

The period when most of the crystallites are fully functional is during the dark adapted state of the eye when they become localized in the shortened middle region of the cell. The vitread withdrawal of rods allows all the pigment epithelium cells at this level to come into contact and thus results in the development of a continuous tangential band of tapetal material throughout the retina. This zone with its increased density of crystallites over that of the l.a. condition provides an extensive reflector of light for the rods. Reflexion is caused by the back-scattering of light from the facets of the numerous crystallites in an apparently random manner, and it is this which is believed to contribute greatly to the eyeshine of d.a. eyes in *A. mitchilli* and *A. hepsetus*. The semi-equatorial notch noted in most crystallites may enhance the effective number of facets developed by the crystallite for the diffuse reflexion of light. Because of their relatively large dimensions and high refractive index, the crystallites probably are efficient in back-scattering incident light.

(*d*) *Melanosomes*

The distribution of melanosomes in l.a. and d.a. eyes is spatially linked with the various tapetal reflectors and indicates a functional relationship between the two kinds of structures. The fact that the melanosomes are positioned behind the guanine platelets and needles of l.a. eyes suggests that they are important in absorbing any stray light not reflected into the cone unit. In this way melanosomes would prevent stray light emitted by one cone unit from entering another unit in the same row. The often high concentration of melanosomes in the lobes and vitread processes further suggests that the melanosomes are important here in preventing the lateral leakage of light between one row of cones and the next.

The two forms of melanosomes found in pigment epithelial cells of *Anchoa* are unmistakable. The closely packed cylindrical melanosomes probably enable the development of a more efficient system for absorbing light than spherical forms where only a few layers of melanosomes can be accommodated – as in the lobes and vitread processes of the pigment epithelial cell of l.a. eyes. The rôle of the melanosomes changes on dark adaptation of the eye. Withdrawing from the reflectors around the cone units they cease to screen the cones. The accumulation of melanosomes in the basal region allows for the greatest amount of tapetal material to be exposed to the path of incoming light and thus for it to serve as a reflector for the rods. The rôle of melanosomes as absorbers of light in d.a. eyes is probably slight as little stray light would be expected to penetrate beyond the broad tapetal layer.

The mode of migration of melanosomes and tapetal material during photomechanical movements of pigment epithelial cells has not been specifically studied in *Anchoa*. In certain teleost melanophores Murphy & Tilney (1974) consider microtubules to be important in the movement of the pigment granules. Current work on frog pigment epithelial cells, on the other hand, has revealed bundles of thin actin – like filaments associated with the membrane surrounding each melanosome in a manner suggesting that the pigment granule moves along these filaments, perhaps in conjunction with a myosin-like protein in the membrane (Dubin & Murray 1975). So far we have not detected microtubules or microfilaments in association with melanosomes or tapetal crystals in *Anchoa*, however, our work has not been directed specifically towards this problem.

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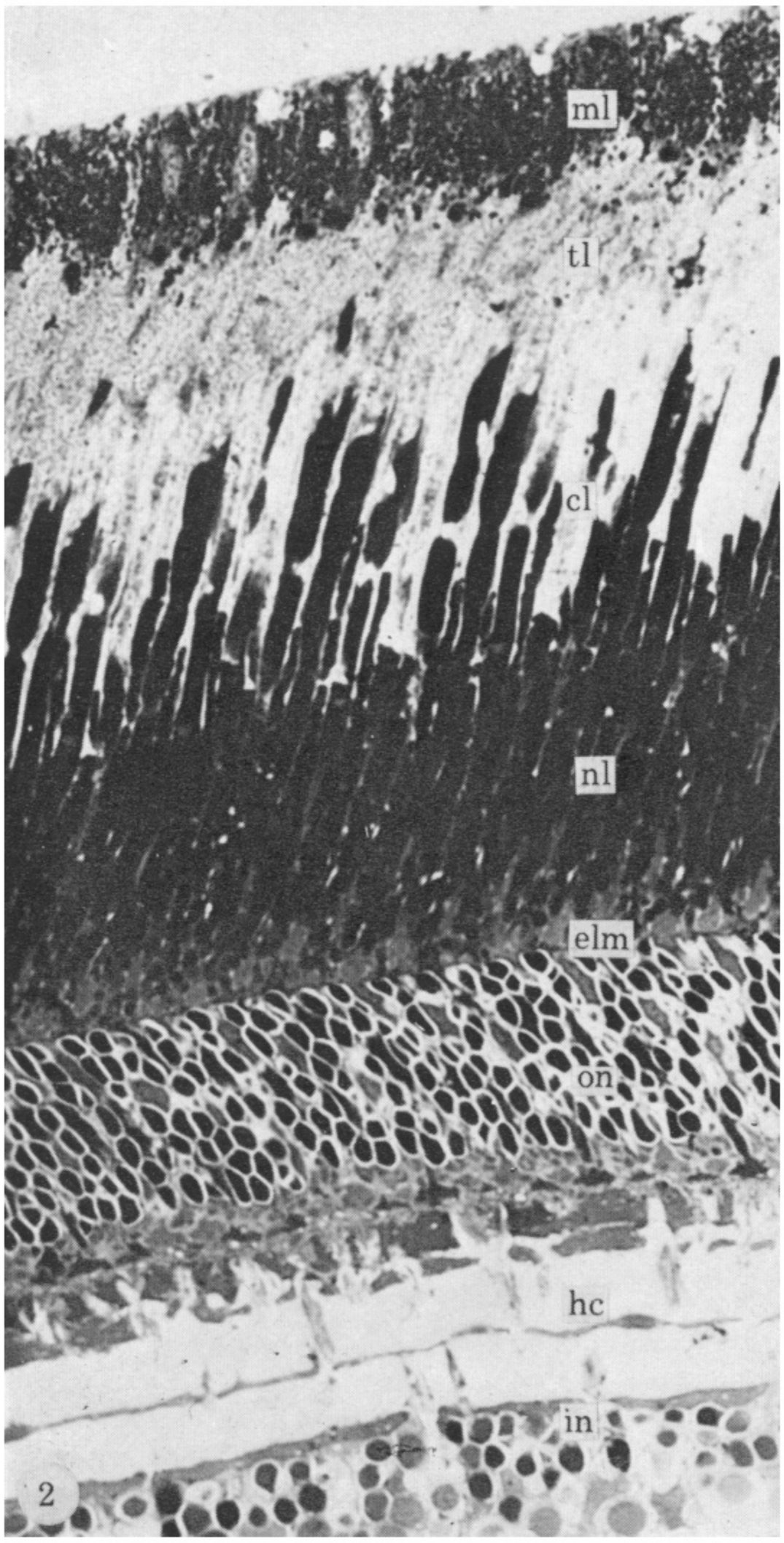
Abbreviations used on illustrations

bc	bifid cone	ml	melanosome layer
br	basal region of pigment epithelium cell	mr	middle region of pigment epithelium cell
c	tapetal crystallite	my	myeloid body
cu	cone unit	n	needle
cl	cone layer	nu	nucleus
d	desmosome	obc	outer segment of bifid cone
ebc	ellipsoid of bifid cone	olc	outer segment of long cone
elc	ellipsoid of long cone	on	outer nuclear layer
elm	external limiting membrane	p	tapetal platelet
hc	horizontal cell layer	pe	pigment epithelium
in	inner nuclear layer	ps	platelet stack
l	lysosome	r	rod
lc	long cone	rl	rod layer
m	melanosome	tl	tapetal layer
mi	mitochondrion	vr	vitread region of pigment epithelium cell

1



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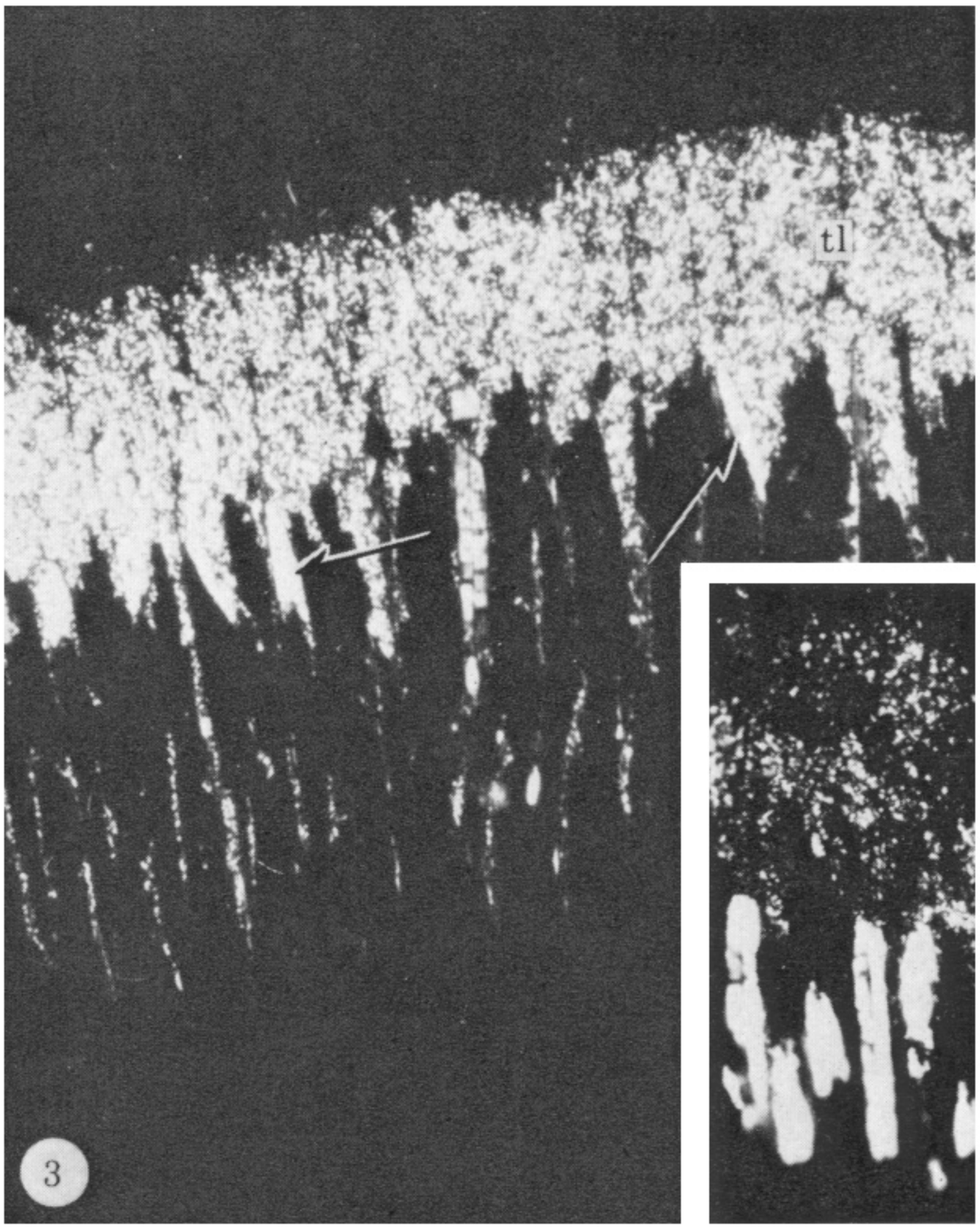

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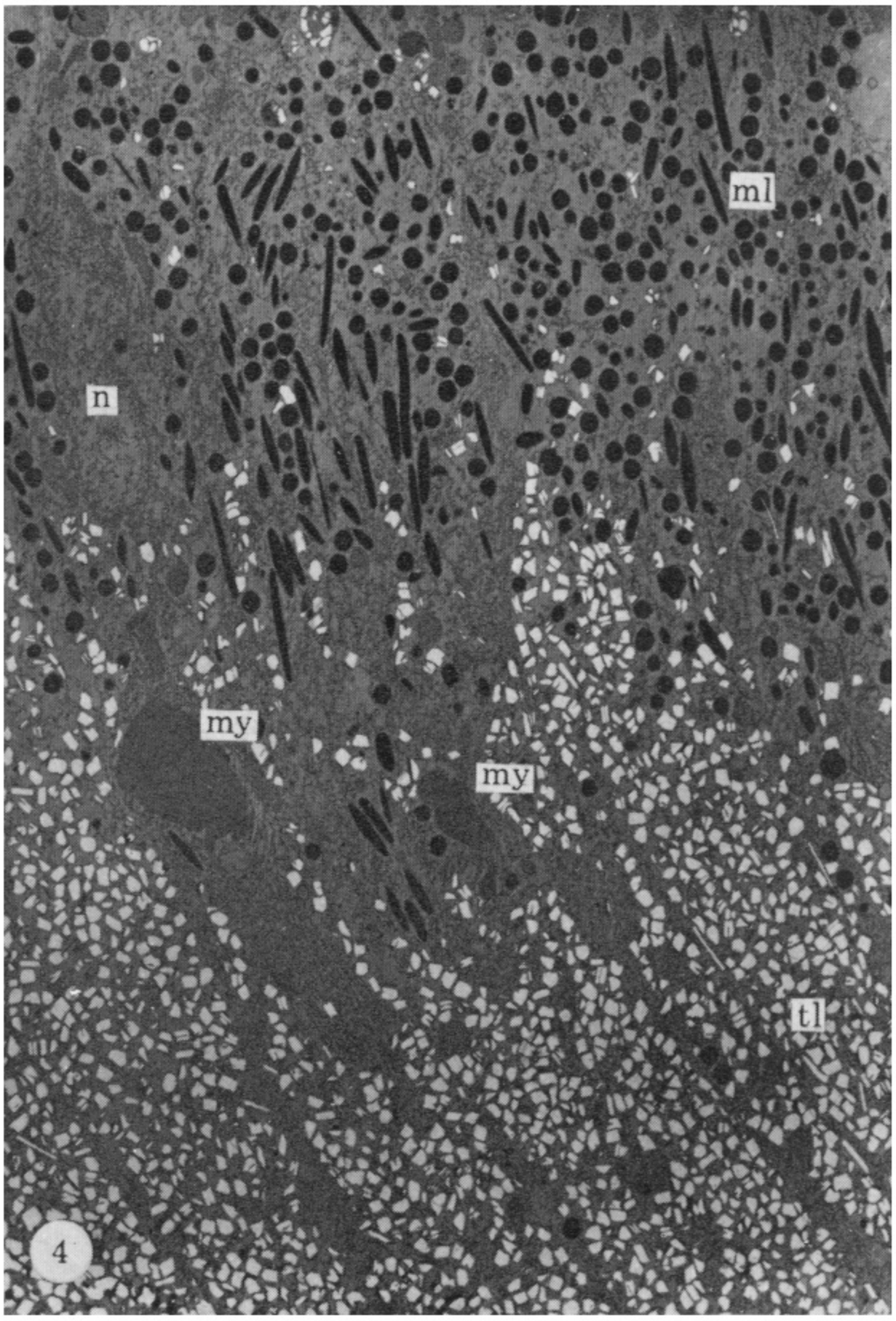

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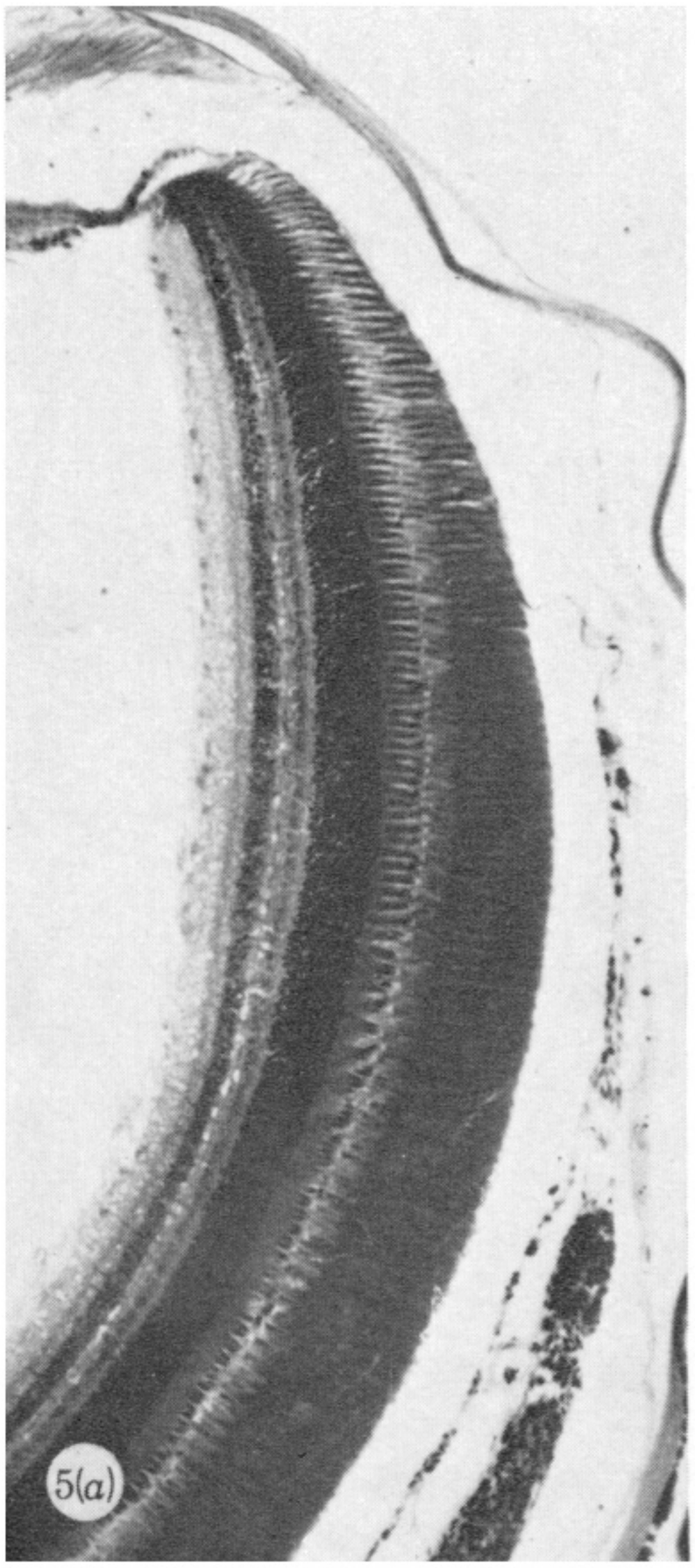
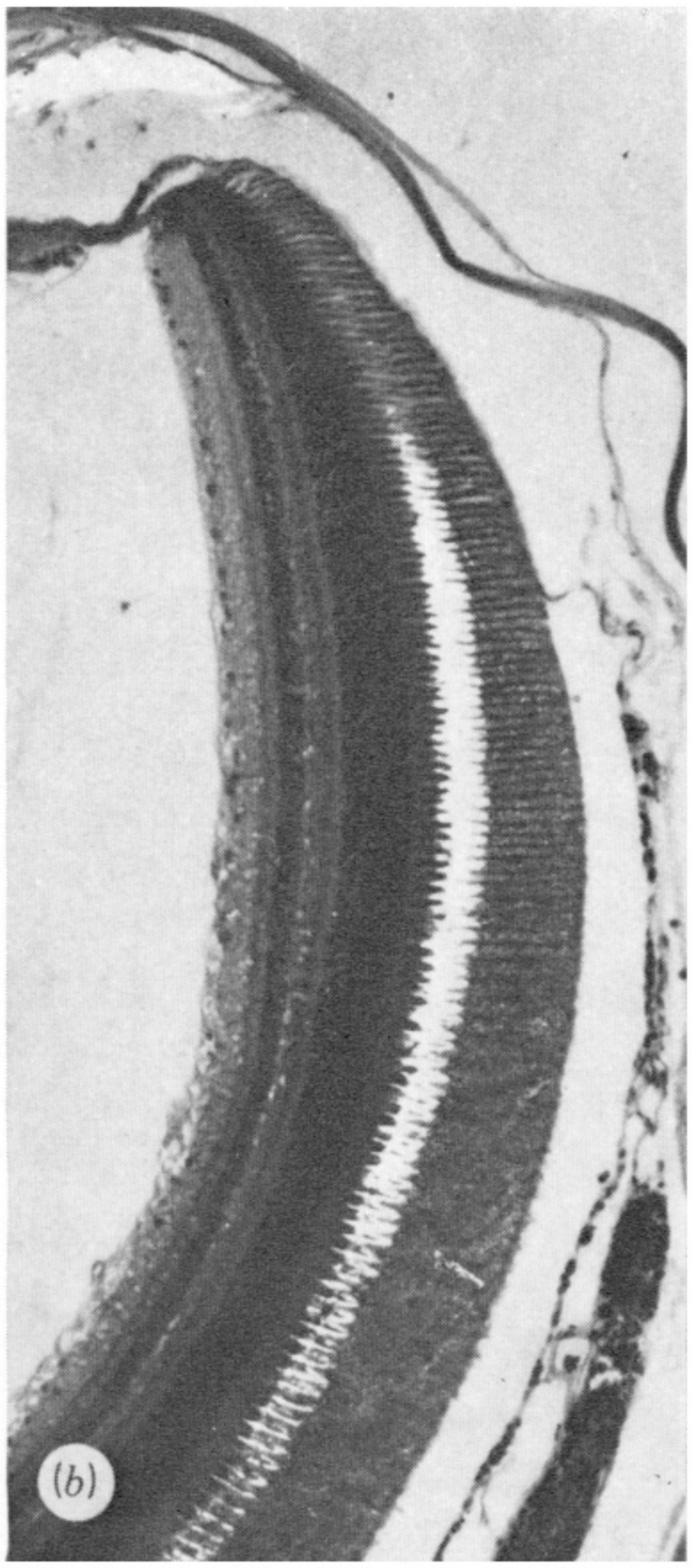
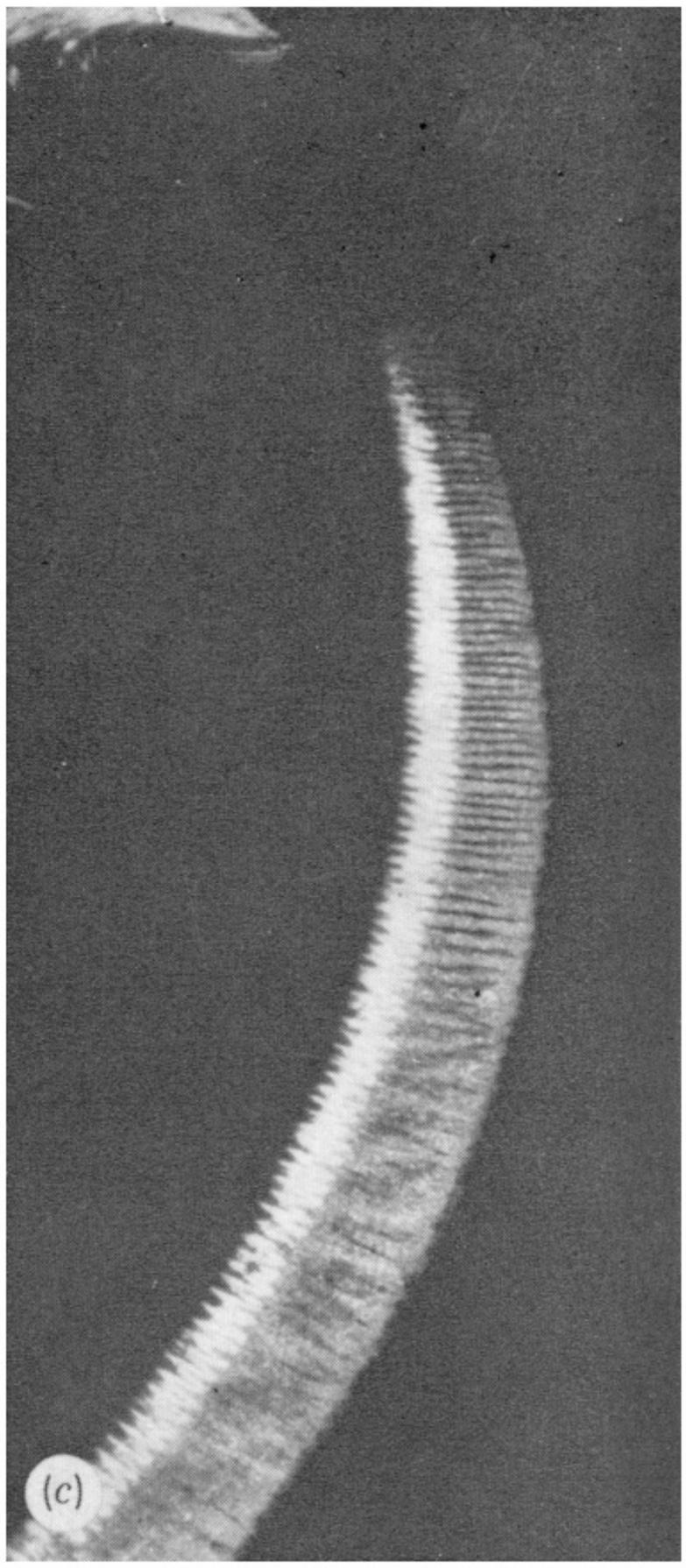
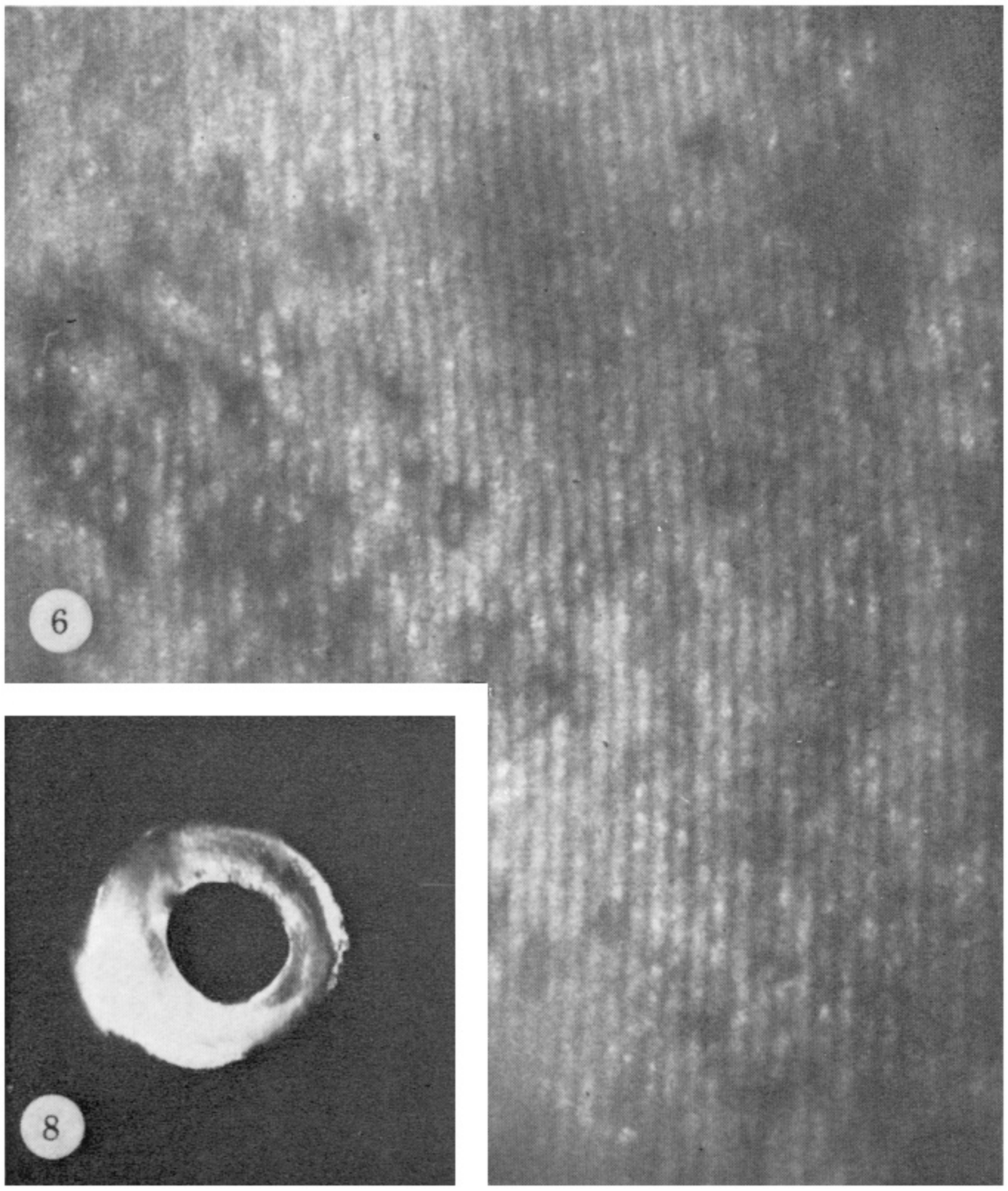
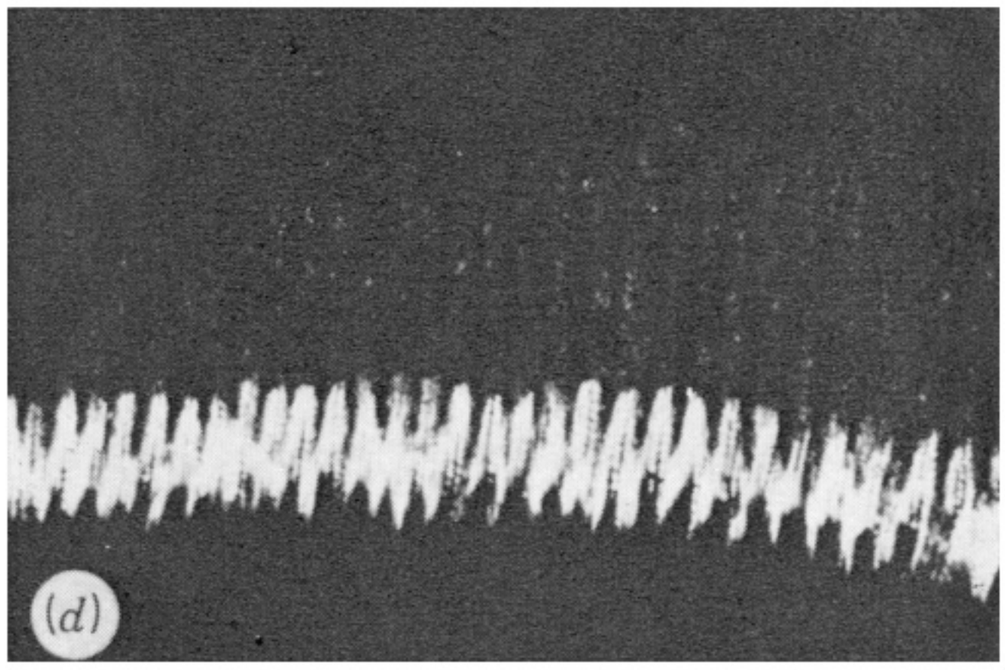
FIGURES 1–4. For description see opposite.

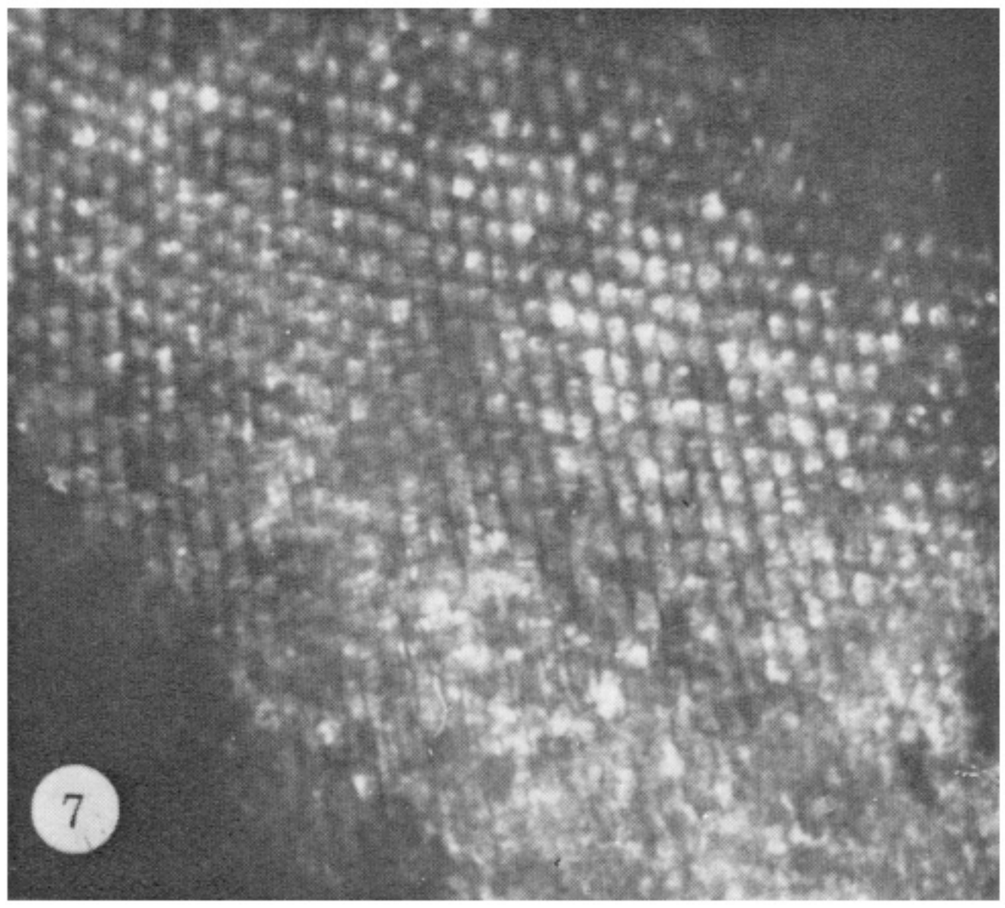












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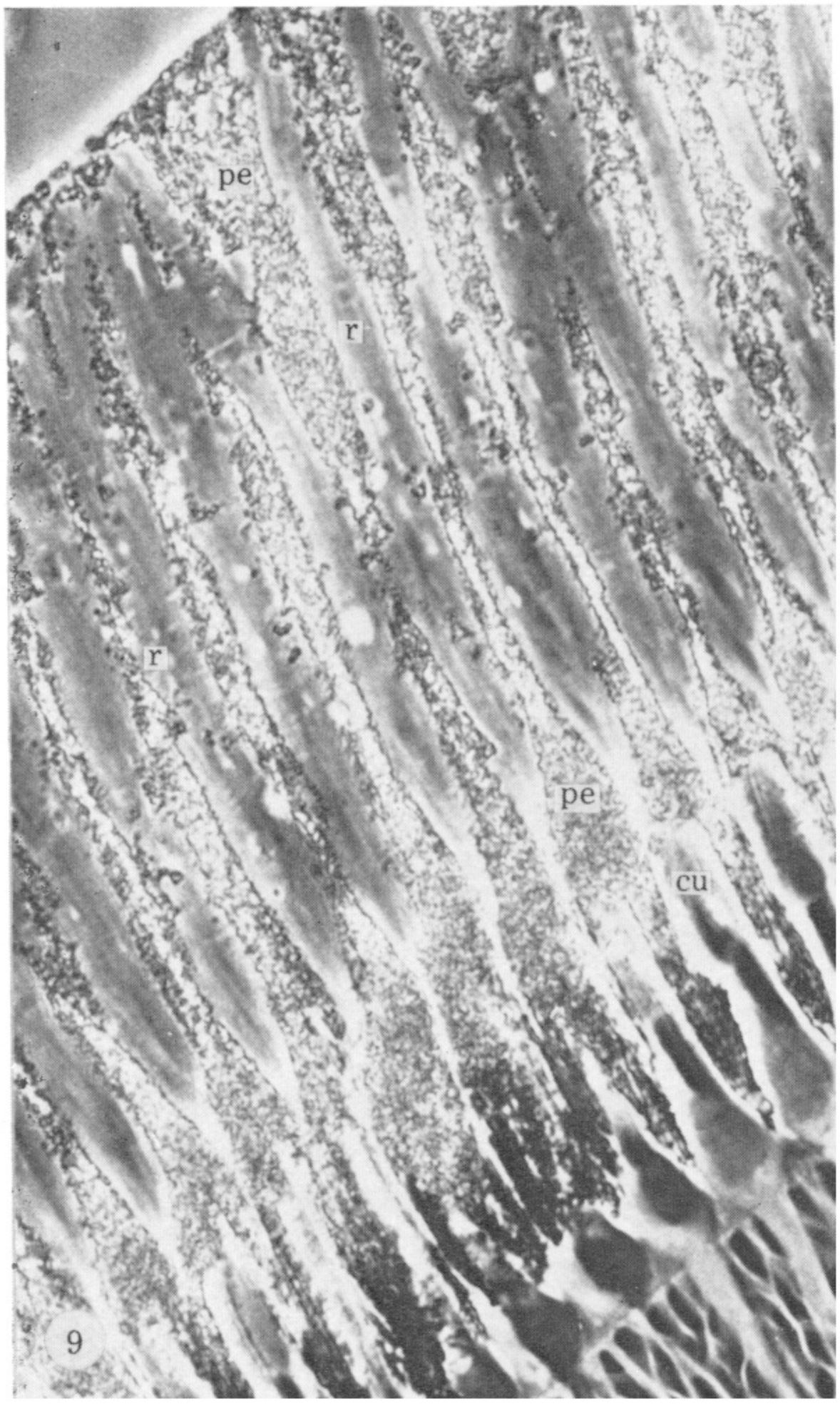

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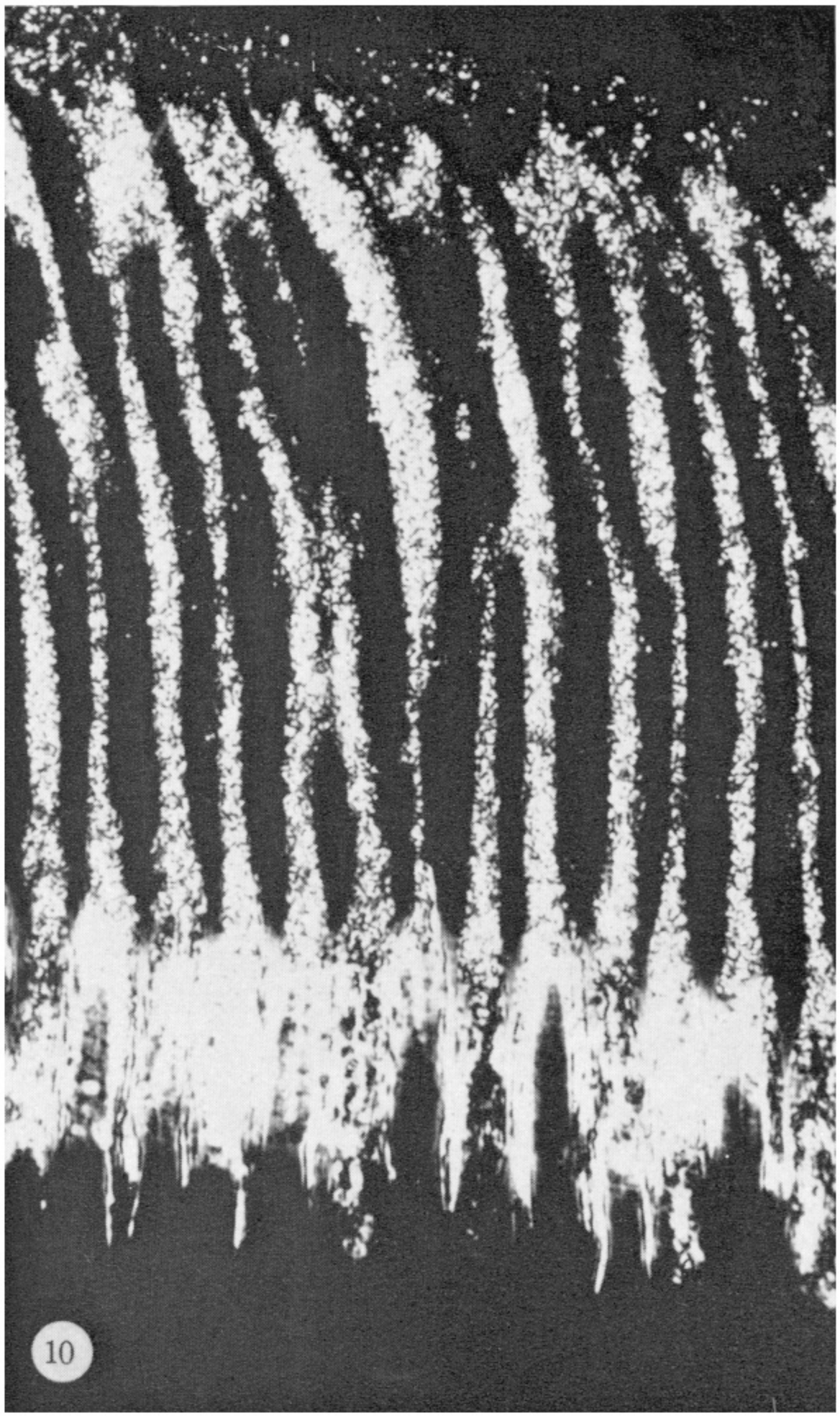

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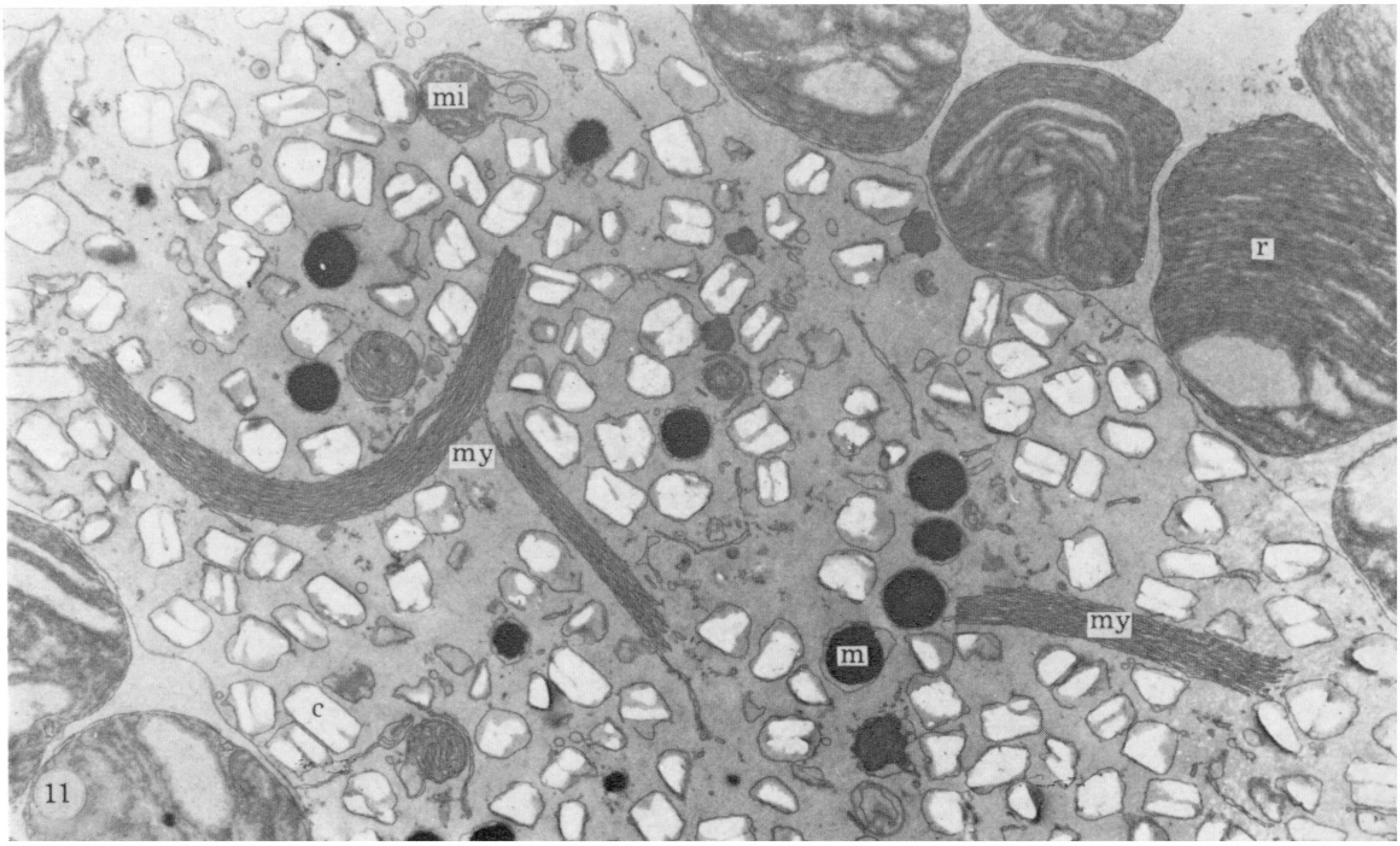

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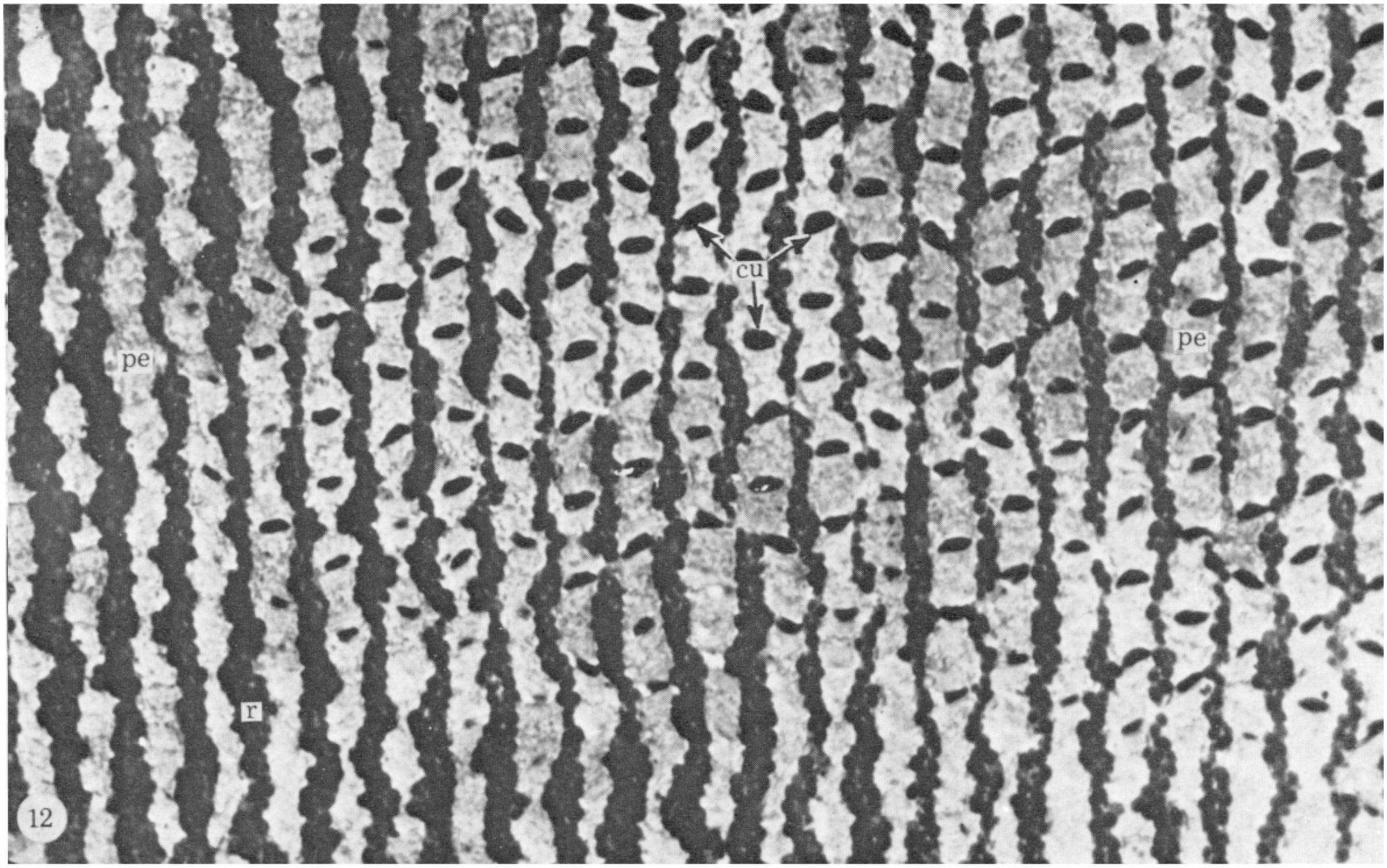
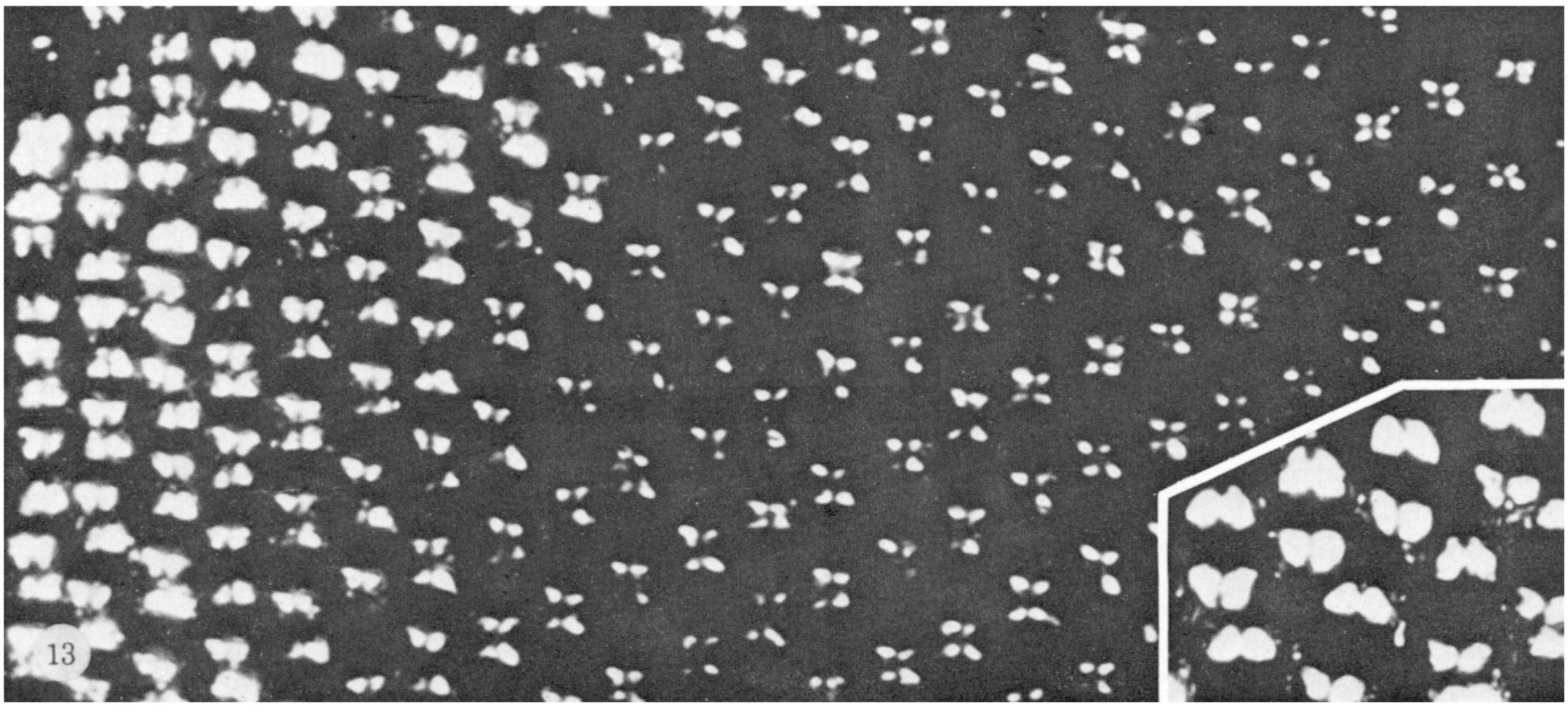
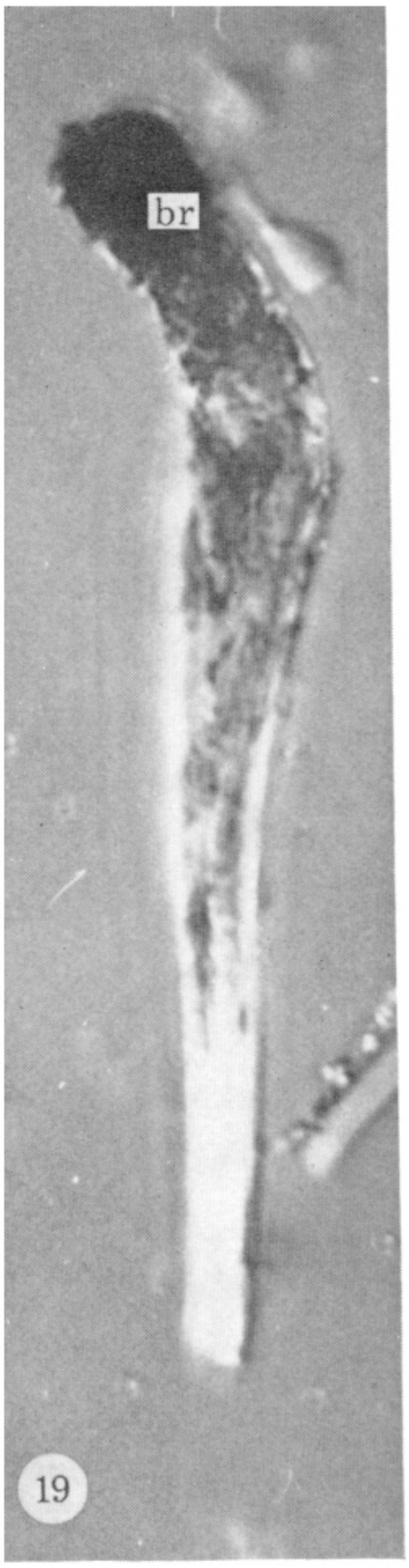
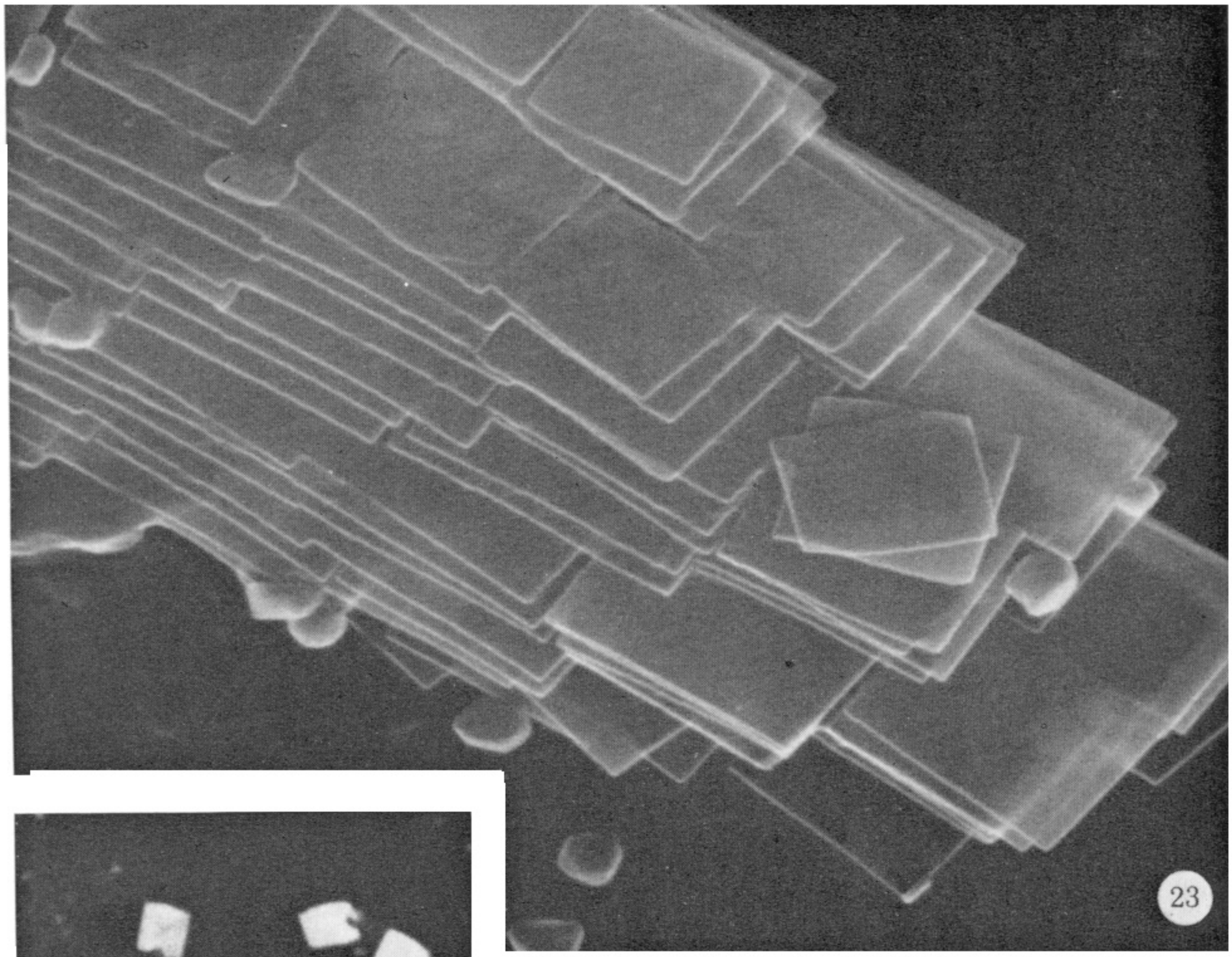
FIGURES 9–11. For description see opposite.

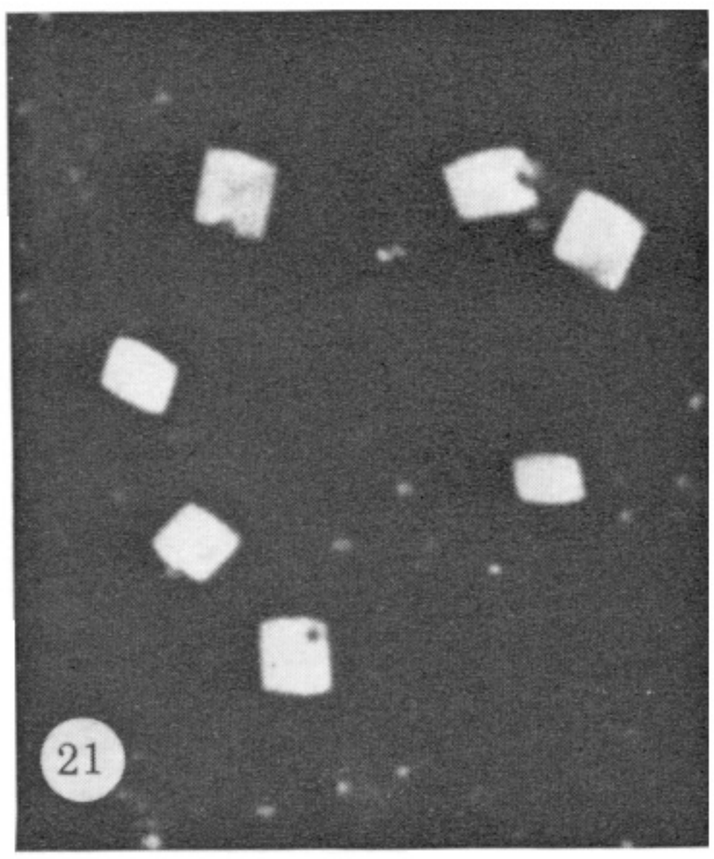


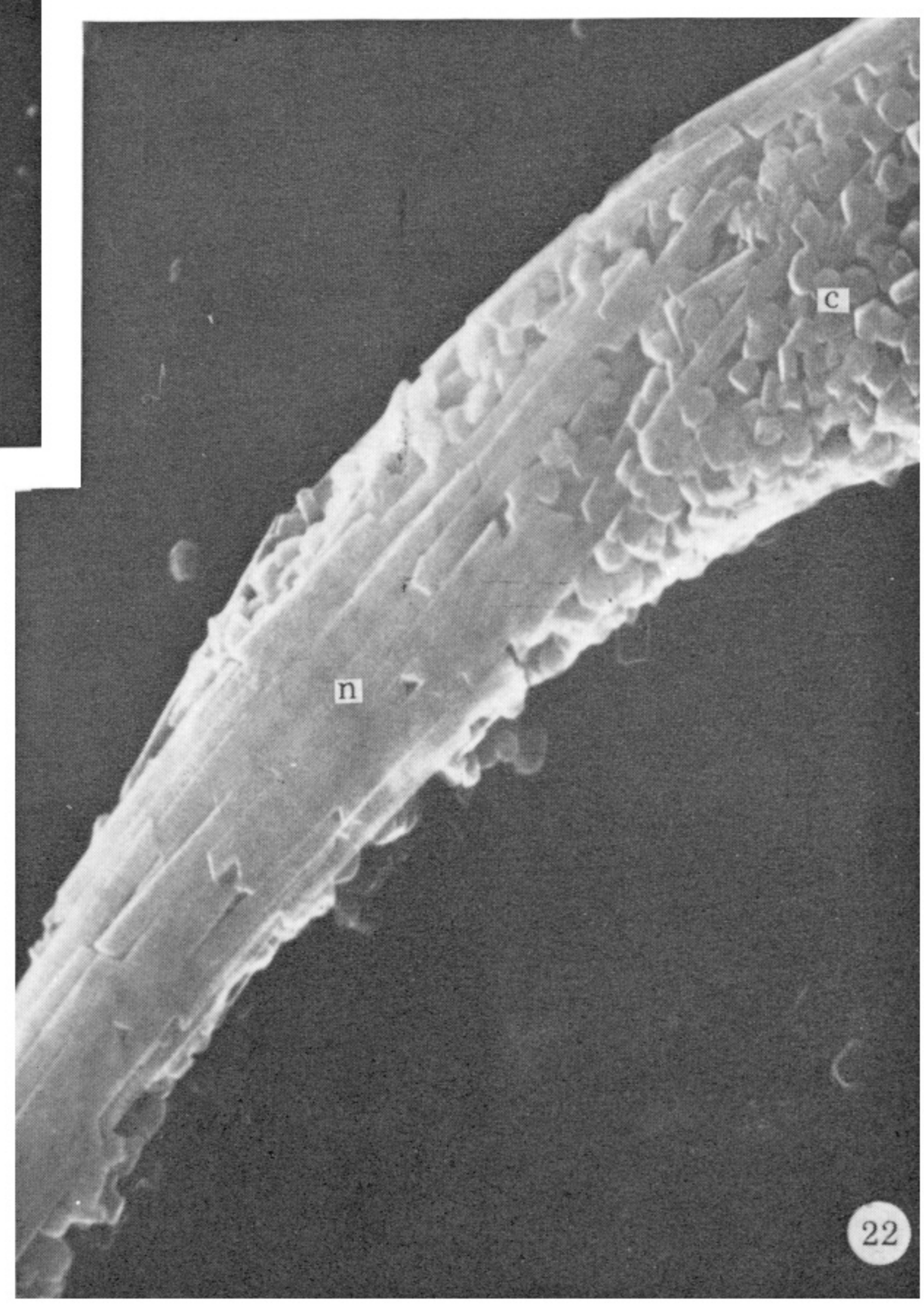


FIGURES 12 AND 13. For description see opposite.

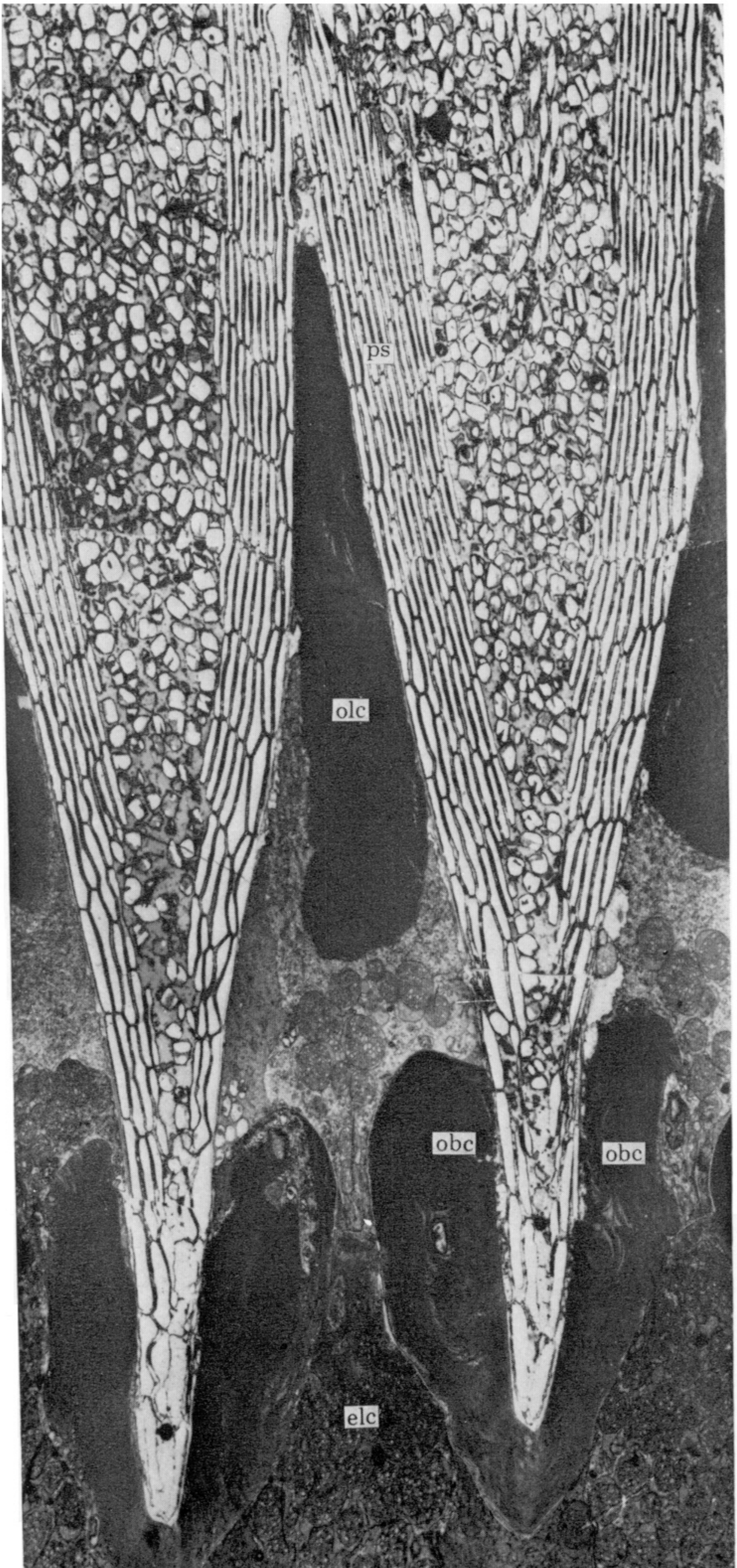








FIGURES 19–23. For description see opposite.



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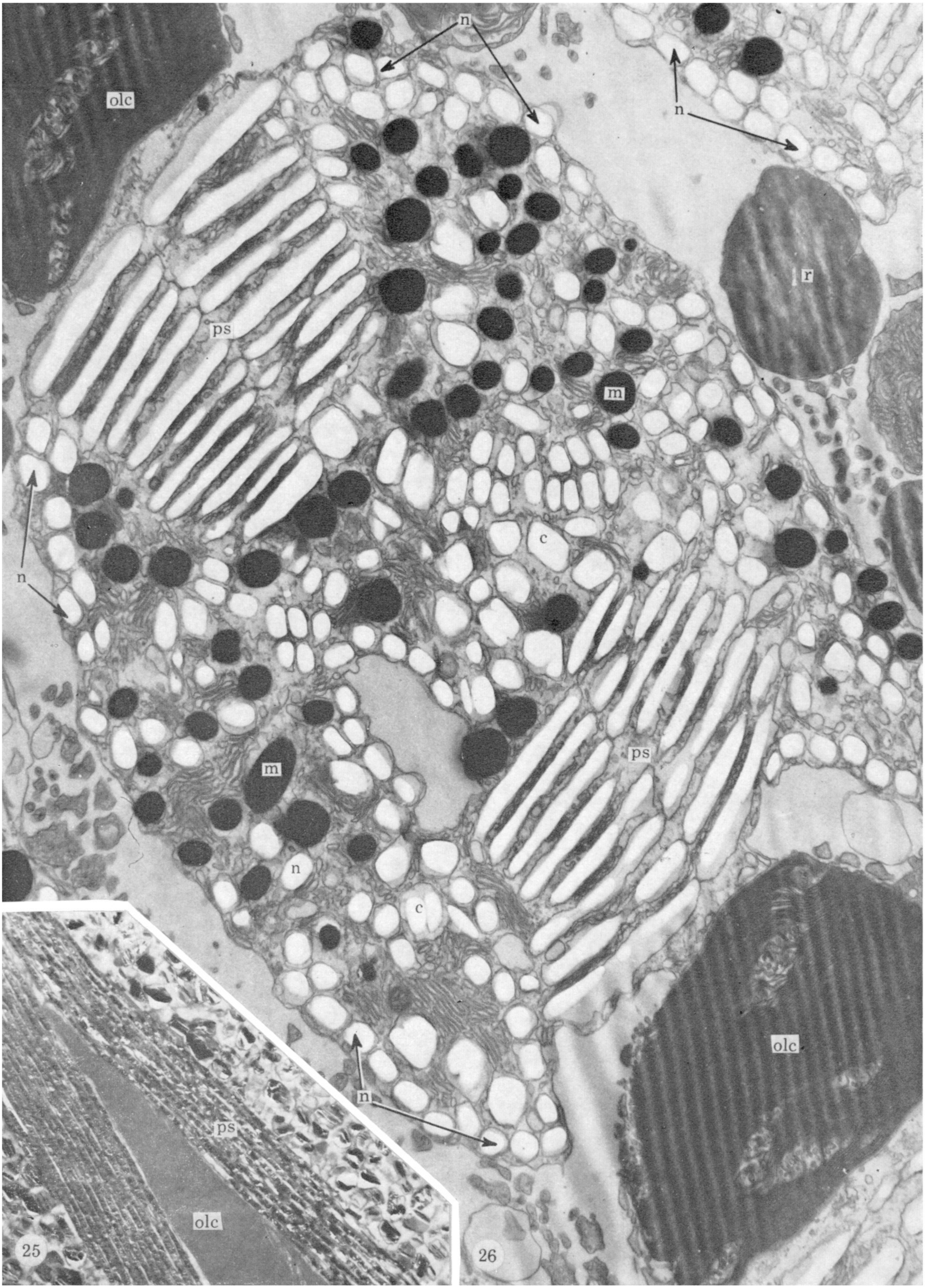
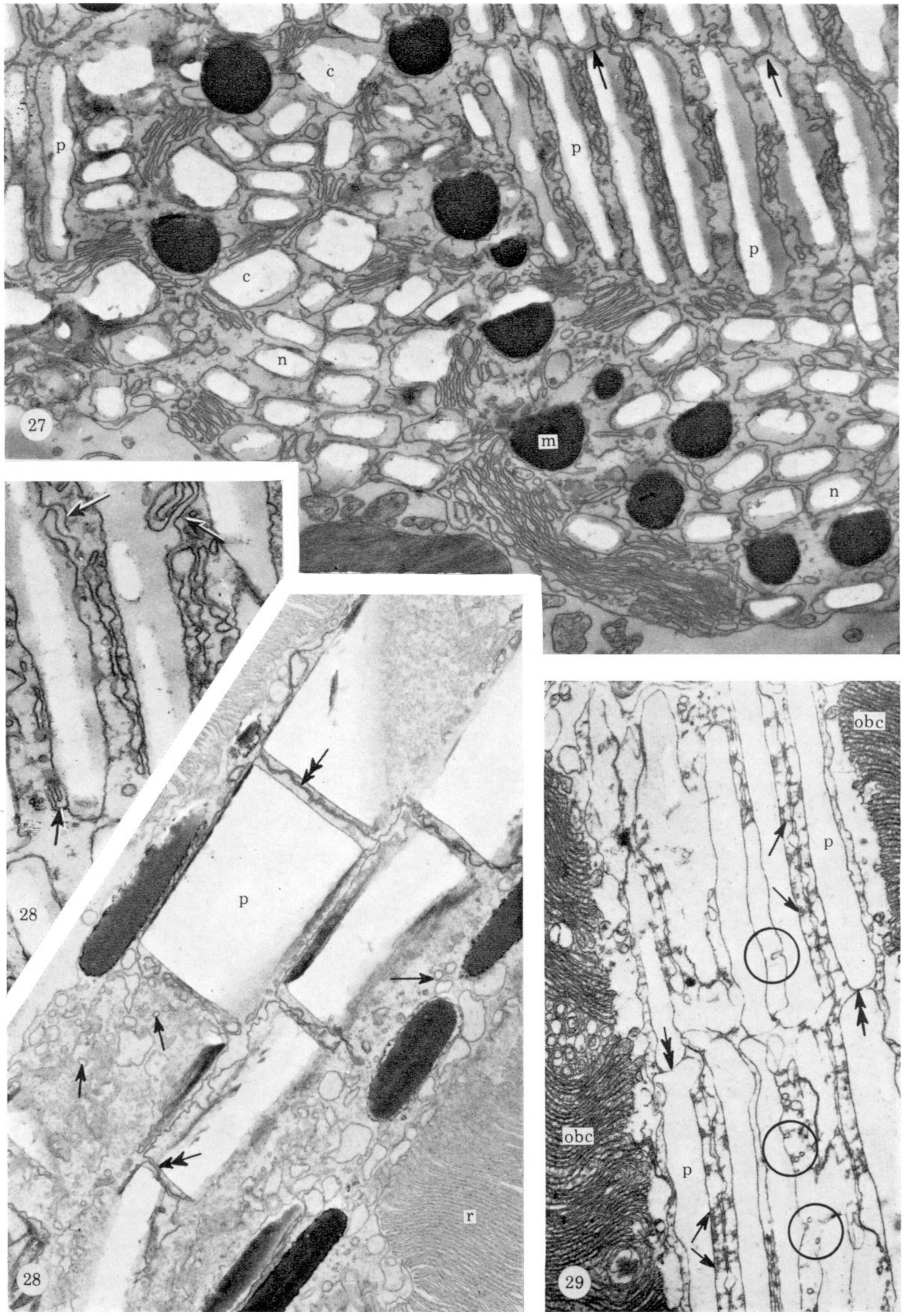
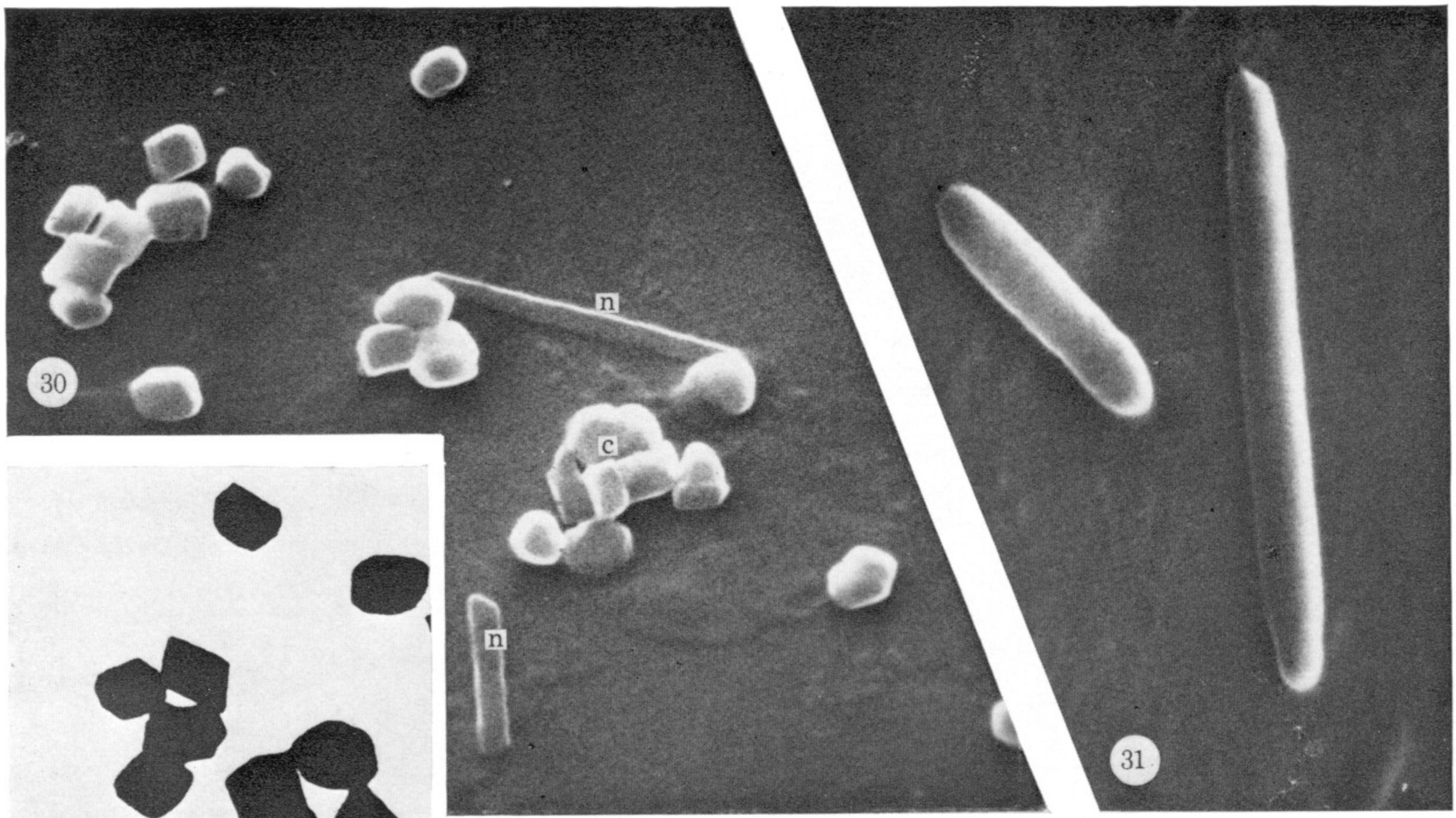
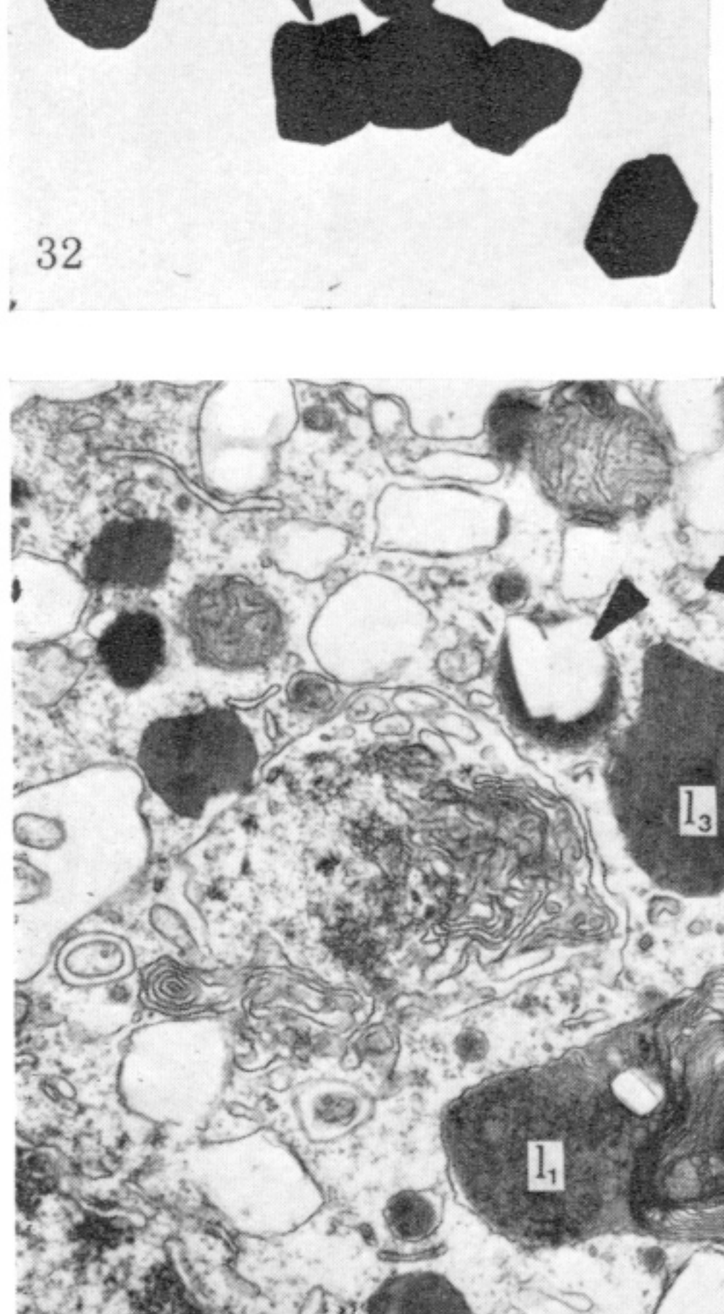


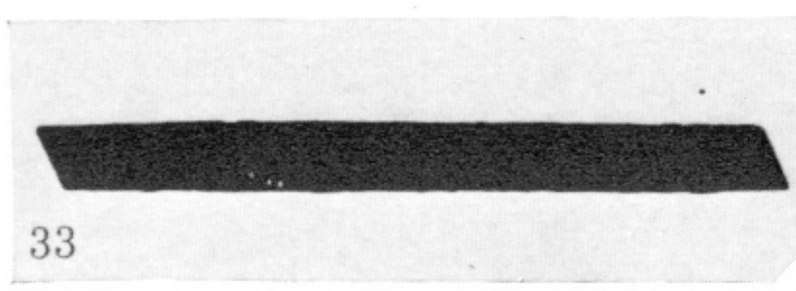
FIGURES 25 AND 26. For description see opposite.

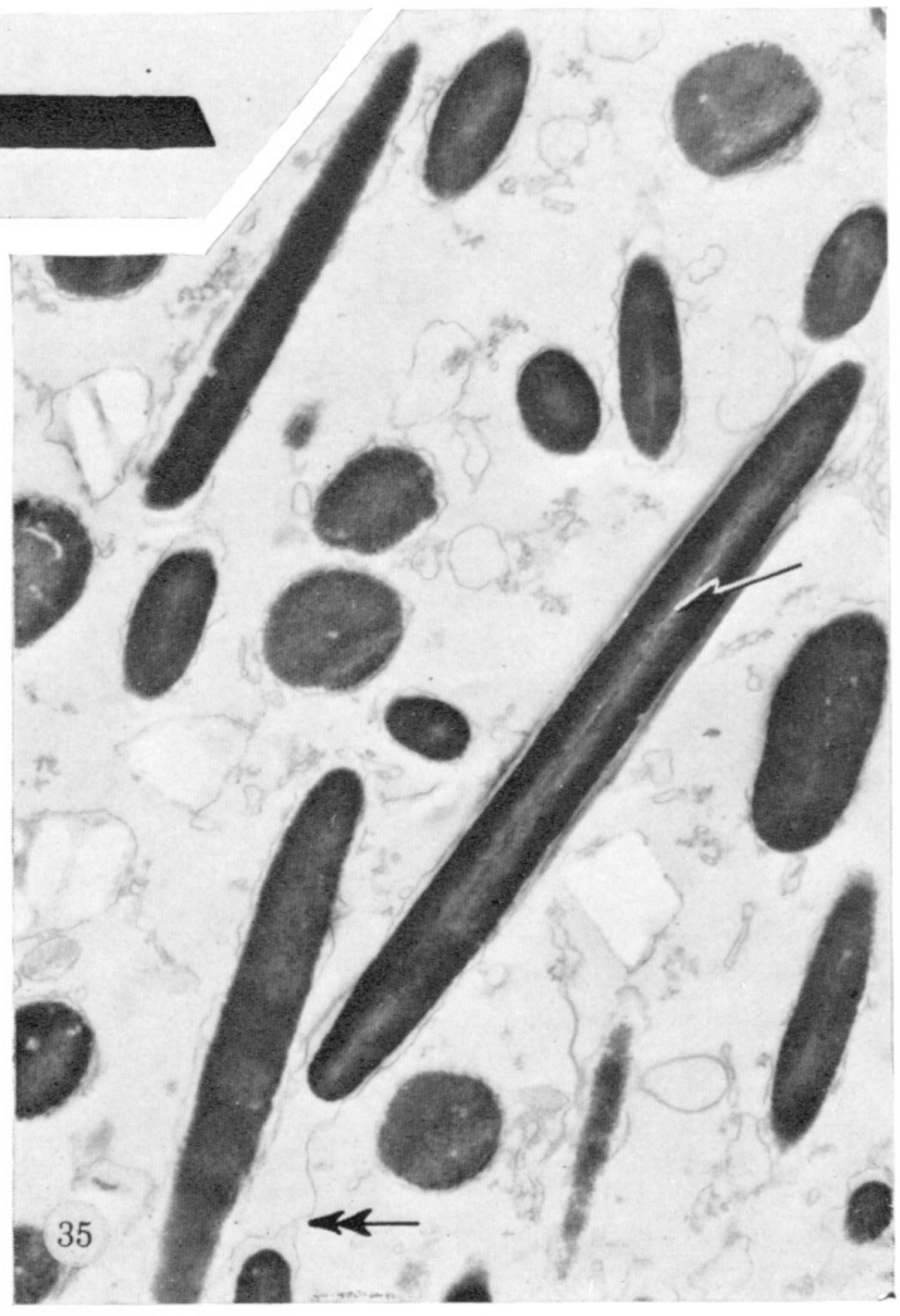


FIGURES 27–29. For description see opposite.

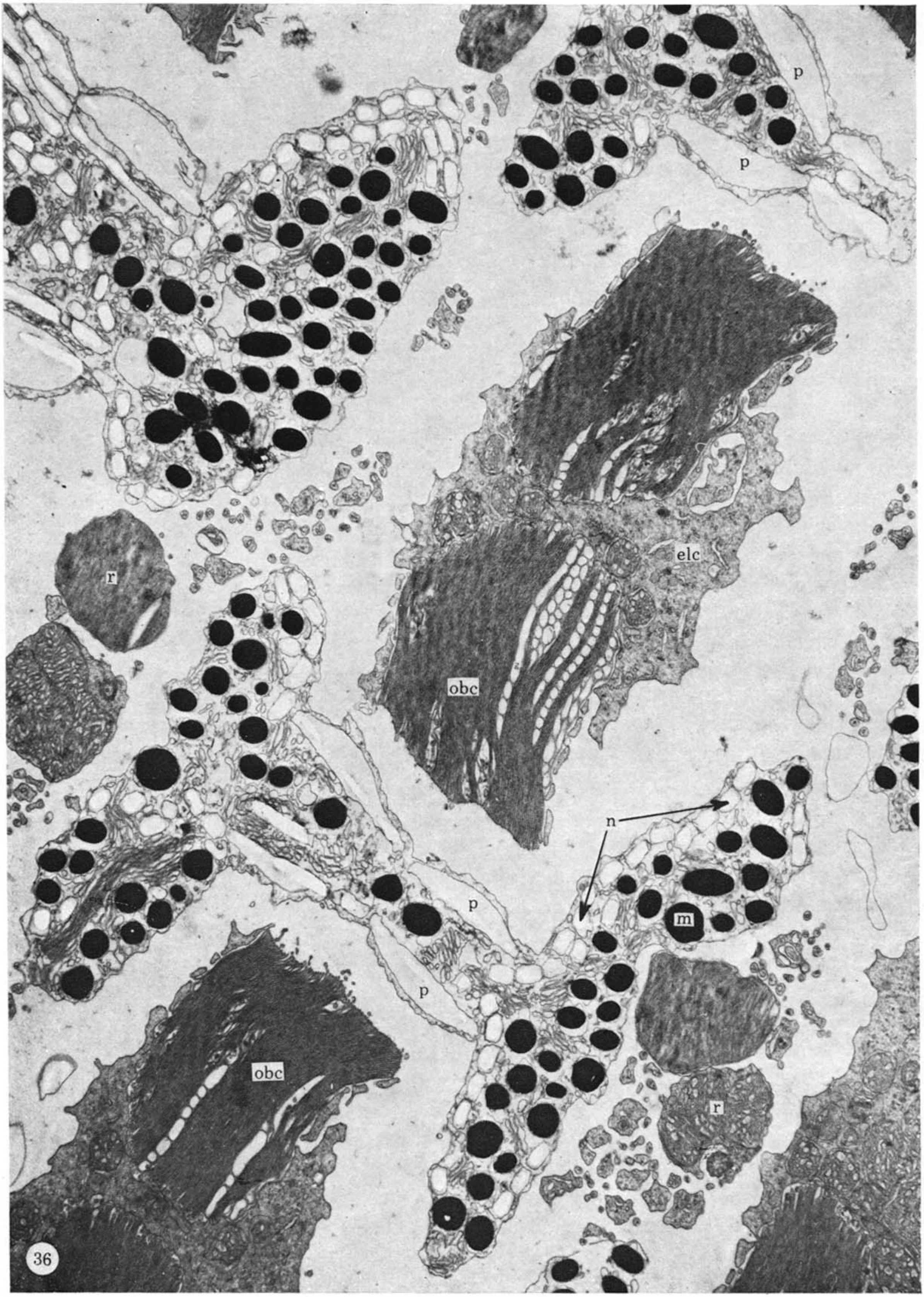








FIGURES 30–35. For description see opposite.



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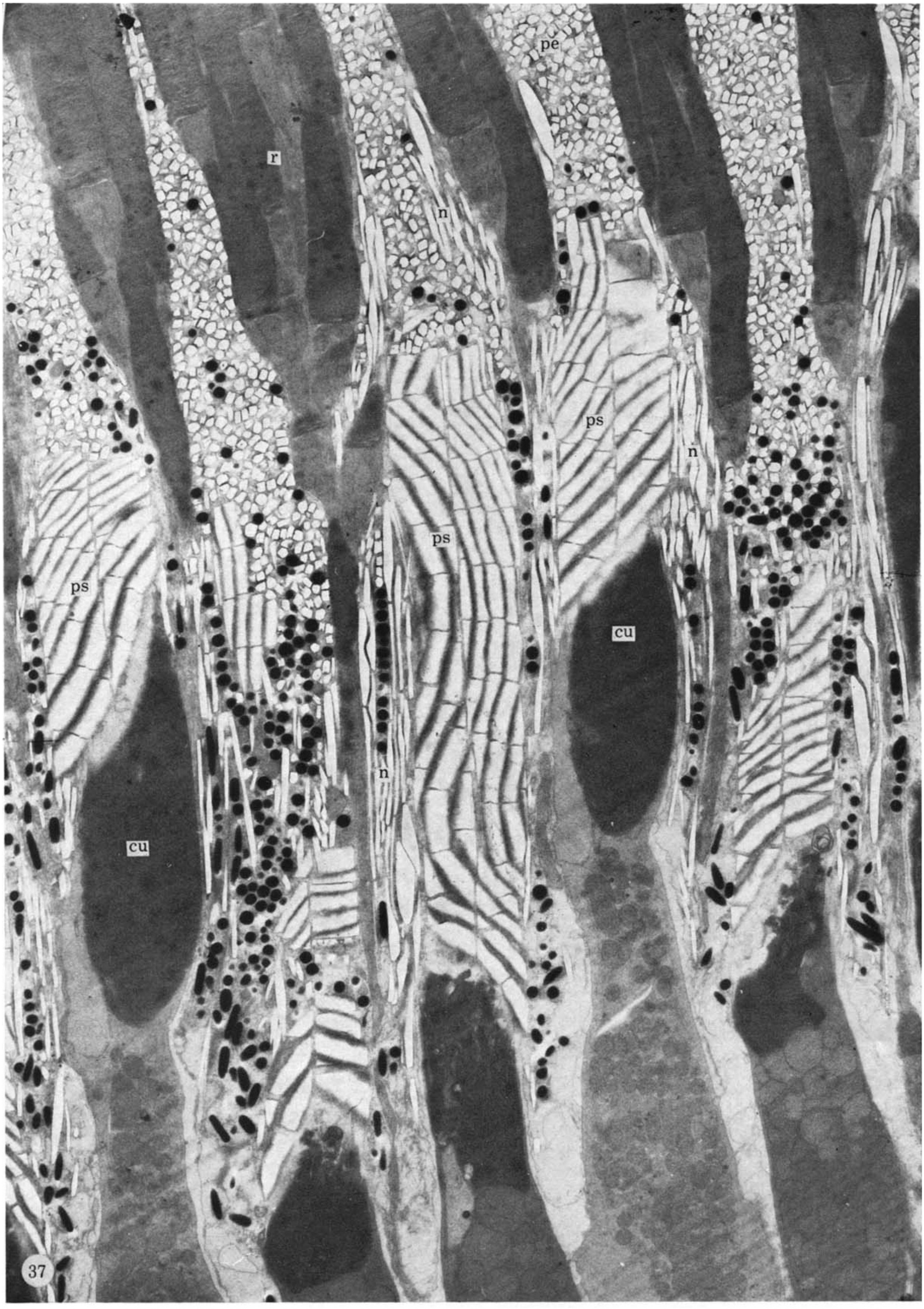

FIGURE 37. For description see opposite.